Continuous separation and manipulation of particles and cells using dielectrophoresis

Lewpiriyawong Nuttawut

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**CONTINUOUS SEPARATION AND MANIPULATION
OF PARTICLES AND CELLS USING
DIELECTROPHORESIS**

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**LEWPIRIYAWONG NUTTAWUT
SCHOOL OF MECHANICAL AND AEROSPACE
ENGINEERING**

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OF PARTICLES AND CELLS USING
DIELECTROPHORESIS**

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Nomenclature

a	Particle radius [m]
$\vec{E}$	Electric field [$V \cdot m^{-1}$]
$\vec{E}_{rms}$	Root mean square of electric field [$V \cdot m^{-1}$]
$\vec{d}$	Distance between + and - dipole charges [m]
F_{DEP}	Dielectrophoretic force [N]
F_{Drag}	Drag force [N]
F_{EOF}	Electroosmotic force [N]
F_{EP}	Electrophoretic force [N]
f	Frequency [Hz]
f_{cr}	Cross-over frequency [Hz]
i	Imaginary unit $\sqrt{-1}$
$\underline{f}_{CM}(\omega)$	Clausius-Mossotti factor
K_s	Surface conductance [nS]
m_p	Particle mass [kg]
$\vec{n}$	Normal unit vector
P	Pressure [$N \cdot m^{-2}$]
$\vec{p}$	Dipole moment [$C \cdot m$]
q	Charge [C]
$\vec{r}$	Position vector [m]
t	Time [s]
$\vec{t}$	Tangential unit vector
T	Temperature [K]
τ	Period of sinusoidal wave [s]
τ_{EO}	Electroosmotic relaxation time [s]
u_{EO}	Electroosmotic velocity [$m \cdot s^{-1}$]
u_{EP}	Electrophoretic velocity [$m \cdot s^{-1}$]

u_p	Particle velocity [$\text{m} \cdot \text{s}^{-1}$]
V_{DC}	DC voltage [V]
V_{AC}	AC voltage [V]
V_{Th}	Threshold voltage [V]
ω	Angular frequency [$\text{rad} \cdot \text{s}^{-1}$]
μ_{EP}	Electrophoretic mobility [$\text{m}^2 \cdot \text{V}^{-1} \text{s}^{-1}$]
ϵ_0	Free space permittivity [$\text{F} \cdot \text{m}^{-1}$]
ϵ_m	Medium permittivity [$\text{F} \cdot \text{m}^{-1}$]
ϵ_p	Particle permittivity [$\text{F} \cdot \text{m}^{-1}$]
σ_m	Medium conductivity [$\text{S} \cdot \text{m}^{-1}$]
σ_p	Particle conductivity [$\text{S} \cdot \text{m}^{-1}$]
ρ	Medium density [$\text{kg} \cdot \text{m}^{-3}$]
ρ_p	Particle density [$\text{kg} \cdot \text{m}^{-3}$]
ϕ	Electric potential [V]
φ	Phase of electric field [rad]
η	Medium dynamic viscosity [$\text{kg} \cdot \text{m}^{-1} \text{s}^{-1}$]
ζ_w	Zeta potential of channel wall [V]
ζ_p	Zeta potential of particle [V]
$\text{Re}[\dots]$	Real part of a complex number
$\text{Im}[\dots]$	Imaginary part of a complex number
$ \dots $	Magnitude operator
$\langle \dots \rangle$	Time average operator
$\nabla \dots$	Del operator
$\nabla^2 \dots$	Laplace operator

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Abstract

Cell manipulation and separation are critical in biomedical diagnosis. Typical techniques such as flow cytometry require costly and bulky instruments. Microfluidics holds promise in minimization and integration of such biomedical routine processes onto a portable and affordable chip. It also offers faster analysis with less sample/reagent volume. Field-Flow Fractionation (FFF) employing dielectrophoresis (DEP) in microfluidics is an emerging technique for continuous manipulation and separation of cells. Although microfluidic DEP-FFF techniques can overcome some FFF-associated problems such as sample band broadening and slow separation, most microfluidic DEP-FFF devices utilize AC electric field generated by metal electrodes fabricated on silicon or glass substrates. This study focuses on implementation of DEP-FFF techniques into polymer microfluidic devices for continuous separation, sorting, and concentration of particles and cells.

The modified PDMS-based H-filter platform with multi-insulating blocks is developed for sorting and continuous separation of particles. The use of a single-channel DC power supply greatly simplifies the device operation. The multi-insulating blocks not only can enhance DC-DEP force but also focus particles in a small region, thereby facilitating particle separation. In order to establish critical guidelines for optimal device configurations, the effects of the number and geometrical structures of insulating blocks on the threshold voltages are investigated. The effectiveness of the proposed technique using combined AC and DC field is experimentally and numerically evident by reducing the threshold voltage in separation. Moreover, a novel microfluidic technique utilizing a

combined AC and DC electric field is also developed for particle and cell concentration under a continuous-flow condition. Findings obtained from both the experiments and simulation show a significant reduction in the threshold voltage by 85.9%. Experimental results suggest that higher buffer concentration, larger particle size and higher ratio of AC-to-DC electric fields improve DEP concentration performance.

In addition, a new PDMS-based microfluidic device with 3D conducting PDMS composites as sidewall electrodes is developed for characterization and separation of particles and cells by AC-DEP. The developed fabrication technique greatly facilitates (i) the integration of the conducting PDMS composite electrodes with PDMS microchannels, and (ii) device assembly by using only oxygen plasma treatment. With these features, the device can be operated at relatively high flow rates without liquid leakage. Unlike conventional 2D planar electrodes, 3D conducting PDMS electrodes can produce 3D electric field that distributes uniformly throughout the entire channel height and varies along the channel lateral direction, thereby giving rise to stronger DEP forces and also allowing for lateral manipulation and observation of particles and cells. The high efficiency (>97%) proves the device capability to effectively separate various samples including submicron particles, micron particles, live and dead yeast cells and bacterial cells under continuous-flow conditions.

Chapter 1 Introduction

1.1 Background and motivation

Sorting, separation and concentration of target cells are of broad importance in a large number of applications. In biomedical diagnosis, separation of target cells from biosamples such as platelets, white or red blood cells from blood is a common practice in modern laboratories and hospitals. To enhance the effectiveness of artificial fertilization, healthy sperm cells must be sorted from unhealthy ones [1]. In environmental monitoring, diagnosis of water quality involves identification, separation and counting of live bacterial cells [2]. In addition, the concentration of samples is a key factor that indicates the efficiency in biochemical reaction and sample detection [3, 4]. In gene expression analysis, a large number of cells are necessary to be concentrated for RNA preparation. For clinical and industrial standards, some of the ruling separation and detection techniques are centrifugation, chromatography, flow cytometry and fluorescence-activated cell sorters (FACS) [5]. Drawbacks associated with existing techniques include (i) non-continuous separation process which leads to longer analysis time, (ii) inefficient collection of separated samples, and (iii) the need for highly trained personnel to operate bulky and costly instruments, and a large volume and modification of samples [5-9].

Given the trend to miniaturize devices from macro to micro scales, Lab-on-a-chip (LOC) or micro-total analysis system (μ TAS) is an fast-emerging interdisciplinary technology that integrates biomedical and chemical processes in a

typical laboratory onto a single chip [10]. In LOC, microfluidics exploits the characteristics of a laminar flow to provide a precise control of fluid in nanolitres and manipulation of thousands of cells through either a pressure gradient or electrokinetics. Due to their miniaturized size, microfluidics-based devices become more portable, require a small volume of samples and shorter analysis time. Thereby, microfluidics has become a promising technology for development of future inexpensive clinical and point-of-care devices. Applications using microfluidics include manipulation, separation and identification of cells, proteins and DNA, drug delivery and rapid diagnosis of complex diseases [11]. Thus, this thesis research focuses on the continuous sorting, concentration and separation of particles and cells in microfluidic devices.

Numerous technologies have been developed to achieve continuous-flow separation of samples. Field-Flow Fractionation (FFF) has been engineered using the principle similar to chromatography but without stationary phase [12]. FFF transports the samples by a pressure-driven flow and fractionates them using an externally applied field perpendicular to the flow. FFF subtechniques are classified by external fields: flow (FIFFF), magnetic (MgFFF), electrical (EIFFF) and dielectrophoresis (DEP-FFF). Among these subtechniques, DEP-FFF is the most promising technique that has exploited microfabrication and microfluidics for continuous-flow separation of samples such as human leukemia (HL-60) cells [13], breast cancer cells [14, 15] and yeast cells [16]. DEP separates cells based on the relative polarizabilities between the cells and their suspending medium in non-uniform electric field [17]. This makes DEP a suitable tool to separate different

cells without pretreatment. Since DEP effects are often produced by AC electric field generated by metal electrodes fabricated on the substrate of channel surfaces, small amount of AC voltages (e.g., from several to tens of voltages) are required and also such AC voltages can be readily programmed through electrical control. However, the separation efficiency of DEP-FFF may be compromised by the overlapping of sample zones due to band broadening and relaxation time [12]. In addition, DEP-FFF suffers from an exponential decay of electric fields from the surface of planar electrodes [18, 19]. The cells located far from such surface are not affected by DEP. To overcome these drawbacks, microfluidic devices with three-dimensional (3D) electrodes have been developed. 3D electrodes can produce electric field uniformly throughout the entire height of device channels, allowing for manipulation of cells located at any height.

With rapid development of soft lithography, polydimethylsiloxane (PDMS) is now widely used as the fabrication material of choice in microfluidic devices, owing to beneficial features: ease of fabrication and bonding, optical transparency, biocompatibility, and low cost [10, 11, 20]. However, it remains difficult to implement AC-DEP in electrode-based microfluidic devices, because adhesion between metallic electrodes and PDMS is poor. Therefore, it would be worthwhile to develop a new fabrication technique that can facilitate the integration of 3D electrodes into a PDMS-based microfluidic device; this will allow AC-DEP to be used in order to achieve continuous cell sorting and separation.

An alternative to electrode-based technique, insulator-based DEP (iDEP) has also been developed for concentration and separation of biosamples such as

virus, bacteria and DNA [21-23]. The iDEP utilizes insulating posts to produce non-uniform electric field [24]. A direct-current (DC) electric field over the insulating posts is generated via external electrodes remotely placed at inlet and outlet reservoirs. This DC-iDEP employs linear electrokinetics: electrophoresis (EP) and electroosmosis (EO) to transport samples and nonlinear electrokinetics: DEP to concentrate or separate the samples. Compared with electrode-based DEP devices, the iDEP offers following major advantages. First, device fabrication is simplified and relatively inexpensive because fabricated metal electrodes are not needed. Second, the device assembly through thermal bonding provides excellent prevention of liquid leakage. Third, the insulating posts are less prone to fouling, mechanically robust, chemically inert and therefore compatible with biomedical applications. Forth, since the only required electrodes are placed remotely through reservoirs, the device can be operated under a DC electric field without gas evolution due to electrolysis. Finally, EP, EO and DEP can be simultaneously utilized for manipulating fluid flow and samples without mechanical moving parts.

The commercially available microfluidic H-filter is a well-known device developed for continuous extraction of analytes without the need of filtration membrane [25]. The device utilizes mass diffusion as a driving force in extracting samples from one fluid stream to the others, and its platform also facilitates easy collection of analytes [25, 26]. However, the separation function of this device can suffer from low efficiency and even fail, especially for large-sized bioparticles such as cells whose mass diffusivity is very low, and the difference of mass diffusivities between cells is not significant. To achieve higher efficiency in continuous cell

separation, the study would also focus on development of new microfluidic H-filter platform which can integrate DC-iDEP as separating force, and the use of combined AC and DC electric field will also be explored.

In terms of sample concentration, most iDEP devices rely on DC electric fields. Thus, in addition to cell separation, this study will also incorporate the use of the combined AC and DC electric field for concentration of particles and cells.

1.2 Objective and scopes

The main objective of this study is to achieve continuous-flow separation and manipulation of particles and cells in PDMS-based microfluidic systems using the principle of DEP-FFF. To accomplish this objective, the scopes of the thesis include as the following:

- (1) Develop a new PDMS-based microfluidic H-filter platform using iDEP for continuous-flow separation and manipulation of particles under (i) a DC electric field and (ii) a combined AC and DC electric field.
- (2) Report a new electrokinetic technique using iDEP generated by combined AC and DC electric field for continuous-flow concentration of particles and cells in a PDMS-based microfluidic device.
- (3) Present a new, robust and cost-effective fabrication technique that can integrate 3D electrodes into a PDMS-based microfluidic device to allow for using AC-DEP for continuous sorting and separation of particles and cells.

1.3 Outline of the thesis

The complete thesis comprises the following main sections:

Chapter 1 introduces the background and motivation of this work. The latest biomedical separation technologies and recent development of microfluidics using DEP for continuous separation of samples are briefly reviewed. The objectives of this thesis research are clearly stated.

Chapter 2 provides a review on the separation techniques in laboratories and medical institutions. Principles of linear and nonlinear electrokinetics are then illustrated. A broad literature review of FFF and microfluidics technologies especially using DEP for continuous separation and manipulation of particles and cells is presented.

Chapter 3 reports new development of a modified PDMS-based H-filter with multi-insulating blocks for flow-through manipulation of particles by size using iDEP under (i) a DC electric field and (ii) a combined AC and DC electric field. Modeling of particle trajectory based on the Lagrangian approach is then presented. Particle sorting and separation are demonstrated. In addition, the chapter also presents the use of iDEP for (i) separation of multiple particles, and (ii) manipulation of single-walled carbon nanotubes (SWCNTs).

Chapter 4 presents a new electrokinetic technique for concentration of particles and cells by using iDEP generated by a combined AC and DC electric

field. Effects of experimental parameters on iDEP concentration of samples are discussed. Numerical modeling based on the Lagrangian approach is presented.

Chapter 5 reports a new fabrication technique that allows 3D conducting PDMS composite electrodes to be embedded into a PDMS-based microfluidic device for continuous separation of particles and cells by using AC-DEP. The fabrication process, separation mechanisms and numerical results are then presented. Finally, manipulation and continuous separation of particles, yeast cells and bacterial cells are demonstrated and investigated.

Chapter 6 summarizes the major findings and contributions of this work. Directions for further studies are briefly outlined.

Chapter 2 Literature Review

2.1 Introduction

A broad body of literature has been conducted to review studies reported on the development of conventional and latest techniques for manipulation and separation of particles and cells. These techniques usually involve in multidisciplinary principles from mechanical, chemical, biological to electrical backgrounds. In laboratories and medical institutions, common separation techniques are micro/ultra-filtration, centrifugation, chromatography, flow cytometry and fluorescence-activated cell sorting (FACS). Micro/ultra-filtration and centrifugation are traditional techniques which rely only on the physical difference in the cells' size, shape and density in separation. However, they do not provide high selectivity in isolating different types of cells (e.g., live and dead cells) [6, 27, 28]. Moreover, collection of the isolated samples in the same centrifuge tube becomes rather inconvenient and inefficient [27, 28].

Chromatography is primarily a separation technique mostly employed for chemical and biomolecular analysis. The technique isolates multiple components from a mixture and also offers quantitative assess of each constituent [6]. This separation process is performed by distributing the components of a mixture between two phases: a stationary phase (e.g., beads or a porous monolithic membrane packed in a column) and a mobile phase (e.g., a solvent). This is known as column chromatography. The mobile phase carries the components under

pressure through the column that has the stationary phase packed or bonded. The component, which has a stronger interaction or affinity with the stationary phase, is retained longer, leaving the other to elute first. However, column chromatography has intricacies during its tedious process, and requires technical skills from a user to frequently change a collection container and fill up the solvent. Thus, these hindrances become disruptive during the whole separation process [6]. Some analysis may require costly and sophisticated instruments. Irreversible adherence of biological samples on the stationary phase was also reported to cause structure deformation and denaturation to the samples [12]. To perform separation in a lesser time and collect the samples more effectively after the process, separation must be performed in a continuous manner.

Flow cytometry and FACS are the most advanced techniques that allow continuous flow-based separation, fast analysis and high selectivity. The separation and analysis are based on the samples' size, shape, density, affinity to antibodies and fluorescent emission for counting, sorting and analysis. Thousands of samples such as blood cells, bacterial cells, and virus cells can be analyzed within seconds. Despite the advantages surpassing other medical-standard techniques, their peripheral equipment is complex and bulky. They are quite costly and require highly trained personnel to operate. In addition, modification of sample surfaces with expensive reagents often is necessary and can potentially alter the properties of cells in subsequent steps of analysis [5, 7-9].

Using the fast emerging LOC technology, biomedical and chemical processes can be integrated in a single chip in which microfluidics is utilized to provide a precise control of fluid and samples. Because of its portable size, a microfluidic device requires a smaller volume of samples and shorter analysis time. These advantages show the potentials in development of future inexpensive clinical and point-of-care devices.

With an inspiration to achieve continuous-flow separation of samples, Field-Flow Fractionation (FFF) was developed using the principle similar to chromatography but has no stationary phase to overcome the aforementioned hindrances [12]. In the development of FFF, external separating field forces can be generated by a gravitational field, flow field, magnetic field or electrical field, depending on the suitability of the field and samples. In the present study, non-uniform electric field is extensively used as an external field in the separation and manipulation of biological samples and particles. The fundamental of electrically-driven manipulation of flow and particles involved electrokinetic phenomena is therefore given before reviewing details of the FFF family. Subsequently, the present chapter reviews the development of other promising separation techniques which are fundamentally based on the principle of FFF using a non-uniform electric field. These techniques are integrated with various kinds of electrodes: planar/double-sided interdigitated electrodes, three dimensional (3D) electrodes, insulators, liquid electrodes and optically-induced electrodes. Advantages and disadvantages of each technique are also provided.

2.2 Electrokinetic phenomena

2.2.1 Electric double layer

Generally, most solid surfaces acquire surface electric charges and thus possess electrical surface potential when brought into contact with an aqueous electrolyte solution [29]. The presence of this charged surface in the aqueous solution influences the distribution of nearby ions. Counterions, ions of opposite charge to that of the surface, are attracted to the surface whereas coions, ions of like charge, are repelled away from the surface. This leads to the formation of an electric double layer (EDL).

In 1924, Stern proposed a model for EDL consisting of *compact* and *diffuse layers* (Figure 2.1 (a)) [30]. The compact layer, usually less than one nanometer, is immediately next to the solid surface where the counterions are strongly attracted and thus immobile. The diffuse layer is next to the compact layer and ranges from several nanometers to sub-micrometers. Since repelled away from the compact layer, ions in the diffuse layer are mobile. The net charge density in EDL is nonzero so as to neutralize the charged solid surface. According to Stern's model, the divided surface between the immobile inner part and the mobile outer part of EDL is named as the *Stern plane*. The *Shear plane*, approximately located between one to two radii away from the surface, is the plane where no-slip velocity boundary condition is assumed. As shown in Figure 2.1 (b), with increasing distance from the charged surface the electric potential varies from the surface potential (ψ_w), to the

Stern potential (ψ_s), and to the zeta (ζ) potential at the shear plane. . The zeta potential is an experimentally determined parameter which characterizes the strength of EDL. Its value can be influenced by a group of factors, such as temperature, concentration and pH of an electrolyte solution, level of surface absorption, surface material etc. Outside the EDL, the electrical potential is zero in the bulk phase.

Figure 2.1: (a) Schematic configuration of the electric double later (EDL) of a negatively charged wall and (b) the potential distribution in EDL [30]

2.2.2 Electroosmosis

In most of cases, a solid surface bears a negative charge when brought in contact with an electrolyte solution. With EDL formed near the charged surface, an applied electric field exerts an electric body force (or columbic force) on excess positive counterions. The motion of the counterions drags the surrounding fluid with them to generate bulk electroosmosis (EO). For a micro-scaled channel, the EDL thickness is on the order of several to ten nanometers for a wide range of ionic concentrations of the solution [31]. Thereby, the existence of EDL is generally considered negligible in a micro-scaled channel. For the cases of very thin EDL, the velocity profile of EO flow in a microchannel is plug-like. The well-known Smoluchowski equation determines the magnitude of flow velocity expressed as [32],

$$\vec{u}_{EO} = -\frac{\epsilon_m \zeta_w}{\eta} \vec{E} \quad (2.1)$$

where $\epsilon_m = \epsilon_m^* \epsilon_0$ is the permittivity of the medium. ϵ_m^* and ϵ_0 are respectively the dielectric constant of medium and the permittivity of free space, η is the viscosity of the medium, E is the applied electric field and ζ_w is the zeta potential of the channel wall.

2.2.3 Electrophoresis

In EO, the stationary phase is a wall surface and the mobile phase is liquid. In contrast, electrophoresis (EP) refers to the motion of an electrically charged particle

in a stationary bulk liquid when subjected to an external electric field [29]. Figure 2.2 shows an EDL formed near the surface of a negatively charged particle, and the corresponding motion of such charged particle is then towards the positive electrode.

Figure 2.2: Electrophoresis of a negatively charged particle in an external electric field (the image is drawn not to scale) [29]

In typical microfluidic applications, the estimated EDL thickness of a microparticle in a 10^{-3} M electrolyte solution is on the order of 10 nm, which is considered relatively thin to the particle size [29]. Without the polarization and retardation under the thin EDL assumption, the EP force acting on a spherical particle of radius a is given by [32]

$$\vec{F}_{EP} = 6\pi a \epsilon_m \zeta_p \vec{E} \quad (2.2)$$

where ζ_p is the particle zeta potential. Under this EP force, the particle moving in a stationary liquid with a EP velocity of $\vec{u}_{EP}$ experiences a drag force given by Stokes' law [32],

$$\vec{F}_{Drag} = f\vec{u}_{EP} \quad (2.3)$$

where $f = 6\pi\eta a$ is the Stokes frictional factor for a spherical particle in a creeping flow. Under the steady state, the force balance between the EP and the drag (frictional) force results in the particle EP velocity

$$\vec{u}_{EP} = \frac{\epsilon_m \zeta_p}{\eta} \vec{E} \quad (2.4)$$

For a negatively charged particle in a negatively charged microchannel filled with an electrolyte solution, the effective particle velocity is simply a combination of EP and EO velocities (equation 2.5). The particle motion is dominated by EP when the zeta potential of the particle is greater than that of the channel wall.

$$\vec{u}_p = -\frac{\epsilon_m (\zeta_w - \zeta_p)}{\eta} \vec{E} \quad (2.5)$$

2.2.4 Dielectrophoresis

Dielectrophoresis (DEP) refers to the motion of a neutrally charged particle in non-uniform electric field as a result of the interaction of induced dipoles in the polarizable particle and the applied non-uniform electric field [17]. Such interaction

is also known as polarization effects. Under a DC or AC electric field, DEP is different from EP as depicted in Figure 2.3 and Figure 2.4 [17]. A positively charged particle and neutral particle are brought into a medium where a uniform DC electric field is generated by two parallel electrodes (Figure 2.3 (a)). While the positively charged particle is induced towards the negative electrode along the electric field line, the neutral particle is simply polarized. The negative charges in the neutral particle are pulled towards the positive electrode and reside in the left portion of the particle whereas the positive charges prefer to stay near the negative electrode on the right portion. This charge configuration represents a dipole. Because the electric forces acting on both portions of the neutral particle are equal in magnitude but opposite in direction, the net force is zero and no motion occurs.

However, the neutral particle begins to move in the non-uniform electric field (Figure 2.3 (b)). In the neutral particle, the number of positive and negative charges is equal. The net force resulting from both sides of the particle becomes non-zero since the electric field is non-uniformly distributed. The higher strength of electric field near the positive electrode causes a larger force to pull the particle towards the positive electrode. For the charged particle, EP still remains and its direction is along the electric field line towards the electrode carrying the charge opposite to that on the charged particle.

Figure 2.3: Motion of a charged particle and a neutral particle in (a) a uniform DC electric field and (b) a non-uniform DC electric field [17]

Figure 2.4 shows the motion of a charged particle and a neutral particle when the polarities of electrodes are alternating. Due to high frequency, the charged particle keeps stationary, while the neutral particle, after the dipole is re-oriented according to the polarities of electrodes, remains the same direction towards the region of higher electric field.

Figure 2.4: Motion of a charged particle and a neutral particle in a non-uniform AC electric field [17]

To study the behaviors of a neutral particle in a non-uniform electric field, the estimation of the net force on an infinitesimal dipole that consists of equal and

opposite charges (+q and -q) is performed [33]. In the spatial coordinate as shown in Figure 2.5, the location of the charge -q is described by the vector, $\vec{r}$, and a distance, $\vec{d}$, apart from the charge +q.

Figure 2.5: Finite dipole model in a non-uniform electric field [33]

Since the electric field is spatially non-uniform, the charges -q and +q will experience different electric fields, $\vec{E}(\vec{r}, t)$ and $\vec{E}(\vec{r} + \vec{d}, t)$, respectively. Then, the total force acting on the infinitesimal dipole can be written as

$$\vec{F}(t) = q\vec{E}(\vec{r} + \vec{d}, t) - q\vec{E}(\vec{r}, t) \quad (2.6)$$

Using Taylor series expansion, the electric field located at the charge +q becomes

$$\vec{E}(\vec{r} + \vec{d}, t) = \vec{E}(\vec{r}, t) + \vec{d} \cdot \nabla \vec{E}(\vec{r}, t) + \dots \quad (2.7)$$

where high-order terms such as d^2, d^3 and so forth are neglected. The equation (2.6) can be simplified as

$$\vec{F}_{DEP}(t) \cong \vec{p} \cdot \nabla \vec{E}(\vec{r}, t) \quad (2.8)$$

where $\vec{p} = q\vec{d}$ is the dipole moment. Due to the infinitesimal dipole size, the distance $\vec{d}$ approaches zero, but it must remain finite. The dipole moment and the force in equation (2.8) will remain finite. It becomes clear that the force on the dipole can only be created when the electric field is non-uniform. Considering the accuracy of equation (2.8), the dipole force is valid under one important assumption, that is, the dimension of the dipole is much smaller than the variation scale in the electric field. This well-known dielectrophoresis assumption is adequate due to the much larger electrode sizes compared to the particle dimensions [17, 33, 34].

Under a sinusoidal AC electric field, $\vec{E}_{ac}(\vec{r}, t)$, the DEP force acting on a dielectric particle can be determined by using the Maxwell stress tensor formulation [35], or alternatively by using the effective dipole moment method [33]. The results from both methods have been shown to yield the identical DEP force expression [36]. However, because the latter is much simpler and provides valid predictive results, it is used to derive DEP force here. The method points out that the effective dipole moment of a neutral particle being polarized by an electric field is defined as the moment of an equivalent free-charge point dipole that is immersed in the same dielectric medium and located at the same point of the particle's center [33]. Assuming that the particle and suspending medium have dielectric permittivities of ϵ_p and ϵ_m , and finite conductivities of σ_p and σ_m , respectively. The general

expression for the complex effective dipole moment of a dielectric sphere with radius a that contains the magnitude and phase information of the effective dipole moment has been given as [33]

$$\vec{p}_{eff} = 4\pi\epsilon_m a^3 \underline{f}_{CM}(\omega) \vec{E}_{ac}(\vec{r}, t) \quad (2.9)$$

where $\underline{f}_{CM}(\omega)$, the Clausius-Mossotti (CM) factor or polarization factor, is a function of electric field frequency and complex permittivities of the particle and suspending medium. It also contains magnitude and phase information of the effective dipole moment.

$$\underline{f}_{CM}(\omega) = \frac{\underline{\epsilon}_p - \underline{\epsilon}_m}{\underline{\epsilon}_p + 2\underline{\epsilon}_m} \quad (2.10)$$

where $\underline{\epsilon}_p = \epsilon_p - i\sigma_p/\omega$ and $\underline{\epsilon}_m = \epsilon_m - i\sigma_m/\omega$ are the complex permittivities of the particle and suspending medium, respectively, $i = \sqrt{-1}$, and $\omega = 2\pi f$ is the radian field frequency. For a submicron-to-micron particle, the particle's conductivity σ_p is the sum of bulk conductivity ($\sigma_{p,bulk}$) and surface conductivity ($2Ks/a$) where $\sigma_{p,bulk} \sim 0$ is usually applied for latex particles and Ks is the surface conductance ranging from 0.2 to 2.1 nS [37, 38]. An integration of the instantaneous DEP force (equation 2.8) with the effective dipole moment (equation 2.9) over a full period of $\tau=2\pi$ yields the time-average AC-DEP force (Appendix A) [33, 39]

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} \int_0^{2\pi} (\vec{F}_{DEP}(t)) \cdot dt = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{rms,ac}^2 \quad (2.11)$$

where $\langle \rangle$ denotes the time-average, $\text{Re}[\underline{f}_{CM}(\omega)]$ is the real part of the CM factor and $-0.5 \leq \text{Re}[\underline{f}_{CM}(\omega)] \leq 1$. $\vec{E}_{rms,ac} = \vec{E}_{ac}/\sqrt{2}$ is the root-mean-square magnitude of the applied electric field. As seen in equation (2.11), the average DEP force is dependent on various parameters including the cube power of particle radius, the gradient of the square of applied electric field, electric field frequency, dielectric permittivity and electrical conductivity of the particle and suspending medium. The magnitude and sign of the DEP force depends on the value of $\text{Re}[\underline{f}_{CM}(\omega)]$. When the particle is more polarizable than the buffer medium (e.g., $\text{Re}[\underline{f}_{CM}(\omega)] > 0$), positive DEP (pDEP) attracts the particle to high electric field region as shown in Figure 2.6 (a). When the medium is more polarizable (e.g., $\text{Re}[\underline{f}_{CM}(\omega)] < 0$), negative DEP (nDEP) repels the particle away from the high electric field region as shown in Figure 2.6 (b).

Figure 2.6: Classification of DEP: (a) positive DEP (pDEP) and (b) negative DEP (nDEP)

To consider the limiting values of $\text{Re}[\underline{f}_{CM}(\omega)]$, the real part of the CM factor (equation 2.10) can be expressed as

$$\text{Re}[\underline{K}(\omega)] = \frac{\omega^2 (\epsilon_p - \epsilon_m)(\epsilon_p + 2\epsilon_m) + (\sigma_p - \sigma_m)(\sigma_p + 2\sigma_m)}{\omega^2 (\epsilon_p + 2\epsilon_m)^2 + (\sigma_p + 2\sigma_m)^2} \quad (2.12)$$

At either low or high frequency regimes, $\text{Re}[\underline{f}_{CM}(\omega)]$ is bounded by

$$\text{Re}[\underline{f}_{CM}(\omega \rightarrow 0)] \rightarrow \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}; \quad \sigma_p = \sigma_{p,bulk} + \frac{2Ks}{a} \quad (2.13)$$

$$\text{Re}[\underline{f}_{CM}(\omega \rightarrow \infty)] \rightarrow \frac{\epsilon_p - \epsilon_m}{\epsilon_p + 2\epsilon_m} \quad (2.14)$$

Equations 2.13 and 2.14 show that when $\omega \rightarrow 0$, $\text{Re}[f_{CM}(\omega)]$ is only related to the conductivities of the particle and suspending medium. Conversely, when $\omega \rightarrow \infty$, $\text{Re}[f_{CM}(\omega)]$ is only associated with the dielectric permittivities of the particle and suspending medium. However, $\text{Re}[f_{CM}(\omega)]$ becomes zero at the cross-over frequency, f_{cr} . As a result, the DEP force vanishes and thus gives no translational motion to the particle. This occurs when the particle and medium are equally polarized. By setting the value of $\text{Re}[f_{CM}(\omega)]$ to zero, the cross-over frequency can be obtained as

$$f_{cr} = \frac{1}{2\pi} \sqrt{\frac{(\sigma_p - \sigma_m)(\sigma_p + 2\sigma_m)}{(\epsilon_m - \epsilon_p)(\epsilon_p + 2\epsilon_m)}} \quad (2.15)$$

Unlike spherical particles, the CM factor of biological cells (e.g., yeast and bacterial cells) becomes complicated and can be computed by modeling the cells as consisting of three concentric layers: cells' walls, cytoplasm and nuclei (Figure 2.7) [40].

Figure 2.7: Model of a biological cell as a concentrically layered particle

The model represents a layered particle in which each layer has different radii and values of electrical conductivity and dielectric permittivity. In this case, the CM factor is expressed as

$$\underline{f}_{CM}(\omega) = \frac{\underline{\epsilon}_{cell} - \underline{\epsilon}_m}{\underline{\epsilon}_{cell} + 2\underline{\epsilon}_m} \quad (2.16)$$

where $\underline{\epsilon}_{cell}$ is the effective complex permittivity of the cell, and $\underline{\epsilon}_m$ is the complex permittivity of the suspending medium. Using the smeared-out sphere approach [41], the effective complex permittivity of the cell can be determined by

$$\underline{\epsilon}_{cell} = \underline{\epsilon}_1 \left[\frac{\left(\frac{r_1}{r_2} \right)^3 + 2 \left(\frac{\underline{\epsilon}_{23} - \underline{\epsilon}_1}{\underline{\epsilon}_{23} + 2\underline{\epsilon}_1} \right)}{\left(\frac{r_1}{r_2} \right)^3 - \left(\frac{\underline{\epsilon}_{23} - \underline{\epsilon}_1}{\underline{\epsilon}_{23} + 2\underline{\epsilon}_1} \right)} \right] \quad (2.17)$$

and

$$\underline{\epsilon}_{23} = \underline{\epsilon}_2 \left[\frac{\left(\frac{r_2}{r_3} \right)^3 + 2 \left(\frac{\underline{\epsilon}_3 - \underline{\epsilon}_2}{\underline{\epsilon}_3 + 2\underline{\epsilon}_2} \right)}{\left(\frac{r_2}{r_3} \right)^3 - \left(\frac{\underline{\epsilon}_3 - \underline{\epsilon}_2}{\underline{\epsilon}_3 + 2\underline{\epsilon}_2} \right)} \right] \quad (2.18)$$

where $\underline{\epsilon}_1$, $\underline{\epsilon}_2$ and $\underline{\epsilon}_3$ are the complex permittivity of cells' walls, cytoplasm and nuclei. Figure 2.8 shows the CM factor as a function of the frequency of the electric field, for yeast and bacterial cells suspended in DI water (2 $\mu\text{S/cm}$) and in a NaCl solution (380 $\mu\text{S/cm}$). The CM factor of yeast cells and *E. coli* is mostly positive when they are suspended in DI water (dashed line) and can be either positive or negative (depending on the electric field frequency) in a 380 $\mu\text{S/cm}$ NaCl solution (solid line). In contrast, the CM factor of latex particles in a 380 $\mu\text{S/cm}$ NaCl solution approaches -0.5 for the entire frequency spectrum, because the latex particles are non-conducting.

Figure 2.8: The CM factor as a function of the frequency of the electric field for a yeast cell (shown in a), an *E. coli* bacterium cell (shown in b) and a latex particle (shown in both a and b). The geometrical and electrical properties of yeast and

bacterial cells are taken from literature [41, 42]. The following properties are used for yeast cells: (radii, in μm) 2.5 for wall, 2.36 for cytoplasm, 2.35 for nucleus; (conductivities, in $\mu\text{S/cm}$) 140 for wall, 0.0025 for cytoplasm, 2000 for nucleus; (permittivities) 60 for wall, 6 for cytoplasm, 50 for nucleus. The properties used for *E. coli* are: (radii, in μm) 1 for wall, 0.98 for membrane, 0.975 for cytoplasm; (conductivities, in $\mu\text{S/cm}$) 500 for wall, 0.0005 for membrane, 1000 for cytoplasm; (permittivities) 60 for wall, 10 for membrane, 60 for cytoplasm.

For the case of DC electric field, the time-average DC-DEP force can be expressed as (Appendix B)

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[f_{CM}(\omega)] \nabla \vec{E}_{dc}^2(\vec{r}) \quad (2.19)$$

In addition to a pure AC or DC electric field, an AC electric field can also be biased with a DC electric field. Under the assumption that the real part of the CM factor for both AC and DC electric field is the same, the DEP force can be derived and expressed as (Appendix C)

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[f_{CM}(\omega)] \nabla (\vec{E}_{dc}^2(\vec{r}) + \vec{E}_{rms,ac}^2(\vec{r})) \quad (2.20)$$

The assumption is generally valid especially when the electric field frequency is below 10 kHz because the CM factor for the AC electric field approaches the value governed by the conductivities as shown in equation (2.13). In another case where spatial changes in the phase $(\varphi_x, \varphi_y, \varphi_z)$ of the sinusoidal AC electric field having

the respective magnitude (E_{x0}, E_{y0}, E_{z0}) are involved, the generalized DEP force can be expressed as [43],

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \left\{ \text{Re}[f_{CM}(\omega)] \nabla \vec{E}_{rms,ac}^2 + \text{Im}[f_{CM}(\omega)] (E_{x0}^2 \nabla \phi_x + E_{y0}^2 \nabla \phi_y + E_{z0}^2 \nabla \phi_z) \right\} \quad (2.21)$$

where $\text{Im}[f_{CM}(\omega)]$ is the imaginary part of the CM factor. Apparently, both equations (2.11) and (2.21) are identical without the variation in the phase of the applied electric field.

2.3 Field-Flow Fractionation (FFF)

Field-Flow Fractionation (FFF), pioneered by Giddings, is a flow-based separation methodology encompassing subtechniques, classified by externally applied fields [12]. The FFF methodology employs a pressure-driven flow to transport samples along an open-end channel and achieves retention of the samples by the action of an externally applied field perpendicular to the flow. With the open-end channel design, FFF allows for continuous separation and its channel walls are not necessary to be active with the samples for retention. Thus, the sample collection becomes faster and more efficient.

2.3.1 FFF operating modes

Normal (or Brownian) mode is the first FFF mode which is suitable for submicron particles ($< 1 \mu\text{m}$ in diameter) and is pertinent to a competition between an

externally applied field and diffusion force (Figure 2.9). As the particles are driven towards the accumulation wall, they build up their concentration gradient which creates diffusion opposing to the direction of the applied field force. The smaller particles having a higher value of mass diffusivity penetrate further into the parabolic flow profile and will be eluted first. The second mode is named as steric mode which is suitable for particles with a diameter above 1 μm . This mode is referred to balancing of an externally applied field force and a repulsive force that resists particles to penetrate the wall upon approaching it. With increasing size of particles, diffusion becomes insignificant. By definition, this mode limits the mean height of particles as one radius above the wall. Hence, because of the permeation of higher mean elevation of larger particles in the parabolic flow profile, the larger particles are eluted first. Therefore, the steric mode offers a reverse order of separation to the normal mode. Finally, similar to steric mode, lift-hyperlayer mode is for large particles ($> 1 \mu\text{m}$ in diameter), but the elevation of particle cloud is beyond the limit of one radius due to the hydrodynamic lift force. As the larger particles have a stronger hydrodynamic lift force against the applied field force, they lift further away from the wall than the smaller ones. Therefore, they obtain higher speeds of migration and achieve the first elution.

Figure 2.9: Equilibrium distribution of particles representing a balance of field-driven and diffusive transport [12]

2.3.2 Flow FFF

Flow FFF (FIFFF) drives a fluid containing samples by a pressure-driven flow and retain (or compress) the samples towards the membrane wall using an external crossflow field. The FIFFF device comprises two clamping blocks between which two porous frits are sandwiched to allow passage of the crossflow stream (Figure 2.10 (a)). A membrane is used as a bottom wall to prevent samples but allow fluid to pass through [44]. Different samples build up different concentration gradients based on the balance of the hydrodynamic force due to the cross flow and diffusivities. It was reported that the time taken for proteins to build up their concentration gradients could be much shortened by introducing the second crossflow through a frit inlet located right after the sample inlet (Figure 2.10 (b)) [45].

Figure 2.10: (a) FIFFF structures [44] (b) with a frit inlet [45]

FIFFF is capable of separating samples such as particles ranging from 0.001 to 50 μm in diameter, synthetic polymers and biological macromolecules in aqueous media [12, 46]. However, this technique remains inefficient because sample concentrations cannot stably formed above the porous membrane through which the crossflow stream always passes. This instability of sample concentration reduces the resolution in separation [12]. In addition, the device requires a series of pumps to supply different flow streams, making the operation complicated.

2.3.3 Magnetic FFF

Magnetic FFF (MgFFF) achieves separation by an external magnetic field perpendicular to a fluid flow. Nowadays, magnetic-based separation techniques increasingly receive attention in biomedical applications. The first theoretical basis of MgFFF was highlighted by Vickrey [47]. A characterization of the metallic oxide particles was later performed in the typical FFF channel design in which the bottom wall is attached to a powerful electromagnetic generator for accumulating the samples [48]. Further investigation was performed to enhance an feasibility of magnetic nanoparticle separation by using a quadrupole magnetic system in which concentric and symmetric magnetic fields are generated in a helical channel [49]. As shown in Figure 2.11 (a), the channel occupies a thin annular space between inner and outer radii, r_i and r_o , respectively. The sample is fed into the thin space of the 500 μm deep helical channel. Figure 2.11 (b) shows the actual helical channel [50]. The use of high gradient magnetic fields across the channel was reported to improve MgFFF efficiency [51]. MgFFF was also performed in a capillary tube [52]. The separation of 10 nm particles were achieved using superconducting magnet [53]. However, the technique is obviously limited to magnetic samples. More information on MgFFF can be found elsewhere [5].

Figure 2.11: (a) MgFFF quadrupole magnetic system [49]. (b) Helical channel fabricated by machining a 500 μm deep helical path into the surface of a 15 cm long Delrin cylinder which can be assembled tightly into a polished stainless steel tube [49, 50]

2.3.4 SPLITT FFF

Not only is the analytic application of small volumes needed but also a preparation of a large number of samples. SPLITT-FFF is generally used for the preparation of samples ranging from milligram to gram amounts. The SPLITT-FFF channel integrates additional splitters at the ends of entrance and exit (Figure 2.12).

Figure 2.12: SPLITT-FFF channel structure and separation mechanism [44]

By controlling the flow rate of the inlets and outlets, inlet splitting plane (ISP) and outlet splitting plane (OSP) are formed. The upper flow named as *feed stream* (a') with a lower flow rate brings samples into the channel and is squeezed by the bottom stream, called *carrier stream* (b') with a high flow rate. To achieve the particle separation, one type of particles must transport across the entire channel width to the exit (b). This means that the applied field such as diffusion or gravity should be strong enough to drive one of the groups across the OSP. The optimization of the applied field strength and channel dimension is thus so important that the device could provide the cleanest separation and the highest mass throughput is feasibly assured in a single run [54, 55]. The applications of SPLITT have been seen in many fields of studies such as environment [56] and oceanography [57]. One of them in biology was the separation of live sperm [58]. The applications of SPLITT are limited to the two-component separation owing to the FFF design with two splitters and outlets, and also cannot achieve narrow fractions in a single run. An attempt to operate the system within a single run can cause overloading and reduce separation efficiency [12].

2.3.5 Electrical FFF

Electrical FFF (ElFFF) was introduced to integrate an external electric field in separating proteins [59, 60]. As illustrated in Figure 2.13 (a), semipermeable membranes of cellulose were used as channel walls which separate positive and negative (platinum) electrodes from the flow. Graphite plates were also used as channel walls and simultaneously electrodes (Figure 2.13 (b)). It was reported that

although the effective dc electric field was discovered to be sufficiently high to retain the proteins, the field strength was estimated to be only less than 1% of the applied electric field due to electrode polarization. Electrode polarization occurs when EDL at the electrode-electrolyte interface, considered as a capacitance, is fully charged by an externally applied voltage. As a result, a small amount of current can only pass through EDL, and the electrical potential is significantly reduced across it [61-65].

Figure 2.13: (a) Separation mechanism of IIEFF [59]. (b) Modified IIEFF channel with graphite electrodes [60]

Several attempts were reported to improve the performance of IIEFF. The application of a pulsed electric field was found to boost the effective electric field strength across an electrolyte medium to be 50 folds stronger in comparison with the case of the dc electric field [62]. Further investigations of the motion of charged nanoparticles under a pulse electric field via planar electrodes (Figure 2.14 (a)) and segmented electrodes (Figure 2.14 (b)) provided significant findings [66]. With the segmented electrodes, the particle motion was governed by DEP which was independent of the polarity change of the applied electric fields, thus resulting in stable and controllable average heights of different subpopulations. In contrast, with

the planar electrodes, the particle motion was governed by EP which was sensitive to the polarity change of electric fields that induced large fluctuating displacement of the particles and high possibility of overlapping of two subpopulations. These important findings turned an attention from the development of EIFFF to DEP-FFF.

Figure 2.14: Schematic diagrams of the motion of charged nanoparticles under a pulsed electric field via (a) planar electrodes and (b) segmented electrodes [66]

2.3.6 Dielectrophoresis FFF

In 1986, the use of DEP in FFF was first theoretically and numerically examined in an annular channel. The study suggests the feasibility of using DEP in FFF (DEP-FFF) by separating ultrahigh-molecular-weight polymers and submicron particles suspended in organic liquids [67]. With the advancement of microfabrication technologies, interdigitated gold parallel and castellated electrodes have been extensively integrated in DEP-FFF devices by using sputtering process and lift-off technique. Due to the miniaturized size of microfabricated electrodes, an electric field gradient and the resulting DEP force are significantly enhanced. DEP-FFF devices are operated under a non-continuous-flow or continuous-flow condition.

Under the non-continuous-flow condition, the particles or cells injected into the DEP-FFF channel are allowed to distribute themselves under pDEP or nDEP before the channel is flushed [16]. The locations of cells depend on the distribution of $E \cdot \nabla E$. As shown in Figure 2.15 (a), the maximum values of $E \cdot \nabla E$ occur at the electrode edges (label B) while the minima of $E \cdot \nabla E$ are at the electrode base regions (label A) [68]. Figure 2.15 (b) shows that at an electric field frequency of 30 kHz, murine erythroleukemia ($\uparrow$) cells experiencing pDEP are directed towards the electrode edges but normal murine erythrocytes ($\uparrow\uparrow$) experiencing nDEP are collected at the electrode base regions.

Figure 2.15: (a) Distribution of $(E \cdot \nabla)E$ and (b) collection of normal murine erythrocytes ($\uparrow\uparrow$) at the electrode base regions and erythroleukemia ($\uparrow$) at the electrode edges [68].

Furthermore, Marx *et al.* also separated viable from non-viable yeast cells [16]. At 10 MHz, the viable cells under pDEP were retained at the electrodes

against the hydrodynamic force while the non-viable cells under nDEP are washed away. Although a series of short-time flushing for cleaner cell separation was employed, the system still must need extra time for the cells to reach equilibrium after each on-off flushing [69]. In addition, other electrode designs were adopted to achieve microparticles and single-cell trapping by using multilayered grid electrode array [70], ring electrodes [71] and combined quadrupole and microwell electrodes [72].

In the continuous-flow condition, particles injected into the FFF channel attain different equilibrium heights based on the balance of nDEP, hydrodynamic lift and sedimentation (or gravitational) forces (Figure 2.16). The particles at the higher equilibrium height having a higher velocity in the pressure-driven flow will be eluted prior to the others. Figure 2.16 also shows the DEP-FFF device with interdigitated microelectrodes that induce spatially non-uniform electric fields necessary for producing DEP forces to levitate the particles [73]. This principle of DEP-FFF was successfully used for the separation of polystyrene latex beads [19] as well as human leukemia (HL-60) cells from peripheral blood mononuclear cells [13].

Figure 2.16: A DEP-FFF device with interdigitated electrodes and particle levitation by a nDEP force [73]

In another scenario, different types of particles may experience different types of DEP forces. The particles experiencing nDEP are levitated above the electrodes while the others undergoing pDEP are attracted to the electrodes. After flushing with a flow, the device elutes the levitated particles but remains the attracted particles which can be subsequently collected after the electric field is turned off. This simple and efficient mechanism has been exploited for separating various biological samples such as viable from non-viable yeast cells [74], erythrocytes from latex beads [75], different types of white blood cells [76] and cultured human breast cancer M4DA-435 cells from normal blood cells [14, 15]. Other flow-through applications using interdigitated microelectrodes also include cell lysis and trapping [77], immobilization of probe beads for affinity assay [78] and cell genetic detection [79].

2.3.7 Band broadening and relaxation time

In practice, two inherent problems of FFF are commonly referred to as band broadening and relaxation time. Figure 2.17 illustrates a complete fractionation of two subpopulations and detector response, showing the number of cells versus elution time. In comparison with the first subpopulation, the second subpopulation having a higher mean-height shall completely be eluted first with the higher velocity as shown in Figure 2.17 (a). The detector signals the first normal distribution of the second subpopulation in the initial elution time followed by an absent distribution due to the complete separation and lastly signals the second normal distribution of the first subpopulation as shown in Figure 2.17 (b).

Figure 2.17: Schematic diagram of (a) complete fractionation and (b) detector response: number of cells versus elution time [12]

First, band broadening is the key factor that causes the overlapping of sample zones during fractionation, thus leading to incomplete fractionation. Two sources of band broadening are described below. The first source is the different displacement velocity of individual particles due to their different locations in the pressure-driven flow profile (Figure 2.18 (a)). As the particles at the top portion of

the first subpopulation are much faster than those at the bottom, the sample band is broadened. If the difference in equilibrium heights of two subpopulations is not significant, the particles at the top portion of the first subpopulation with the relatively high local velocity would catch those at the bottom portion of the second subpopulation. Thus, the first sample band has overlapped the second one, and this is unfavorable upon collection or detection.

The second source is diffusive or random dispersion of samples. Although the equilibrium heights of two subpopulations are so different that they transport without overlapping, the random dispersion due to sample diffusion in an axial direction (flow direction) may contribute to band broadening (Figure 2.18 (b)). Figure 2.18 (c) exemplifies the detected signals showing the overlapping of samples, as highlighted by a red circle, for the separation of granulocytes from monocytes in DEP-FFF [44, 80].

Figure 2.18: Schematic illustrations of band broadening due to (a) different displacement velocities (b) random dispersion of samples, and (c) the overlapping of samples: granulocytes and monocytes in DEP-FFF [80]

Besides band broadening, the second hindrance is that the FFF device in practice often requires certain time for injected samples, initially distributed entirely across the channel thickness, to reach their steady-state exponential form before they are eluted. Such time is referred as relaxation time or stop-flow time in FFF [12]. The channel length and/or field strength can be increased to shorten the relaxation time to allow complete fractionation of all subpopulations.

2.4 DEP-FFF for lateral separation

In addition to band broadening and relaxation time, the DEP-FFF device using planar electrodes also encounters two key drawbacks. First, a DEP force is only strong near the surface of planar electrodes but decays exponentially at a further distance [18, 19]. As a result, the manipulation of cells far away from the electrode surfaces becomes ineffective. Second, the DEP-FFF device structure with an outlet usually set in a vertical direction is not compatible with most 2D on-chip microfluidic networks for subsequent or parallel analysis processes [81].

Dürr *et al.* developed a microfluidic device with electrode pairs (or so called deflectors) embedded on both bottom and top channel substrates [82]. The deposition of double-sided electrodes allows an electric field to distribute along the channel height (Figure 2.19 (a)). Figure 2.19 (b) illustrates the lateral separation of microparticles by size, using a pair of electrodes inclined with the flow direction. The larger particles experiencing larger repulsive DEP forces are pushed to outlet 2 while allowing the smaller to pass through the electrodes. Besides the simple pair of straight electrodes, similar developments including trapezoidal and slanted electrode arrays and a triangular electrode pair were also reported to achieve separation of microparticles and cancer cells [83-86]. Park *et al.* [84] recently proposed a 3D asymmetric fan-shaped electrode system that can produce electric fields of continuously varying magnitudes along the transverse direction of a channel to increase sensitivity of cell sorting in comparison with the typical double-sided electrode systems. With these abilities to manipulate cells laterally, the

compatibility of the device with other 2D on-chip analysis processes becomes simpler and straightforward.

Figure 2.19: (a) Electric field distribution along the channel height. (b) Lateral size-dependent separation with a pair of electrodes inclined with the flow direction [82]

In a more advanced electrode system, electrode pairs are aligned under different angles with respect to the direction of flow as shown in Figure 2.20. Kim *et al.* [87] achieved the lateral separation of multiple bacterial cell types with distinct synthetic DEP tags in multi-target DEP activated cell sorter (MT-DACS) assisted by a hydrodynamic focusing. Similar investigations utilizing DACS with/without hydrodynamic focusing were also reported to achieve separation of microparticles and platelets from dilute blood samples [88-92]. Other applications include focusing of nanoparticles [93], sorting and separation of microparticles [83] and cells [94], trapping of microparticles [95]. However, since the technique relies on electrodes on both bottom and top substrates, an increase in the channel height for high throughput is limited since cells at the middle of the channel height will not be affected by DEP force. It is noted that operation of these devices in parallel can increase system throughput.

Figure 2.20: Separation of multiple bacterial cell types with synthetic DEP tags [87]

Recently, a number of DEP-based devices have been developed to integrate 3D electrodes in a microfluidic channel using various techniques such as heavy doping of silicon, pyrolysis of SU8, electroplating of gold or platinum, and manual insertion of etched copper electrodes. Using the 3D electrodes, an electric field is generated uniformly along the entire channel height direction. Thus, cells situated at any height can experience DEP force, thereby enabling high throughput and allowing lateral separation. Iliescu *et al.* developed a microfluidic device in which heavily doped 100 μm thick silicon was used as DEP electrodes and simultaneously a channel (Figure 2.21) [96]. The doped silicon was sandwiched by two glass substrates through anodic bonding process, thus yielding a fully enclosed device without fluidic leakage. The device was successfully tested to separate live from dead yeast cells, although still under the non-continuous flow [96-99]. A numerical study on a temperature rise due to applied electric field reveals that the use of 3D electrodes reduce 8-10 time temperature change as compared to planar electrodes [100, 101]. It is due to the fact that both silicon wafer and glass are good thermal

conductors. Although providing advantages over the double-sided electrode technique, the Si-based technique still remains two main fabrication problems. First, handling of such thin wafer likely complicates microfabrication processes. Second, as the channel height depends on the thickness of silicon wafer, it is hard to obtain a desired thickness (e.g., 10-50 μm), which are the typical range of microfluidic heights. Otherwise, the cost could be very high. In addition, Park *et al* [102] reported a similar design based on carbon microelectromechanical system (C-MEMS) that turns the SU8 photoresist microstructures to 3D electrodes by pyrolysis. Although the technique was able to extract nanofibrous carbon from canola oil, it still exploits expensive SU8 photoresist [102, 103].

Figure 2.21: A microfluidic chip with heavily doped silicon as electrodes and channel walls

Wang *et al.* [104] proposed an alternative design by integrating 3D electrodes along the sidewall of a SU8 microfluidic channel for lateral separation of particles and cells. The 3D electrode formation was achieved by electroplating of gold on a silicon substrate coated with a SU8 thin layer, served as an insulating channel floor (Figure 2.22 (a)). As this technique controls the channel height by

photoresist-coating process, adjustment of the channel height becomes flexible and plausible for high throughput, unlike the above two techniques. As shown in Figure 2.22 (b), the lateral separation is enabled by two sets of interdigitated sidewall electrodes which are imposed with different electric field frequencies and voltages [105]. Both types of cells experience different DEP forces, based on their dielectric properties. The equilibrium location of a white cell (point A) is determined from the balance of DEP forces (F_1 and F_2) from DEP electrode#1 and #2, respectively. When DEP force F_1 is larger than DEP force F_2 , the white cell is pushed away from the DEP electrode#1 until the cell reaches point C where F_1 and F_2 are equal. Similarly, an equilibrium location of a gray cell is determined by DEP forces from both sides which push the cell near the DEP electrode#1. Thus, these two cells can be separated continuously in different outlets. The device was successfully used to achieve focusing, sorting and separation of beads and biological cells such as mouse neural stem cells and human kidney cells [81, 104, 105]. In term of choice of materials, titanium was also reported to be integrated as sidewall electrodes in a DEP device for particle manipulation [106]. With the development of 3D electrodes by electroplating, an exponential decay of electric fields along the channel height can be ultimately avoided and the adjustment of channel height is flexible. However, these microfluidic devices are usually made from glass or silicon and still suffer from significant complications in assembling to prevent liquid leakage [104].

Figure 2.22: (a) 3D gold electrodes electroplated along the sidewall of a SU8 microchannel [104]. (b) Lateral separation principle by coupled DEP forces [105].

The microfabrication including formation of 3D electrodes and device assembly was later simplified by Cetin *et al.* [107] and Kang *et al.* [108]. The fabrication technique relies on a manual insertion of 3D etched-Cu electrodes in a PDMS-based microfluidic device, using soft lithography with a SU8 master (Figure 2.23). The device assembly between a glass cover and PDMS channels using oxygen plasma treatment is then simple. In this technique, particles are transported by hydraulic pressure and separated by AC-DEP via the 3D electrodes. As DEP forces occur only near the tip of triangular electrodes (Figure 2.23 (a)), or around the corners of the PDMS block (Figure 2.23 (b)), the technique offers minimum exposure of electric fields on cells and reduces negative problems related to Joule heating. Despite these simplicities, the technique remains ineffective and inconvenient for fabrication because of the following reasons. First, the etching process for patterning Cu electrodes cannot guarantee the exact dimension of electrodes to match with PDMS channels, and thus always leaves a gap between the electrodes and PDMS walls. Such gap can cause fluid leakage and even cell loss during separation. Second, similar to the case of heavily doped silicon, adjustment

of channel height is constrained by the availability of Cu sheet thickness. Third, when insertion of multiple Cu electrodes is necessary for enhancing DEP forces and thus particle velocity, it becomes difficult for batch fabrication.

Figure 2.23: PDMS-based devices with manually inserted Cu electrodes for continuous separation of (a) white blood cells from yeast cells [107], and (b) the 10 μm particles from yeast cells [108]

2.5 Electrorotation and traveling wave dielectrophoresis

Previously, DEP-based devices transport the particle by using a pressure-driven flow and achieve the levitation and the lateral separation of particles by using DEP force. However, such DEP force is associated only with the real part of the induced

dipole moment. This section reviews unique two techniques which utilize the imaginary part of the induced dipole moment ($\text{Im}[\underline{\vec{p}}_{eff}(\omega)]$) as an additional parameter, giving rise to electrorotation and traveling wave dielectrophoresis.

Electrorotation (ROT) arises as a result of rotational torque acting on a polarized particle exposed to a rotating electric field (Figure 2.24 (a)). Since a cosine wave always leads a sine wave by 90 degrees of phase, the resulting electric field rotates anti-clockwise. As shown in equation 2.22, the torque is proportional to $\text{Im}[\underline{\vec{p}}_{eff}(\omega)]$ and the electric field strength [109, 110]. This imaginary part is also referred to as the out-of-phase component which determines whether the induced dipole moment leads or lags the applied electric field [43]. When $\text{Im}[\underline{\vec{p}}_{eff}(\omega)] > 0$, the induced dipole moment leads the electric field, causing the particle to rotate in an opposite direction to the rotating field. The direction of rotation reverses when $\text{Im}[\underline{\vec{p}}_{eff}(\omega)] < 0$ [111].

$$\Gamma(\omega) = -\text{Im}[\underline{\vec{p}}_{eff}(\omega)]E \quad (2.22)$$

ROT has been widely used in measurement of dielectric and conductive properties of latex spheres such as surface conductance and zeta potential [38], human leukocyte subpopulations [112], cell membrane [113], and breast cancer cell lines [114]. Fuhr *et al.* [115] also demonstrated the separation of plant protoplasts using a ROT system in which cells having distinct membrane capacitances were observed to rotate in different directions and then separated.

Figure 2.24: Electrode configurations for (a) electrorotation [33] and (b) traveling wave dielectrophoresis [116]

Alternatively, the electrode arrays can be aligned side by side as shown in Figure 2.24 (b). Instead of rotating, the resulting electric fields travel from left to right or vice versa, depending on the phase changes assigned to the electrode arrays [116]. If $\text{Im}[\underline{\vec{p}}_{eff}(\omega)] > 0$, the induced dipole moment leads the electric field, causing the particle to translate in the direction opposing to that of the travelling field (or from right to left). The translational direction reverses if $\text{Im}[\underline{\vec{p}}_{eff}(\omega)] < 0$. This mechanism is known as traveling wave dielectrophoresis (twDEP), which can be used to achieve continuous separation of particles. twDEP employs the conventional DEP force related to $\text{Re}[\underline{\vec{p}}_{eff}(\omega)]$ to levitate particles from the electrodes (or attract particles to the electrodes), and the twDEP force related to $\text{Im}[\underline{\vec{p}}_{eff}(\omega)]$ to generate a translational motion in the direction either parallel or anti-parallel to the traveling wave propagation (V). The translational twDEP velocity of a particle is proportional to the square of the electric field strength and in

turn determines the speed in separation process [111]. The technique was validated by the separation of non-viable and viable yeast cells in a mannitol solution [111, 116]. At 35 kHz, the non-viable cells were attracted to the electrodes by p-DEP while the viable cells were levitated and transported by the traveling wave. Furthermore, the separation of erythrocytes from leukocytes was also achieved, although both blood cells experienced levitation by nDEP due to their different speeds [117]. Other twDEP-based applications include the separation of latex beads [118] and the size-based separation of DNA molecules [119]. A detailed theoretical study of electrokinetic behaviors of a particle in traveling waves can be found elsewhere [120].

Although twDEP enables continuous separation of various kinds of particles and cells, the twDEP performance in separation is limited because the speed in separation relies on the square of the electric field strength. To speed up the separation process, stronger electric field strength can be increased but it may heat the medium and thus damage the cells. This problem can be resolved in DEP-FFF devices which utilize a pressure-driven flow to accelerate the particle velocity. In addition, with planar electrode structure, twDEP has a problem associated with the exponential decay of electric fields above the electrode surfaces as mentioned previously.

2.6 Moving DEP

Moving DEP (mDEP) was recently developed in an attempt to fractionate cells as in DEP-FFF and simultaneously to transport cells as in twDEP. However, unlike twDEP, mDEP employs a DEP force which is associated only with the real part of the induced dipole moment in transporting and fractionating cells by sequentially activating an electrode (or a number of electrodes) at a time [121]. In Figure 2.25, the dotted circle represents a cell at the initial time prior to energizing the electrode while the patched and solid circles illustrate the cells after experiencing pDEP and nDEP, respectively. Figure 2.25 (a) illustrates the cell transportation under pDEP from left to right. Because the cell under pDEP is attracted to the high electric field gradient region, when the electrode on the right is sequentially energized from $t=0$ to $t=2T$, the moving electric field pulls the pDEP cells from left to right. In contrast, Figure 2.25 (b) illustrates a transportation of cells under nDEP. Since the nDEP cell is repelled from the high electric field gradient region, when the electrode on the left is sequentially energized from $t=0$ to $t=2T$, the moving electric field pushes the nDEP cells from left to right.

Figure 2.25: Particle transportation by (a) pDEP and (b) nDEP. (c) Fractionation of cells under pDEP (patched circle) and nDEP (solid circle)

Using these two mechanisms, mDEP successfully fractionated viable and non-viable yeast cells (Figure 2.25 (c)) [121]. At $t=0$, the second electrode was energized to initiate the fractionation of both cell types. At $t=T$, the second and third electrodes were energized to hold a viable yeast cell by pDEP and repel a non-viable cell by nDEP, thus separating both cell types with a two-electrode distance. Once the three electrode distance is achieved at $t=2T$, the transportation mode is then activated at $t=3T$ by moving three energized electrodes from left to right. The nDEP cells will be collected first and leave the pDEP cell to be collected the last. The analytical solutions for electric potential and electric field suggested that the

channel height and both electrode size and gap must be minimized to obtain for efficient fractionation [122]. The trajectory of cells was further numerically studied to understand the cell motion under mDEP, drag, buoyancy and gravitational forces [123]. Although cell fractionation by mDEP simplifies twDEP as it does not require the variation in the phase of the applied electric field, mDEP can only fractionate cells in a stationary fluid. Similar to twDEP, further improvement in terms of flow-through separation and the decay of electric fields from planar electrode surface is still needed.

2.7 Insulator-based dielectrophoresis

All aforementioned techniques are dependent on metallic electrodes for generating non-uniform electric field. Instead, Cummings *et al.* [24] proposed an alternative technique named insulator-based dielectrophoresis (iDEP). The iDEP principle is based on coupled phenomena between linear electrokinetics: EP and EOF, and nonlinear electrokinetics: DEP. The glass-etched microchip contains arrays of insulating (quartz) posts so as to induce non-uniform electric field necessary for DEP [24]. As shown in Figure 2.26 (a), a DC electrical potential is applied across the insulating post arrays through two external electrodes distantly located at inlet and outlet reservoirs, allowing the chip to be operated under DC electric field without severe problems of electrolysis.

Figure 2.26: (a) Glass-etched channel structures of iDEP chip [23] and (b) non-uniform electric field near circular quartz posts. (c) Triangular PDMS-based constriction used for DNA trapping and (d) separation of blood cells and *E. coli* [124].

The electrical current given by the surface integral of current density can be expressed as

$$I = \int \vec{i} \cdot \vec{n} dA = \int \sigma \vec{E} \cdot \vec{n} dA = \sigma EA \quad (2.23)$$

where $\vec{i} = \sigma \vec{E}$ is the current density equivalent to Ohm's law, $\vec{n}$ is a unit normal vector along the current direction, and the cross-sectional area is A . Equation 2.23 implies that the electrical current in any cross section of the channel must be conserved. Since the channel walls and posts are electrically insulating, the electrical current does not go through their surfaces. The electric field strength at a smaller gap must be stronger, as being squeezed, than that at a larger gap to conserve the electrical current. This produces high and low electric field regions around the posts (Figure 2.26 (b)). Furthermore, as the posts are of 3D structure, the electric fields do not decay along the channel height, thus allowing lateral manipulation of cells effectively.

In EK principle, EO and EP are linearly dependent on the local electric field strength E while DEP is dependent to the gradient of the square of the electric field ∇E^2 . Thus, depending on the applied electric field strength, two flow regimes: streaming DEP and trapping DEP can be observed and switchable. Streaming DEP can be activated when DEP overcomes diffusion but is dominated by linear EK flow whereas trapping DEP occurs when DEP overcomes both diffusion and linear EK flow [24]. The DC-iDEP microchip with arrays of quartz posts was successfully used to achieve concentration of spores, virus and bacteria in water with concentration factors of the order of 3000 $\times$ [21], and selective separation of single- and double-stranded DNA [22] and two species of live bacteria in water [23]. Furthermore, Chou *et al.* also developed an iDEP chip, made of a glass substrate bonded with PDMS, for trapping DNA (Figure 2.26 (c)) and separation of blood cells and *E. coli* (Figure 2.26 (d)) [124]. The chip consists of a 500 μm wide main

channel and triangular constrictions, whose gap is 1 μm wide, to produce non-uniform electric field. In addition, some researchers reported to achieve continuous separation by using 3D insulating ridges in faceted prism microchannels [125]. Although DC-iDEP enables the concentration of cells in an inexpensive insulator-based device, it still requires rather high electric field strength to achieve the task because of coupled linear and nonlinear electrokinetics. The requirement of DEP to dominate EP and EO by relying solely on increment in the applied electric field strength becomes a limitation of this technique.

The concept of iDEP was developed further by integrating an oil droplet obstacle or a PDMS hurdle in a microfluidic device to induce non-uniform electric field needed for lateral DEP [126-128]. Figure 2.27 (a) and (b) show a superposition of a series of consecutive images of moving particles. As shown in Figure 2.27 (a), an adjustable oil droplet was controlled by a syringe at reservoir B. High DC voltages of 400V and 160V were respectively applied at reservoir C and A, whereas reservoir D was electrically grounded. This causes electrokinetic focusing in which the flow from branch C becomes stronger than that from branch A. Thus, all particles (5.7 μm and 15.7 μm) from reservoir A can be focused near the oil droplet where the electric field is strong. As DEP force depends on particle size, the larger particles experiencing larger DEP force were repelled farther away from the droplet than the smaller one.

Figure 2.27: Continuous separation using (a) an oil droplet [126] and (b) a PDMS-based block [127]

Subsequently, Kang *et al.* proposed a similar design by using a PDMS block (Figure 2.27 (b)) [127, 128]. It is advantageous that the block possesses sharp corners which in turn produce higher electric field gradients and thus stronger DEP forces, compared to the oil droplet. The 15 μm particles experiencing larger DEP force were directed into reservoir D while the 5 μm particles left the channel through reservoir A. The variation of voltages also affects the trajectory of particles. In another design, a device with a triangular PDMS hurdle was also used to separate white blood cells (of 5 μm) from other smaller cells [129]. Despite the success, the technique relies on multichannel DC voltages to achieve focusing and separation thus making the operation more complicated. Moreover, due to the coupled linear and nonlinear electrokinetics, it becomes a limitation of the device that can only use the DC voltage to achieve the separation [130]. This could lead to the use of high electric field strength in manipulation of samples. Thus, a new technique is needed to involve a single channel DC voltage power supply, and also lower voltages are

required to make the operation simpler and favorable to cells sensitive to high field strength.

2.8 Liquid electrode AC dielectrophoresis

On the basis of the iDEP, a new insulator-based channel geometry with embedded planar electrodes was proposed to achieve lateral DEP separation of particles [131]. As shown in Figure 2.28 (a), an electric field is produced by planar metallic electrodes (in black) deposited in access channels perpendicular to the active area in the main channel. The boundary plane between the access and main channel is defined as a liquid electrode where an equipotential surface is assumed to occur. The potentials at the liquid electrodes are different from those applied at the metallic electrodes due to the potential drop across the access channel. The patterned SU8-based insulator is responsible for shaping electric fields, thus resulting in a non-uniform electric field needed for DEP force. By adjusting the applied voltages V_1 and V_2 , the particle location at the exit can be controlled. When two pairs of electrodes on opposite sides of the main channel are assigned with the potential of the same magnitude, particle focusing can be achieved by the DEP forces equally generated from both sides (Figure 2.28 (a)). If the potential at the upper electrodes is larger than that at the lower electrodes, the particle is pushed near the side of the lower electrodes or vice versa. An integration of arrays of liquid electrodes into a microfluidic device has successfully been used to achieve focusing, sorting and continuous separation of particles, live/dead yeast cells, *Babesia bovis*-infected red blood cells by balanced DEP forces at multiple

frequencies (Figure 2.28 (b)) [132-136]. However, the use of SU8 as channel walls can be costly for disposable applications. The use of inexpensive materials like PDMS can be optional to make this technique more promising.

Figure 2.28: (a) Schematic of a microfluidic channel for cell manipulation using liquid electrodes [131] and (b) 3D schematic of a separation device using multiple frequencies [134].

2.9 Optically-induced dielectrophoresis

Different from using metallic electrodes or insulating posts, Chiou *et al.* [137] proposed using optoelectronic tweezers (OET) for high-resolution manipulation of single cells as well as massively parallel manipulation of cells. OET employs a photosensitive surface sandwiched by indium tin oxide (ITO)-coated or conductive glass substrates. As shown in Figure 2.29, the photosensitive surface consists of three thin layers: a 50-nm n+ hydrogenated amorphous silicon (a-Si:H) layer, an 1 μm undoped a-Si:H layer, and a 20-nm silicon nitride layer. Top and bottom ITO-

coated glasses are applied with an AC electric field. When the photosensitive areas are projected with light, the impedance of projected areas is reduced, thus yielding virtual electrodes for DEP manipulation. The projection of optical images (e.g., circular or parallel electrode patterns) on the photosensitive areas is achieved by combining a light source from a light-emitting diode and a digital micromirror display (DMD) through a 10× objective lens. The sufficient intensity of light to activate virtual electrodes is found 100,000 times lower than that used in the laser-based technique such as optical tweezers. Thus, the technique clearly becomes benevolent towards cell applications. Other methods used to produce optical images can be achieved by using liquid crystal display (LCD) projector [138], len-less direct image [139], LCD integrated with condenser lens [140]. OET has been also used to manipulate various kinds of samples such as human B cells (lymphocytes) [137], DNA [141] and CNT [142].

The OET platform offers high resolutions in manipulating single cells. However, in terms of continuous separation, it may suffer from an exponential decay of electric fields from the photosensitive surface like planar electrodes. The use of ITO-coated glass substrates may also lead to the need of high voltage supply as the light-intensity-dependent impedance of the photosensitive layer is not excellent, as compared to metallic electrodes. Furthermore, the use of high intensity of light is not desirable for fluorescence-based applications in which the differentiation of different cells stained by fluorescent dyes is not possible because of bleaching.

Figure 2.29: Optoelectronic tweezers structures for DEP manipulation of particles and cells via optically-induced, virtual electrodes [137]

2.10 Summary

This chapter provides a broad literature review on various techniques for sample sorting, separation and concentration. As inspired to solve the hindrances faced by chromatography and flow cytometry, DEP-FFF has been developed by combining microfabrication and microfluidic technologies to achieve continuous-flow separation of particles and cells with non-uniform electric field induced DEP force. The main drawbacks of DEP-FFF have subsequently led to a number of promising techniques using 3D electrodes which successfully solve the problems associated with the exponential decay of electric field along the channel height. However, some of these techniques do not provide flexibility in adjusting the channel height

and involve expensive photoresist-based electrodes. Others still need the complicated fabrication to prevent liquid leakage and are not suitable for batch fabrication.

To improve these limitations, a new PDMS-fabricated microfluidic device using 3D conducting PDMS composites as sidewall electrodes (which will be presented in Chapter 5) is developed for characterization and separation of particles and cells in this study. A simple fabrication technique is proposed to allow the channel's height to be adjustable, and to greatly facilitate (i) the integration of 3D conducting PDMS electrodes into PDMS microchannels and (ii) the device assembly by using only oxygen plasma treatment which efficiently prevents liquid leakage at high flow rates.

Although insulators such quartz and PDMS are inexpensive materials of choice in DC-iDEP, the device still requires a multichannel voltage power supply, making the operation more complicated. Moreover, due to the coupled linear and nonlinear electrokinetics, it becomes a limitation of the device that can only use the DC voltage to achieve the separation. This also led to the use of high electric field strength in manipulation of samples as discussed earlier. As inspired by these limitations, this study also developed the modified PDMS-based H-filter platform with multi-insulating blocks for particle sorting and separation by size using only a single-channel DC power supply (which will be presented in Chapter 3). The integration of multi-insulating blocks facilitates the controlled manipulation of particles, and the use of a single-channel DC power supply can greatly simplify

the device operation. In comparison with the pure DC voltage, the use of combined AC and DC voltage not only allows the users to separate particles using either a DC or AC component but also can reduce the threshold voltage for particle sorting (Chapter 3) and concentration of particles and cells (Chapter 4).

Chapter 3 Dielectrophoretic Manipulation of Particles in a Modified H-Filter with Multi-Insulating Blocks[†]

3.1 Introduction

The commercially available microfluidic H-filter is widely recognized to offer continuous extraction of analytes using mass diffusion as a driving force. Due to its H-shaped platform, this original H-filter greatly facilitates collection of analytes [25, 26]. However, its separation function suffers from low efficiency and even fails, especially for large-sized samples such as cells whose mass diffusivity is very low, and the difference of mass diffusivities between the cells is not significant.

This chapter presents a modified PDMS-based H-filter platform with multi-insulating blocks to achieve flow-through separation of particles by size relying only on a single channel DC power supply. The device transports particles by exploiting EO and EP, and manipulates the particles by utilizing DEP. The PDMS blocks fabricated in the main channel of the modified H-filter induce local non-uniform electric fields, which exert negative DEP forces on the particles. The use of multi-insulating blocks not only enhances the DEP forces, but it also increases the

[†] The works related to sorting and separation of particles in the H-filter has been published in *Biomicrofluidics*, 2008. 2(034105) [DOI: 10.1063/1.2973661]

controllability of the motion of the particles, facilitating their manipulation and separation. Experiments were conducted to demonstrate the controlled flow direction of particles and the separation of particles by size by adjusting the applied voltages under two different input modes: (i) a DC electric field, and (ii) a combined AC and DC electric field. Under the combined AC and DC electric field, linear and nonlinear electrokinetics can be decoupled. This technique provides a frequency-dependent AC electric field for controllable manipulation of particles. More importantly, the studies show that the technique can minimize the threshold DC voltage for particle separation.

Furthermore, numerical modeling of particle trajectory based on the Lagrangian approach is developed to elucidate the electrokinetic and hydrodynamic forces acting on a particle. The velocity field of an EO flow obtained experimentally using micro-particle image velocimetry (μ -PIV) technique is compared with the numerical simulation. Moreover, the effects of the number, width and gap of insulating blocks on threshold voltages are studied to determine the optimal configuration. Finally, the device is used to perform two promising applications: (i) separation of multiple particles simultaneously by size and type using hydrodynamics and AC-DEP[‡], and (ii) manipulation of single-walled carbon nanotubes using a combined AC and DC electric field.

[‡] The works presenting the separation of multiple particles simultaneously by size and type using hydrodynamics and AC-DEP are in preparation for submission to *Electrophoresis*.

3.2 Experiment

3.2.1 Design and fabrication of the modified H-filter

Figure 3.1 (a) depicts the structure and dimensions (in mm) of the modified H-filter. Three insulating blocks which are $240\text{ }\mu\text{m}$ high and $150\text{ }\mu\text{m}$ wide are fabricated in a $300\text{ }\mu\text{m}$ main channel. All channels have a depth of $60\text{ }\mu\text{m}$. A scanning electron microscopy (SEM) image of the fabricated H-filter channels and the actual H-filter chip made of patterned PDMS and a glass slide are shown in Figure 3.1(b) and (c), respectively.

Figure 3.1: (a) The modified H-filter dimensions, (b) the SEM image and (c) the modified H-filter chip [143].

This PDMS-based H-filter was fabricated using the standard photolithography and soft lithography [144]. As shown in Figure 3.2, photolithography is the process to transfer a pattern from a transparent mask to a negative photoresist (SU-8), and soft lithography is to transfer the pattern from SU-8 to PDMS. The fabrication starts by cleaning a silicon wafer in piranha solution, a

mixture of H_2SO_4 (70%) and H_2O_2 (30%), for 20 minutes, and rinsing with deionized (DI) water. The wafer was spun dry and left on a hotplate at 200°C for 5 minutes to remove the humidity on its surface. As shown in Figure 3.2 (a), SU-8 was spin-coated on the wafer at a final speed of 2,000 rpm for 30 seconds to form a thickness of $60\text{ }\mu\text{m}$ [145]. After soft-baking at 65°C (6 minutes) and 95°C (20 minutes), the SU-8 layer was exposed to UV light for 40 seconds. In Figure 3.2 (b), post-baking at 65°C (1 minute) and 95°C (5 minutes) was performed by using a hot plate to enhance the cross-linking of the exposed portion of SU-8. Figure 3.2 (c) shows a 6-minute developing process that allowed the unexposed SU-8 to be removed, and developed the exposed SU-8 as a master of microchannels. The wafer with the SU-8 master was rinsed thoroughly with isopropyl alcohol (IPA) and DI water, and then spun dry.

In soft lithography, Sylgard 184 Silicone Elastomer or PDMS, mixed with the base and curing agent at the ratio of 10:1, was thoroughly stirred and poured on the wafer. After bubbles generated from the stirring were removed in a vacuum chamber for 40 minutes, PDMS was cured in an oven at 80°C for 1 hour (Figure 3.2 (d)). The cured PDMS was peeled off from the wafer. Four reservoirs of 5 mm in diameter were punched for inlets and outlets. The irreversible bonding between the PDMS channels and a clean glass slide was performed by oxygen plasma treatment, followed by heating at 80°C for 10 minutes (Figure 3.2 (e)). The bonded PDMS-based device (in Figure 3.2 (f)) was left in air for 48 hours to ensure that the zeta potential of the PDMS channel walls was reduced and stable prior to experiments

[146, 147]. As a result, during the operation of the device, the particle EP is expected to be stronger than the fluid EOF.

Figure 3.2: Photolithography: (a) Spin coating, soft-baking and UV exposure of SU-8 photoresist, (b) Post-baking and (c) Developing process. Soft lithography: (d) PDMS curing, (e) Oxygen plasma treatment and (f) A PDMS-based device.

3.2.2 Sample preparation and experimental setup

Latex polystyrene microspheres (Duke Scientific, USA) of 5 μm, 10 μm and 15 μm in diameter were selected in our experiments, as their sizes are comparable to biological cells such as red and white blood cells. The original number concentration of particles in deionized (DI) water was approximately $10^7/\text{ml}$. Prior

to experiment, the particle suspension was diluted with a 10^{-3} M NaHCO_3 buffer with a volumetric ratio of 1:10. This particle-buffer mixture was introduced at inlet C (Figure 3.1 (a)) while other reservoirs were filled with the buffer solution only. After electrodes were placed in the four reservoirs (but before the application of an electric field), the buffer level in each reservoir was balanced using a 50 μl precision syringe until no noticeable particle motion was observed a microscope.

Particle's motion was captured at a rate of 15 frame per second (fps) through a CCD camera (Samba, EZ-140 c, image resolution of 696×520 pixels) attached to an epifluorescence microscope (Zeiss, Axiostar Plus FL, Germany) under a 5x objective lens. Since an image contains many particles, only one representative particle was selected for an analysis of particle's trajectory. The trajectory was constructed using image processing software (ImagePro® Plus).

The device was operated under various voltages produced by either one of the two different power sources: (i) in the DC electric field mode, a DC power supply (Stanford Research, USA) was used; and (ii) in the combined AC and DC electric field mode, a function generator (Agilent 33250A, USA) was connected with a customized high voltage amplifier (OptroBio, Singapore), with the DC-biased, AC voltage signal monitored by an oscilloscope (CombiScope® HM 1008, USA).

In addition, to obtain the fluid velocity field in a microfluidic channel, the μ -PIV technique was used to measure the velocity of $0.93 \mu\text{m}$ fluorescent tracer particles (Duke Scientific, USA). The fluorescent particles have a maximum

excitation wavelength of 540 nm and a maximum emission wavelength of 610 nm. The original concentration 2×10^9 particle/ml in DI water was diluted with a 10^{-3} M NaHCO_3 buffer solution in a volumetric ratio of 1:10. A mercury lamp (Zeiss, mbq 52 ac) connected to the epifluorescence microscope (Zeiss) with an appropriated filter set (excitation of 546 nm and emission > 590 nm) was used to illuminate the fluorescent tracer particles. Two sequentially captured images were then evaluated with PIVview software (PivTec GmbH) to obtain the velocity field of the tracer particles.

3.3 Modeling of particle motion

Under applied DC voltage at the reservoirs A and B with the reservoirs C and D grounded, a DC electric field generated in the modified H-filter is schematically illustrated in Figure 3.3 (a). Near the insulating block corner, the physical forces exerting on a negatively charged particle are attributed to the EP, EO, DEP and drag forces as illustrated in Figure 3.3 (b). It is assumed that these forces on a particle can be decoupled [32]. Since both the channel walls and the particle are negatively charged, the EO and EP forces are in the opposite direction. When the EP force is stronger than the EO force, the particle in fact transports along an electric field line against the EO flow towards the positive electrode as shown in Figure 3.3 (b). When the particle travels near the insulating block where a non-uniform electric fields exists, the particle also experiences a negative DEP force to push the particle away from the block corner.

Figure 3.3: Schematic diagrams of (a) electric field lines in the modified H-filter and (b) physical forces on the particle near the block's corner [143]

To track the particle motion, a model based on the Lagrangian approach is developed. Then the instantaneous particle location $\vec{r}_p = x_p \vec{i} + y_p \vec{j}$ can be obtained by integrating the particle's velocity $\vec{u}_p = d\vec{r}_p/dt$ over a period of time t from the initial state,

$$\vec{r}_p(t) = \vec{r}_0 + \int_0^t \vec{u}_p(t') dt' \quad (3.1)$$

where $\vec{r}_0$ is the initial location of a particle. In a fixed frame, the particle motion governed by Newton's second law is approximately described with a two-dimensional model. The following assumptions are made: (i) sedimentation due to particle gravity is negligible as the density of particle (1.05 g/cm^3) is close to that of the solution, (ii) the zeta potential values of particles and channel walls are constant, (iii) the electrical and thermophysical properties (such as electrical permittivity and viscosity) of the buffer are constant due to negligible Joule heating effect in a PDMS/glass channel filled by a low-concentration $10^{-3} \text{ M NaHCO}_3$ solution [148],

(iv) the electric field distribution in the modified H-filter is two-dimensional because both top and bottom walls are electrically insulating, and (v) the coupled particle-channel interactions are not included [149]. Accordingly, the equation of particle's motion based on the Lagrangian approach can be described by Newton's second law:

$$\Sigma \vec{F} = m_p \frac{d^2 \vec{r}_p}{dt^2} \quad (3.2)$$

where $\vec{r}_p$ represents the position of particle center. $m_p = (4/3)\pi a^3 \rho_p$ is the particle mass while a and ρ_p are the particle radius and density, respectively.

$\Sigma \vec{F}$ represents the net external force exerting on the charged particle. For a general case of an arbitrary external force $\vec{F}_{Ext}$ acting on a particle, the particle moving with a velocity $\vec{u}_p$ in a fluid having a velocity $\vec{u}_{flow}$ experiences a hydrodynamic drag force which is proportional to its velocity relative to the fluid, $\vec{F}_\eta = f(\vec{u}_{flow} - \vec{u}_p)$ [32, 37]. The equation of particle motion becomes

$$m_p \frac{d\vec{u}_p}{dt} = \vec{F}_{Ext} + f(\vec{u}_{flow} - \vec{u}_p) \quad (3.3)$$

Assuming that the particle is initially at rest, the solution of equation (3.3) is

$$\vec{u}_p = \left(\vec{u}_{flow} + \frac{\vec{F}_{Ext}}{f} \right) \left(1 - e^{-(f/m_p)t} \right) \quad (3.4)$$

The expression gives the particle velocity after $t > 0$. The exponential term describes the acceleration phase of the particle motion and has a characteristic time constant $\tau = m_p / f \sim O(a^2 \rho / \eta)$. For times much longer than τ , the particle reaches its terminal velocity [37]

$$\vec{u}_p = \vec{u}_{flow} + \frac{\vec{F}_{Ext}}{f} \quad (3.5)$$

For a particle of 10 μm in diameter suspended in a buffer solution with a density of 1050 kg/m^3 and a dynamic viscosity of $9 \times 10^{-4} \text{ kg} \cdot \text{m}^{-1} \text{s}^{-1}$, the characteristic time is on the order of 10^{-4} s . This time scale is smaller than that of the variation of the external forces and that of experimental observation. Thus, the particle always appears to move with the terminal velocity as shown in equation (3.5) in experiments [37, 150].

Substituting the EO velocity, the EP and DC-DEP force from equations (2.1), (2.2) and (2.19), the terminal velocity of a charged particle under a DC electric field in the modified H-filter is expressed as

$$\vec{u}_p = \vec{u}_{EO} + \frac{\vec{F}_{EP} + \vec{F}_{DEP}}{f} \quad (3.6)$$

$$\vec{u}_p = -\frac{\epsilon_m \zeta_w}{\eta} \vec{E}_{dc} + \frac{\epsilon_m \zeta_p}{\eta} \vec{E}_{dc} + \frac{c \epsilon_m a^2 \text{Re}[f_{CM}(\omega)] \nabla \vec{E}_{dc}^2}{3\eta} \quad (3.7)$$

where c is the correction factor accounting for the perturbation of the particle size in the electric field [127]. As for the case of combined AC and DC electric field, the

AC electric field mainly contributes to the DEP force but not to the particle transport as it suppresses the EO flow and causes no EP motion. Simultaneously, the DC electric field can be independently used to transport the particle and also contribute to the DEP force. Using the DC-biased, AC-DEP force from equation (2.20), the expression becomes

$$\vec{u}_p = -\frac{\epsilon_m \zeta_w}{\eta} \vec{E}_{dc} + \frac{\epsilon_m \zeta_p}{\eta} \vec{E}_{dc} + \frac{c \epsilon_m a^2 \operatorname{Re}[\underline{f}_{CM}(\omega)] \nabla(\vec{E}_{dc}^2 + \vec{E}_{rms,ac}^2)}{3\eta} \quad (3.8)$$

Equation (3.8) indicates that the DEP force is decoupled from the EO and EP forces. In other words, the DEP force can be enhanced without increasing the velocity of the particle, thereby reducing the voltage required for manipulation of particles.

To solve equations (3.7) and (3.8), both electric field and EO velocity field are required. The typical thickness of an EDL is in the range of 1 to 100 nm while the characteristic hydraulic diameter of microfluidic channels is on the order of 10 to 100 μm . With such significant difference, the electrical potential distribution and the EO velocity profile within the EDL region become insignificant. Thus, the electrical potential distribution in the microchannel can be governed by Laplace's equation [32], expressed as

$$\nabla^2 \phi = 0 \quad (3.9)$$

Furthermore, under such thin EDL assumption, the EO flow can be considered to be induced by a moving wall with a slip velocity, and thus the EO velocity field is

governed by the Navier-Stokes equation and the continuity equation, respectively expressed as

$$\rho(\vec{u} \cdot \nabla \vec{u}) = -\nabla p + \eta \nabla^2 \vec{u} \quad (3.10a)$$

$$\nabla \cdot \vec{u} = 0 \quad (3.10b)$$

The boundary conditions for equations (3.9) and (3.10) are summarized in Table 3.1. For Laplace's equation, the electric field normal to the wall must be zero since all channel walls are insulating. The symbol, $\vec{n}$, is the unit vector whose direction is normal to the insulating walls. To transport the negatively charged particle from inlet C to either outlet A or B, a voltage of V_{DC} is applied at outlets A and B while inlets C and D are electrically grounded. In the combined AC and DC electric field mode, V_0 is the AC output voltage, and V_{DC} is the DC component. As for the Navier-Stokes equation, all channel walls are set to have a Smoluchowski slip velocity (equation 2.1). The unit vector, $\vec{t}$, is tangential to the insulating walls. In the EO flow, there is no external pressure applied at any reservoirs. With the stated boundary conditions, the electric and velocity fields were numerically solved by a finite-element method using COMSOL MultiphysicsTM.

Table 3.1: Boundary conditions for Laplace's equation and Navier-Stokes equation

Governing equations	Boundary conditions		
	Along the walls	Inlet C and D	Outlet A and B
Laplace	$\vec{E} \cdot \vec{n} = 0$	$\phi = 0$	$\phi = V_{DC}$ or $\phi = V_{DC} + V_0 \sin(\omega t)$
Navier-Stokes	$\vec{u} \cdot \vec{t} = (\epsilon_m \zeta_w / \eta) \vec{E} \cdot \vec{t}$	$P = 0$	

3.4 Results and discussion

In the simulation, the zeta potential of hydrophobic PDMS walls at room temperature was set to -23.5 mV [146]. The zeta potential values of the particles 5 μm , 10 μm and 15 μm in a 10^{-3} M NaHCO_3 buffer solution were chosen as -55.1 mV, -71.6 mV and -93.1 mV, respectively. In the DEP force term, $c=0.5$ was considered to account for the perturbation of the particle size in the electric field [127]. The buffer solution density, dynamic viscosity and electrical permittivity were $1000 \text{ kg}\cdot\text{m}^{-3}$, $9 \times 10^{-4} \text{ kg}\cdot\text{m}^{-1}\text{s}^{-1}$ and $6.9 \times 10^{-10} \text{ C}\cdot\text{V}^{-1}\text{m}^{-1}$, respectively.

3.4.1 Effect of applied voltages

The superimposed trajectories of the 10 μm and 15 μm particles under the applied DC voltages ranging from 60 to 140 V are presented in Figure 3.4 (a) and (b). Starting from arbitrary locations on the right and passing through the area of

insulating blocks, the particles were pushed away from the blocks' corners and finally were confined in a small region of the gap between the block and the channel wall. Depending on the magnitude of the applied voltage, the particles can be sorted into either the upper or lower reservoir.

Figure 3.4: Experimental investigation of the voltage effect on the trajectory of (a) 10 μm particles and (b) 15 μm particles. Comparison of the experimental and simulated trajectories of (c) 10 μm particles and (d) 15 μm particles [143].

According to equation (3.7), the EP and EO forces are linearly proportional to the electric field strength, $\vec{E}$. However, the magnitude of the DEP force depends on the gradient of the square of electric field, $\nabla|\vec{E}|^2$. An increase of the applied

voltage causes the particle to move faster, and more importantly results in a greater repulsive DEP force that pushes the particle away from the block. Hence, the particle's trajectory can be shifted into the upper reservoir once the applied voltage reaches a threshold voltage, V_{Th} , below which the particle is directed into the lower reservoir. As shown in Figure 3.4 (a) and (b), the threshold voltages V_{Th} for sorting the 10 μm and 15 μm particles were 120 and 85 V, respectively. For any voltages above the V_{Th} , the particles were transported towards the upper reservoir. As suggested by equation (2.19), the 15 μm particle required a lower threshold voltage since it acquired a larger DEP force compared to the 10 μm particle under the same applied voltage. To validate the trajectories, the initial locations of the particles in these experiments were used in the simulation. Figure 3.4 (c) and (d) show reasonable agreement between the experimental (dotted lines) and simulation (solid lines) results.

3.4.2 Size-dependent separation of particles

According to equation (2.19), the DEP force is proportional to the cubic power of a particle's radius. Particles of different sizes will thus experience a significant difference in the DEP force. Such mechanism is utilized to perform the separation of particles by size in the modified H-filter. For demonstration purpose, a mixture of two sized particles (e.g., 5 μm and 15 μm) was placed in the inlet C while an equal DC voltage was imposed to the inlets C and D. Making use of the experimental findings presented in Figure 3.4 (a) and (b), 85 V was applied. Such a voltage reaches the threshold voltage of the 15 μm particles, but is still below the

threshold voltage of the 5 μm particles. As shown in Figure 3.5 (a), the 15 μm particles are successfully separated from the 5 μm particles. Using the same voltage, the device also successfully separated the 15 μm particles from the 10 μm particles (Figure 3.5 (a)). These experimental observations are in good agreement with the simulations as shown in Figure 3.5 (c) and (d).

Figure 3.5: Separation at 85 V for (a) 5 μm and 15 μm particles and (b) 10 μm and 15 μm particles. Comparison of experimental and simulation results for (c) 5 μm and 15 μm particles and (d) 10 μm and 15 μm particles [143].

3.4.3 Reduction of threshold voltage

As demonstrated in the previous section, a rather high applied voltage is required so as to intensify the repulsive DEP force for deflecting the particle's trajectory. High voltages however not only may produce Joule heating effects, but also can generate bubbles due to electrolysis. Furthermore, in biomedical applications, such high voltages could also jeopardize those cells. To reduce the applied voltage, the utilization of a combined AC and DC electric field is proposed. Moreover, the AC component presents an added parameter for the control of the particle motion.

Figure 3.6 (a) and (b) show the experimental investigation of the 10 μm and 15 μm particles' trajectories under a combined AC and DC electric field at 2 kHz. The experimental results are in reasonable agreement with the simulation results as shown in Figure 3.6 (c) and (d). Both results indicated that the use of the combined AC and DC electric field can reduce the threshold voltages required for sorting the 10 μm by 13.33% and the 15 μm particles by 24.7%. For example, the required threshold voltage for the 10 μm particle was decreased from 120 V in the DC field mode to $90+20\sin(\omega t)$ V (or 104 V, RMS of V_{ac} plus V_{dc}) in the combined AC and DC electric field mode. Correspondingly, the electric field strength at the block gap is lowered from 580 V/cm to 520 V/cm. Similarly, for the 15 μm particles, the threshold voltage was reduced from 85 V to $50+20\sin(\omega t)$ V (or 64 V). In addition, Lee *et al.* reported that an electric field strength of 21 kV/cm at 2 MHz is required for lysis of yeast cells and *E. coli* in a microchannel [151]. However, the highest

electric field strength used in the modified H-filter is only 520 V/cm through the application of $90+20\sin(\omega t)$ V at 2 kHz. Therefore, the proposed mode of the combined AC and DC electric field will be useful and safe in applications of living cell separation.

Figure 3.6: The experimentally observed trajectory of (a) 10 μm particles and (b) 15 μm particles under (i) a DC voltage and (ii) a combined AC and DC electric field. Comparison of simulation and experimental results of (c) 10 μm particles and (d) 15 μm particles [143].

Furthermore, scaling analysis suggests that the characteristic response time of a charged particle to an applied electric field is of order [152]

$$\tau_{\text{EP}} = O(a^2 \rho_p / \eta) \quad (3.11)$$

For a typical particle of 10 μm in diameter and 1,050 kg/m^3 in density, the particle electrophoretic response time is of order $\tau_{\text{EP}} = O(10^{-5})$ s. In the same fashion, the characteristic response time of an EO flow to the applied electric field can be estimated from [152]

$$\tau_{\text{EO}} = O(D_h^2 \rho / \eta) \quad (3.12)$$

For a typical microchannel of 100 μm in hydraulic diameter, D_h , we can find the EO relaxation time of order $\tau_{\text{EO}} = O(10^{-2})$. Since the electric field frequency of 2 kHz was used in the experiment, the particle EP should be able to follow the applied AC field component but not the liquid EO flow. In other words, the AC component of the EO flow is suppressed. However, as our CDD camera could only record images at a rate of 15 fps, a steady particle motion still was observed during the experiment, but a change in the particle velocity due to AC electric field was noted.

Moreover, although the experimentally observed particle trajectories were in reasonably good agreement with the theoretical predictions, the zeta potential values used in the theoretical predictions can only be considered as a fitting parameter. As previously given in Section 3.4, the zeta potentials of the 5 μm , 10 μm and 15 μm particles in the simulation were chosen as -55.1 mV, -71.6 mV and -93.1 mV, respectively. Obviously, such dependency of the particle zeta potential on size does not comply with the conventional electrokinetic theory. Thus, it should be pointed out that several factors were not included in the present model, and they

include (i) pressure head built up due to the unequal levels of the buffer solution at the reservoirs, (ii) hydrodynamic and electrokinetic interactions between the particle and the channel wall, (iii) heterogeneous electrokinetic effects such as discrete surface charges and non-uniform zeta potential, and (iv) roughness and inclined edges of the channel walls. Therefore, further improvement of the theoretical model is required in the present studies.

3.4.4 Electroosmotic flow field

As EO flow is an important component in EK transport, the numerical simulation and μ -PIV technique were employed to obtain the EO velocity field. Subjected to the boundary conditions shown in Table 3.1, the simulated EO velocity field induced by 50 V is shown in Figure 3.7 (a). Since outlets A and B are assigned with a higher voltage than inlets C and D, two EO streams transport from the outlets to the inlets. The EO velocity profiles at channels linked from outlets A and B exhibit plug-like flows but are distorted at the T-junction near the blocks. The EOF velocities satisfying the conservation of mass are increased when passing the gap between the blocks and the channel wall.

Using the μ -PIV technique, the velocity measurement of the 0.93 μm fluorescent tracer particles subjected to 50 V was performed to obtain the EO flow pattern (Figure 3.7 (b)). A qualitative comparison between the simulation and the experimental results indicates reasonable agreement in the EO flow patterns. The μ -PIV measurement showed that the tracer particles transported in the same direction of the EO flows. This implies that the tracer particles have a lower electrical zeta

potential compared to the channel walls. As both the channel walls and the particles were negatively charged, EO was opposite to EP. Therefore, the magnitude of the velocity field obtained from the μ -PIV measurement was smaller than that from the simulation. In addition, DEP of the tracer particles was not considered in the simulation, and it also contributed to the discrepancy. However, due to limitation of the frame speed of our CCD camera, the μ -PIV measurement of the EO velocity field under the DC-biased, AC field mode was not performed [152].

Figure 3.7: The velocity field of EO flow at the T-junction near insulating blocks by (a) simulation and (b) μ -PIV measurement [143]

3.4.5 Effect of the number of insulating blocks

In this analysis, the effect of the number of insulating blocks on the threshold voltage required for particle separation is conducted numerically and experimentally. The number of insulating blocks is increased from one to five while all the blocks remain the same dimensions ($150\text{ }\mu\text{m}$ wide x $240\text{ }\mu\text{m}$ high) as previously presented in the experiments. The results show that the threshold voltages for both particles of $10\text{ }\mu\text{m}$ and $15\text{ }\mu\text{m}$ decrease as increasing the number of blocks (Figure 3.8). Because the DEP forces are generated around the corners of the blocks, the increasing number of blocks thus favorably provides enhanced effect of DEP on the particles.

Figure 3.8: Effect of the number of insulating blocks on threshold voltage

3.4.6 Effect of the gap of insulating blocks

Furthermore, the effect of the gap between insulating blocks on the threshold voltage for the particle separation was studied through numerical simulation of the modified H-filter with five insulating blocks. Simulations were performed with the 300 μm wide main channel having insulating blocks (150 μm wide x 240 μm high) and the varied gaps of 50 μm , 150 μm , 300 μm , 400 μm and 450 μm were used. The simulation results clearly exhibit that for the 15 μm particle, the threshold voltage decreases when the gap becomes larger, for example, from 50 μm to 300 μm as shown in Figure 3.9. As expected, the larger gap provides more space for electric field to bend around the corners of each block, thus allowing higher degree of non-uniform electric field and promoting the DEP forces in the system. However, the threshold voltage remains unchanged when the gap is wider than the main channel (>300 μm). This suggests that this non-uniform electric field around the block corners no longer varies. Therefore, these results show that the most optimal gap should be as wide as the main channel.

Figure 3.9: Effect of the gap between insulating blocks on threshold voltage

3.4.7 Effect of the width of insulating blocks

To examine the effect of the block width on the threshold voltage, the block width was varied from 50 μm , 150 μm and 300 μm while the 300 μm wide block gap and the 15 μm particle were remained in the simulation. The simulation results reveal that the device requires lower threshold voltages when the width of insulating blocks decreases as illustrated in Figure 3.10. In such case, the electric field around a smaller block varies more rapidly as compared to that around a larger block, therefore increasing the electric field gradient and improving DEP forces. This causes the threshold voltage to reduce with decreasing block widths.

Figure 3.10: Effect of the width of insulating blocks on threshold voltage

3.4.8 Separation of multiple particles

This section extends the application of the modified H-filter from the simple size-dependent separation of two components. Instead, the modified H-filter was employed to separate multiple particles by size and type, simultaneously. This application is of importance in biomedical diagnosis in which cells of interest need to be identified and separated from a mixture containing more than two different sizes and types of bioparticles. In this demonstration, instead of using electrokinetics, the device employs a combination of hydrodynamics through a pressure gradient to transport particles and AC-iDEP to separate them continuously. This alternative operation in fact demonstrates that the device is versatile such that separation can be performed by either electrokinetics or hydrodynamics.

In experiment, fluorescent latex particles of $2\ \mu\text{m}$ and latex particles of $5\ \mu\text{m}$ and $10\ \mu\text{m}$ (Duke Scientific, USA) were suspended in a $13\ \mu\text{S/cm}$ NaHCO_3

solution. To prepare a sample, 0.02 ml (of the original concentration in DI water) of 2 μm particles, 1 ml of 5 μm particles and 2 ml of 10 μm particles were added in a micro-centrifuge tube. The sample was centrifuged at 13300 rpm for 3 minutes first and then transferred into 1 ml of a 13 $\mu\text{S}/\text{cm}$ NaHCO_3 solution. The process was repeated three times to complete. To generate AC electric field, the same set of equipment in the previous sections was used to provide an amplified AC voltage that was imposed externally to pt wires through reservoirs.

As previously shown in equation (2.13), at low electric field frequency regions (e.g., < 10 kHz), $\text{Re}[f_{CM}(\omega)]$ is determined by the electrical conductivity of particles and their suspending medium. Taking the surface conductance into consideration ($K_s \sim 1.2$ nS) [37, 38], $\text{Re}[f_{CM}(\omega)]$ for the particles of 2 μm , 5 μm and 10 μm can be respectively computed as +0.22, -0.1 and -0.27, suggesting that 2 μm particles experience pDEP, but both 5 μm and 10 μm particles experience nDEP.

According to the numerical analysis on the effects of the block gap and width on the threshold voltage, the optimal gap should be as wide as the main channel, and the smaller block the lower threshold voltage needed. Dimensions of the modified H-filter with three outlets for separation of multiple particles are illustrated in Figure 3.11. Here, five insulating blocks (50 μm wide and 80 μm high) with a 100 μm wide gap were fabricated in a 100 μm wide main channel. Since 5 μm and 10 μm particles are expected to experience nDEP forces which repel them towards the outlets C and D, the relatively wider outlet D was designed to provide

more space to prevent 5 μm particles from moving into the outlet C. For 10 μm particles exerting sufficient nDEP forces, the device can separate them into the upper outlet C.

Figure 3.11: Dimensions of a modified H-filter for separation of multiple particles.

In addition, as shown in equation (2.11), since the magnitude of DEP force is proportional to the gradient of the square of electric field, numerical analysis on electric field distribution and the y-component gradient of the square of electric field near the insulating blocks was also performed. For the block gap of 25 μm (Figure 3.12 (a)) and 100 μm (Figure 3.12 (b)), the simulated electric field distribution shows that electric field intensity is high at the constrictions between the blocks and the wall with the local electric field maxima at the corners, but the intensity appears to be significantly lower at surrounding areas.

When a 25 μm wide gap is designed, the insulating blocks are located too closely (Figure 3.12 (a)). The electric field intensity at the top portion of the block

gap sandwiched by two constrictions becomes relatively high due to the surrounding high field intensities produced by the two constrictions as compared to the case of the 100 μm wide gap (Figure 3.12 (b)). This spatial difference in such electric field intensities across the block gap (along the vertical direction) leads to an unwanted nDEP force which pushes the particle downwards and reduces the wanted nDEP force effects induced at the block corners as schematically illustrated in Figure 3.12 (a). To support this simulation interpretation, the y-component nDEP force is analyzed by computing the y-component gradient of the square of the electric field ($\nabla_y |\vec{E}|^2$) along the x direction from the centre of the first block to that of the second block (as labeled by **a** and **a'** in Figure 3.12 (a)). In Figure 3.12 (c), for the 25 μm wide gap, $\nabla_y |\vec{E}|^2$ expectedly reaches its highest values at the right corner of the first block and the left corner of the second block, implying dominant nDEP forces for particle separation. However, the negative value of $\nabla_y |\vec{E}|^2$ appears at the block gap and it can be interpreted as the downwards nDEP force which is unfavorable for the separation. On the other hand, the negative values of $\nabla_y |\vec{E}|^2$ gradually and completely disappear for the wider gap of 50 μm and 100 μm , respectively. These results suggest that at 100 μm , the gap becomes as wide as the main channel, and therefore the electric field has enough space to fully distribute after compressed in the constriction. Hence, the electric field intensity at the block gap is negligible and the negative value of $\nabla_y |\vec{E}|^2$ is not generated. It is also found that in comparison with the 100 μm wide gap, the negative value of

$\nabla_y |\vec{E}|^2$ for the 25 μm wide gap noticeably increases up to 158 % although a slight increase in the maximum value of $\nabla_y |\vec{E}|^2$ of about 2.4% is noted. Furthermore, Figure 3.12 (d) shows that as the block gap increases more than the main channel width ($>100 \mu\text{m}$), no noticeable change of $\nabla_y |\vec{E}|^2$ can be observed. Therefore, from these simulation results, it is recommended that to avoid the unwanted DEP force, the block gap should be as wide as the main channel. This conclusion is consistent with the numerical analysis given in the section 3.4.6.

Figure 3.12: (a-b) Simulation results of electric field distribution and fluid streamlines near two 50 μm wide insulating blocks spaced (a) 25 μm and (b) 100 μm apart. The y-component gradient of the square of electric field representing nDEP force (from the centre of the first block and that of second block as labeled by **a** and **a'** in Figure 3.12a) (c) for the block gap of 25 μm , 50 μm and 100 μm and (d)

for the block gap of 100 μm , 150 μm and 200 μm . It is noted that the nDEP force arrows in Figure 3.12 (a) and Figure 3.12 (b) are not drawn in scale.

The effect of insulating block width on DEP force is also examined by computing the value of $\nabla_y |\vec{E}|^2$ for three block widths of 50 μm , 75 μm and 100 μm while the block gap of 100 μm is remained. The simulation results in Figure 3.13 show that the smallest (50 μm wide) insulating blocks produce the highest value of $\nabla_y |\vec{E}|^2$. In this case, the electric field around the smaller blocks varies more rapidly, thus increasing higher electric field gradients as compared to those around the larger blocks. In addition, it is found that the block width has no effect on the negative value of $\nabla_y |\vec{E}|^2$ as long as the block gap is as wide as the main channel.

Figure 3.13: The y-component gradient of the square of electric field for three block widths of 50 μm , 75 μm and 100 μm with the block gap of 50 μm remained.

Figure 3.14 (a) shows two symmetric streams of a flowing sample particle mixture (bottom) and a buffer solution (top). With no applied electric field, all the particles were directed to the outlet E (Figure 3.14 (b)). By applying a sufficient or threshold AC voltage of 635 V_{AC} with a frequency of 5 kHz, the continuous-flow separation was achieved successfully (Figure 3.14 (c)). 10 μm particles were pushed into the outlet C as they underwent stronger nDEP forces due to their larger size in comparison with the 5 μm particles. The 5 μm particles were then separated from other samples to the outlet D. As for 2 μm fluorescent particles exerted by pDEP forces, the device attracted them near the blocks as seen by the concentrated particle stream flowing to the outlet E. To reduce the threshold voltage, an increase in the number of blocks is a straightforward approach to implement.

Figure 3.14[†]: Particle transport by a pressure-driven flow at (a) inlets and (b) outlets in the absence of an applied electric field. (c) Simultaneous separation of particles of 2 μm , 5 μm and 10 μm by size and type achieved under 635 V_{AC} with a frequency of 5 kHz.

3.4.9 Trapping of single-walled carbon nanotubes (SWCNTs)

Nowadays, single-walled carbon nanotubes (SWCNTs) are widely recognized as building blocks in future nanoscale electronics due to their exceptional electrical and mechanical properties [153, 154]. Metallic SWCNTs could be used for leads in a nanoscale circuit whereas semi-conducting SWCNTs could function as nanoscale Schottky-type field-effect transistors [155]. Therefore, to realize these applications, manipulation and separation of metallic from semi-conducting SWCNTs become necessary. In addition, some researchers also utilized the SWCNTs in enhancing the capture of *E. coli* in a DEP-based chip [156], and simultaneous detection of Hepatitis B virus and α -fetoprotein in a micro-piezoelectric immunoassay chip [157]. In this section, a PDMS-based device with multi-insulating blocks is used to demonstrate proof-of-concept trapping of SWCNTs using a combined AC and DC electric field for possible biosensor applications. The investigation of the trapping by using different shapes and alignments of insulating blocks was also carried out.

[†] These results are in preparation for submission to *Electrophoresis*

To prepare the suspension of SWCNTs, 12 mg of raw SWCNTs from the HiPco process was suspended in 10 ml of DI water containing 0.05 g of the surfactant sodium dodecyl sulfate (SDS) under 4 hour sonication. After sonication, the sample was centrifuged for one hour at 50,000 g. The upper portion of the supernatant was decanted. The resulting suspension has a mass concentration of roughly 1 mg/ml. The sample was injected into the device, and the liquid flow was balanced by a precision syringe before applying a voltage (Figure 3.16 (a)-(d)).

First, effect of a pure AC voltage on trapping was studied. Figure 3.15 (a) shows a uniformly dispersed suspension of SWCNTs with no applied AC voltage. By applying 635 V_{AC}, experimental results showed that the SWCNTs were attracted by pDEP to the block corners and formed chains due to dipole-dipole interactions in front of the blocks as shown in Figure 3.15 (b) [33]. The 2 minute trapping (or concentration) was also accompanied with depletion of the SWCNTs at the area between the blocks.

Figure 3.15: (a) A well dispersed suspension of SWCNTs under no applied AC voltage. (b) Trapping of SWCNTs using pDEP induced by insulating blocks under a pure AC voltage of 635 V at 5 kHz for 2 minutes

With a combined AC and DC electric field, the left reservoir was applied with a voltage of $635\sin(\omega t)+126$ V while the right reservoir was electrically grounded. Figure 3.16 shows the SWCNT suspension before ((a)-(d)) and after the application of the electric field ((e)-(h)), and compares the trapping performance with different block patterns. It was experimentally observed that an EO flow induced by the DC voltage transported from the positive to ground terminal (left to right), indicating that the zeta potential of channel walls is higher than that of the SWCNTs. When moving with the EO flow through the area of insulating blocks, the SWCNTs were trapped at the corners of each block against the EO flow (Figure 3.16 (e)-(h)). Moreover, compared to the case of pure AC voltage, the use of DC-biased voltage was observed to greatly promote the formation of SWCNT chains.

These formed chains in turn induced local non-uniform electric field around themselves and extended DEP effects in the bulk solution [156]. Shortly, a clear accumulation of SWCNTs started to occupy the structures of the insulating blocks.

Figure 3.16: Trapping of SWCNTs in a PDMS-based device with different block patterns under no applied voltage (a)-(d), and under $635\sin(\omega t)+126$ V at 5 kHz for 3.5 minutes (e)-(h).

3.5 Summary

The modified H-filter platform with multi-insulating blocks is developed for a flow-through separation and manipulation of particles by size. As relying only on a single channel voltage power supply, the device greatly simplifies the operation. The particle transport and manipulation are efficiently controlled by means of electrokinetics. The insulating blocks enhance DEP forces and simultaneously focus

particles, thus increasing the controllability of particle motion and facilitating the manipulation and separation.

By simply adjusting DC voltages, sorting and separation of the particles by size can be successfully achieved. The application of a combined AC and DC electric field shows its potential in reducing the threshold voltage required for separation. The modeling based on the Lagrangian approach is developed to simulate particle trajectories under both operation field modes, and the results are in reasonable agreement with the experimental results. In addition, the EO flow field was also experimentally and numerically obtained and analyzed.

Moreover, the study of the effects of the number and geometrical structure of insulating blocks on the threshold voltages can lead to the optimal configurations for particle separation with a minimum threshold voltage. Using such findings, the continuous separation of multiple particles by size and type simultaneously is demonstrated by using hydrodynamics and AC-DEP. This demonstration shows the versatility and flexibility in operating the device, which is not only limited to electrokinetics. Finally, the utilization of insulating blocks is also extended to trap SWCNTs for possible applications in biosensing.

Chapter 4 Electrokinetically-Driven Concentration of Particles and Cells by Dielectrophoresis Using Combined AC and DC Electric field[†]

4.1 Introduction

The concentration of samples is a key factor that indicates the efficiency in biochemical reaction and sample detection [3, 4]. For example, in gene expression analysis, an adequate number of cells are necessary for RNA preparation. For water quality monitoring, culturing and other time-consuming routines are inevitable to enrich bacterial subpopulations for detection. Conventionally, the DEP-based technique has employed metallic electrodes to induce a non-uniform electric field required for producing DEP force for sample concentration [94, 158, 159]. Alternatively, Cummings and Singh proposed an insulator-based DEP (or iDEP). The iDEP utilizes insulator posts to alter the paths of the applied electric field, thus generating non-uniform electric fields around the posts. The iDEP can concentrate samples at low frequencies with no electrolysis and offers simple device fabrication techniques compatible to rapid prototyping: injection molding or soft lithography [24, 160]. This iDEP principle has been successfully applied to concentrate

[†] The works presented in this chapter have been submitted to *Microfluidics and Nanofluidics*.

submicron particles, DNA, bacteria and protein [22, 23, 161, 162]. However, the technique typically required a very high DC electric field due to the coupled phenomena between linear and nonlinear electrokinetic transports [130, 160].

This chapter presents a new electrokinetic technique for concentration of particles and cells using iDEP generated by combined AC and DC electric field. This is achieved in a PDMS microfluidic channel with a contraction to induce a non-uniform electric field required for producing repulsive DEP forces exerting on particles and cells. Concentrations of particles of 5 μm , 10 μm and 15 μm were experimentally conducted under combined AC and DC electric field to analyze the effects of buffer concentration, particle size and threshold voltages on DEP concentration. Experiments and numerical simulations based on the Lagrangian approach revealed that by applying combined AC and DC electric field, the total field strength required to concentrate (or trap) particles of 15 μm was significantly reduced by 85.9% as compared to the applied DC field strength. In addition, this proposed technique was also successfully used to concentrate *Saccharomyces cerevisiae* (yeast) cells.

4.2 Experiment

4.2.1 Design and fabrication of the device

Figure 4.1 depicts a PDMS-glass microfluidic device fabricated by the photolithography technique for patterning a SU-8 mold and the soft lithography for replica of PDMS microchannels. The detailed fabrication can be found in Chapter 3.

The PDMS channels with punched two reservoirs were bonded with a cleaned glass cover through oxygen plasma treatment. The assembled device was left in air for 48 hours to ensure stable zeta potential of the PDMS channel walls prior to experiment [143, 147]. The device structure consists of a 1000- μm wide main microchannel with a contraction to connect a 60- μm wide narrowed channel. Reservoir A is for particle supply and reservoir B is for particle collection. The entire PDMS channel is 8 mm long and 60 μm deep. The contraction design enables applied electric field to be nonuniformly distributed, thereby giving rise to electric field gradient with local maxima at the corners of the narrowed channel.

Figure 4.1: A PDMS-glass microfluidic device for concentration of particles and cells

4.2.2 Sample preparation and experimental setup

Two types of samples were used in experiments, namely (i) Polystyrene latex microspheres (Duke Scientific) of 10 μm and 15 μm in diameter with a number concentration of 5×10^5 particles/ml and (ii) Baker's yeast cells (*Saccharomyces*

cerevisiae). To prepare particle suspensions with a concentration of 5×10^5 particles/ml, 0.5 ml of latex microsphere suspensions with an original number concentration of 1×10^6 particles/ml was suspended into 1 ml of NaHCO_3 buffer solutions of three different concentrations of 10^{-4} , 10^{-3} and 10^{-2} M, by centrifugation and resuspension at 5000 rpm in three consecutive times. The corresponding electrical conductivities of three NaHCO_3 buffer solutions are 13, 95 and 897 $\mu\text{S/cm}$, respectively. To prepare the yeast cell suspension, 5 mg of Baker's yeast (dry form) was suspended in 2 ml of DI water for re-activation for 1 hour at room temperature. The suspension was washed three times with DI water to remove any added preservative before suspending it into 2 ml of 250 mM mannitol solution (M-9546 D-mannitol, Sigma-Aldrich) by centrifugation and resuspension at 5000 rpm three times more. The yeast sample was finally suspended in 2 ml of 600 $\mu\text{S/cm}$ NaHCO_3 solution for DEP experiments.

Prior to experiments, the microchannel was treated with a 5% bovine serum albumin solution for an hour to alleviate particle/cell adhesion on channel surfaces. Platinum wire electrodes were put into Reservoirs A and B to produce required electric field. Before applying voltages, no motion of particles or cells was ensured by balancing the buffer level in each reservoir. Two types of experiments were conducted for comparison: one was to supply pure DC voltages with a DC power supply (Stanford Research), and the other was to provide combined AC and DC voltages with a function generator (Agilent 33250A) amplified by a customized voltage amplifier (Optrobio, Singapore). The motion of particles and cells was

recorded using a CCD camera connected with an inverted microscope (Nikon, TE-2000U).

4.3 Mechanisms of particle concentration under combined AC and DC electric field

Figure 4.2 (a) presents the concentration mechanisms under a DC electric field. Here a negatively charged particle is induced towards a positive terminal by assuming that the EP force is stronger than the EO force. Furthermore, in the neighborhood of the contraction region, the change of channel geometry causes the electric field variation which results in a repulsive DEP force acting on the particle as shown in Figure 4.2 (a). In electrokinetic transport, the EO and EP force, termed as electrokinetic (EK) force ($\vec{F}_{EK}$), acting on a charged spherical particle of radius a under an electric field $\vec{E}$ is given by [32]

$$\vec{F}_{EK} = \vec{F}_{EO} + \vec{F}_{EP} = 6\pi\eta a \vec{U}_{EO} + 6\pi\epsilon_m a \zeta_p \vec{E} \quad (4.1)$$

Under the steady state, a force balance among EK and DEP and Stokes drag force give a terminal velocity of the charged particle in the DC field expressed as

$$\vec{u}_p = \frac{\vec{F}_{EK} + \vec{F}_{DEP}}{f} \quad (4.2)$$

$$\vec{u}_p = -\frac{\epsilon_m \zeta_w}{\eta} \vec{E}_{dc} + \frac{\epsilon_m \zeta_p}{\eta} \vec{E}_{dc} + \frac{c\epsilon_m a^2 \text{Re}[f_{CM}(\omega)] \nabla \vec{E}_{dc}^2}{3\eta} \quad (4.3)$$

On the right-hand side of equation 4.3, the first and second terms present the EO and EP velocities which are linearly proportional to applied electric field strength, and the third term is due to the DEP velocity which is dependent on the gradient of the square of the electric field. Equation 4.3 also suggests that when a sufficiently high DC electric field is applied, the DEP velocity can balance the EO and EP velocities, resulting in zero terminal velocity $\vec{u}_p = 0$, where particles with zero velocity are likely to be accumulated for concentration. However, since the EO and EP velocities are enhanced simultaneously with increasing DC field, this shows that under a pure DC field, the phenomena of EO, EP and DEP are coupled, thereby resulting in required very high DC field for achieving particle concentration in iDEP based microfluidic devices.

Figure 4.2: Concentration mechanisms under (a) a pure DC electric field and (b) a combined AC and DC electric field

In contrast, Figure 4.2 (b) illustrates the concentration mechanisms under combined AC and DC electric field. The AC electric field mainly contributes to a DEP force not to the particle transport because it suppresses an EO flow and has no

effect on particle EP motion. While the DC field still generates DEP force, it mostly determines EO and EP related particle motion. As a result, the particle DEP velocity controlled by the AC field can be decoupled from the EO and EP velocities controlled by the DC field. The terminal velocity of the particle under combined AC and DC electric field can be expressed as

$$\vec{u}_p = -\frac{\epsilon_m \zeta_w}{\eta} \vec{E}_{dc} + \frac{\epsilon_m \zeta_p}{\eta} \vec{E}_{dc} + \frac{c \epsilon_m a^2 \text{Re}[f_{CM}(\omega)] \nabla(\vec{E}_{dc}^2 + \vec{E}_{rms,ac}^2)}{3\eta} \quad (4.4)$$

Equation (4.4) shows the DEP velocity which depends on both the AC and DC field. Therefore, by increasing the AC electric field, the concentration can be achieved at a relatively lower voltage because the AC field has no effect on the EO and EP related particle velocity.

To determine the particle's velocity, the electric field ($\vec{E} = \nabla \phi$) and the EO velocity field must be obtained beforehand. Under the assumption of thin EDL of micro-scaled channels, the electric potential (ϕ) governed by Laplace's equation is given by

$$\nabla^2 \phi = 0 \quad (4.5)$$

The boundary conditions are that the electric fields normal to electrically insulating channel walls are zero and the channel's outlet and inlet are assigned with an electric potential and electric ground, respectively. Under the steady state, the EO velocity field governed by the Navier-Stokes (NS) equation and the continuity equation is expressed as

$$\rho(\vec{u} \cdot \nabla \vec{u}) = -\nabla p + \eta \nabla^2 \vec{u} \quad (4.6)$$

$$\nabla \cdot \vec{u} = 0 \quad (4.7)$$

The boundary conditions for the NS equation are that all the insulating walls are assigned with a slip velocity (by using the Smoluchowski equation), which is tangential to the channel walls, and zero external pressure is assigned at both reservoirs.

4.4 Device performance

Device performance is determined by concentration factor (CF) and removal efficiency (RE). CF indicates the ability of concentrating particles while RE indicates the efficiency of removing particles from a buffer solution [163]. These parameters are given by

$$CF = \frac{C_{Con}}{C_0} \quad (4.8)$$

$$RE = \frac{C_0 - C_{Escape}}{C_0} \times 100\% \quad (4.9)$$

where C_0 is the rate at which particles flow in the main channel, C_{Con} is the rate at which particles are concentrated when an electric field is applied, and C_{Escape} is the rate at which particles pass through the contraction under applied electric field.

4.5 Results and discussion

4.5.1 The CM factor plot

The CM factor for the particles of 5 μm , 10 μm and 15 μm with a surface conductance (K_s) of 1.2 nS [37, 38], suspended in the buffer solutions of 10^{-4} , 10^{-3} and 10^{-2} M (13, 95 and 897 $\mu\text{S/cm}$, respectively), are plotted in Figure 4.3. According to the CM factor described in equation 2.13, to generate a repulsive DEP force necessary for sample concentration, the medium must be more conductive than the particle. At low frequency (e.g., <10 kHz), the CM factor increases in magnitude with a negative sign when the size of particles and buffer concentration increases, thus indicating stronger DEP repulsion. At 10^{-2} M, the CM factors of all sizes of the particles nearly reach their theoretical maximum value at -0.5. This suggests that the conductivity of their suspending medium has completely dominated that of particles which mainly contribute from the surface conductance and particle's size as shown in equation 2.13.

Figure 4.3: The CM factor for various particles of 5 μm , 10 μm and 15 μm in three different NaHCO_3 solutions of 10^{-4} , 10^{-3} and 10^{-2} M (with corresponding electrical conductivities of 13, 95 and 897 $\mu\text{S/cm}$, respectively)

4.5.2 Comparison of threshold voltage for particle concentration

Experiments were conducted to study threshold voltage for particle concentration under (i) a pure DC field, and (ii) combined AC and DC field. Figure 4.4 (a) shows an image of concentrated 15 μm particles in a 10^{-4} M NaHCO_3 buffer solution under a markedly high DC threshold voltage of 3400 V (corresponding to an electric field of 4250 V/cm). In this experiment, the negatively charged particles were induced towards the positive electrode at high speed and were concentrated by

the DC-DEP forces. However, when large electrical potential gradient is applied across a microchannel, the generated electrical current results in a significant temperature rise in the electrolyte. Adverse effects produced by excess temperature rises inside the channel are boiling of electrolyte and samples, bubble formation, denaturation of biosamples and breakdown of the device [164, 165]. This phenomenon is known as Joule heating. As Joule heating is proportional to the second power of the current density, the current density in the narrowed channel generates more heat than that in the wide channel [166]. As a result, Figure 4.4 (a) shows burning PDMS in the narrowed channel due to Joule heating after applying 3400 V for 30 seconds. As reported by Ge *et al*, the determination of liquid temperature distribution in microchannels can be achieved by using the rhodamine B based thermometry technique [167]. As a comparison, Figure 4.4 (b) shows an image of concentrated 15 μm particles in a 10^{-4} M NaHCO_3 solution under a relatively low combined AC and DC voltage of $30\text{ V} + 635\sin(\omega t)$ with its frequency of 5 kHz (corresponding to a total electric field of 479 V/cm). It is noted that $635\sin(\omega t)$ and 5 kHz are the maximum AC amplitude and frequency produced by our amplifier). The high AC frequency of 5 kHz was chosen to suppress EO flows and eliminate particle EP. By using lower AC amplitude, the trapping of particles was experimentally found not effective because some particles could pass the contraction although DC voltage was applied. The comparison of Figure 4.4 (a) and (b) shows the use of combined AC and DC field can lead to a significant reduction in threshold electric field strength by 85.9%.

Figure 4.4: Concentrations of 15 μm particles under (a) a pure DC voltage of 3400 V and (b) $635\sin(\omega t)+30$ V at 5 kHz

To explain the observed particle concentration location shown in Figure 4.4 (a) and (b), the variation of particle velocity along the channel axial direction was numerically computed under a pure DC field (by using equation (4.3)) and under combined AC and DC field (by using equation (4.4)), and the respective computed particle velocity results are depicted in Figure 4.5 (a) and (b). In the simulation, the zeta potentials of 15 μm polystyrene particles and the PDMS/glass microchannel in a 10^{-4} M NaHCO_3 solution were set as $\zeta_p = -93.1$ mV and $\zeta_{wall} = -23.5$ mV. In Figure 4.3, the CM factor is -0.33 for 15 μm particles in a 10^{-4} M NaHCO_3 solution under a frequency of 5 kHz. The correction factor $c=0.5$ was considered as a fitting parameter accounting for the perturbation of the particle size (of 15 μm) in the electric field. This c value is close to $c=0.4$ reported by Kang *et al.* [127]. Figure

4.5 (a) and (b) show that the velocities of a 15 μm particle under both electric field modes are nearly constant in the main channel, then reduce to zero upon approaching the contraction area (where the corners are located at 4000 μm), and finally increase to much high values in the narrowed channel. Moreover, under both the applied pure DC field and the combined AC and DC field, the numerically predicted locations of particle zero velocity shown in Figure 4.5 (a) and (b) reasonably match to the experimentally observed locations as shown in Figure 4.4 (a) and (b).

In addition, to quantify the threshold voltage required for achieving particle concentration, the forces exerting on a 15 μm particle were computed. The electrokinetic forces (represented by square symbols) were calculated using equation (4.1) and the DEP forces (represented by circular symbols) were calculated using equation (2.20). The computed resulting forces versus applied DC voltages for (i) an applied pure DC field and (ii) a combined AC and DC field ($635\sin(\omega t)$ at 5 kHz) are shown in Figure 4.5 (c) and (d), respectively. As expected, the electrokinetic forces are linear proportional to applied DC voltage and the DEP forces are scaled quadratically with applied DC voltage. Furthermore, above a threshold voltage the DEP forces are larger than the electrokinetic forces, suggesting that particle concentration can be realized. The predicted threshold voltages are 3250 V for the case of applied pure DC field and 65 V for the case of combined AC and DC field. These numerical simulations support the experimental results shown in Figure 4.4 (a) and (b) that by applying 3400 V or $635\sin(\omega t)+50$ V, the 15 μm particles can be concentrated. Moreover, the concentration location is

numerically predicted to be at $7.5\ \mu\text{m}$ (in the horizontal direction) and $10\ \mu\text{m}$ (in the vertical direction) away from the corners. This yields a reasonably close match to the experimentally observed location, which is about $15\ \mu\text{m}$ (a particle's diameter) (in the horizontal direction) and $10\ \mu\text{m}$ (in the vertical direction) away from the corners as shown in Figure 4.4 (a) and (b).

Figure 4.5: Numerical simulation of the terminal velocity of a $15\ \mu\text{m}$ particle under (a) $3400\ \text{V}$ and (b) $635\sin(\omega t)+30\ \text{V}$. The EK and DEP forces exerting on a $15\ \mu\text{m}$ particle at the concentration location under various (c) DC voltages and (d) a fixed AC voltage of $635\sin(\omega t)\ \text{V}$ with a variation of DC voltages from 20 to $80\ \text{V}$

4.5.3 Effects of buffer concentration and particle size

Under combined AC and DC electric field, experiments involving 10 μm and 15 μm particles in three different NaHCO_3 solutions of 10^{-4} , 10^{-3} and 10^{-2} M were conducted to investigate the effects of buffer concentration and particle size on DC threshold voltage. As shown in Figure 4.3, an increase in buffer concentration leads to a more negative CM factor, giving rise to a stronger repulsive DEP force. As a result, the particles are also allowed to transport with a higher velocity induced by a higher DC threshold voltage. Our experimental results agreed with this theoretical prediction that under a fixed AC voltage of $635\sin(\omega t)$ at 5 kHz, the DC threshold voltages for both particle sizes increased with increasing buffer concentrations as illustrated in Figure 4.6. Furthermore, since the DEP force is dependent on the cubic power of a particle radius, the larger particle undergoing a larger DEP force will be allowed to transport with a higher speed. Thus, Figure 4.6 shows that in the same buffer concentration, the DC threshold voltage for 15 μm particles is always higher than that for 10 μm particles.

Figure 4.6: Effects of buffer concentration and particle size on the DC threshold voltages with a fixed AC voltage of 635 V at 5 kHz

4.5.4 Determination of threshold voltage for particle concentration

Threshold voltage or the limiting condition that determines whether particles can be concentrated in the present device was investigated by varying applied AC and DC voltages. Figure 4.7 denotes that any combined AC and DC field falling above the curve can achieve particle concentration. For example, corresponding to an AC voltage of 635 V (i.e., the maximum output AC voltage by our power supply), the DC threshold voltages for attaining concentration of 10 μm and 15 μm particles in a 10⁻² M NaHCO₃ solution were found as 38 and 108 V, respectively. Exceeding such limiting conditions with any higher DC voltages resulted in failure of particles concentration, that is, particles could escape to pass through the narrow channel.

Similarly, when the AC voltages were reduced, DEP became weaker. The DC voltages must be decreased to retain the particle transport. Therefore, such optimal AC and DC voltage ratio is important to be identified. In addition, the results also show that the AC and DC voltage ratio (the slope) of 10 μm particles is steeper than that of 15 μm particles. Such difference was caused by the effect of particle size. The smaller particles experienced less DEP forces and therefore required higher AC and less DC voltages to achieve concentration.

Figure 4.7: The mapping of AC and DC threshold voltages under combined AC and DC field for 10 μm and 15 μm particles in a 10^{-2} M NaHCO_3 solution. The mapping suggests any applied DC-offset, AC field with either smaller DC voltage or larger AC voltage (compared to the threshold voltages shown in the figure) can achieve particle concentration.

4.5.5 Concentration factor and removal efficiency

Device performance was assessed by using the concentration factor CF (Figure 4.8 (a)) and the removal efficiency RE (Figure 4.8 (b)). Experiments were conducted for concentration of 10 μm and 15 μm particles in a 10^{-2} M NaHCO_3 buffer solution under their maximum DC threshold voltages: $635\sin(\omega t)+38$ V and $635\sin(\omega t)+108$ V, respectively. For 15 μm particles, the CF can reach as high as about 69 folds, but the RE is only 66%. In the case of 10 μm particles, the CF and the RE are 17 folds and 97%, respectively. These results can be understood as follows. Under combined AC and DC field, the AC component contributes only to DEP force for concentrating particles but has no effect on EO and EP. However the DC component, while still generating DEP force, mostly determines EO and EP related particle motion, suggesting a larger DC voltage can speed up the particle concentration but meanwhile can potentially cause particles to escape from the contraction region (without being concentrated). Therefore, the relatively high CF for 15 μm particles is resulted from stronger DEP forces (scaled to the cubic power of particle size) and faster transport velocity (due to a higher DC voltage of 108 V), compared to 10 μm particles under a DC voltage of 38 V. As a result, more 15 μm particles (thus higher throughput) can be concentrated. However, it was observed that with a large number of particles concentrated in the contraction region, the efficiency in DEP concentration deteriorated with time. Some particles could escape from a DEP virtual wall, thus lowering down the RE as time elapsed. On the other hand, since the transport velocity of 10 μm particles is much lower, their RE could

remain high. To further improve CF and RE, one can increase AC voltage and/or decrease DC voltage.

Figure 4.8: Device performance for 10 μm and 15 μm particles: (a) concentration factor and (b) removal efficiency

4.5.6 Concentration of 5 μm yeast cells

As shown in Figure 4.9, the present device was utilized for concentration of 5 μm yeast cells in a 600 $\mu\text{S}/\text{cm}$ NaHCO_3 buffer solution under combined AC and DC voltage of $8 \text{ V} + 635\sin(\omega t)$ at 5 kHz (the corresponding field strength is 571 V/cm). The experiment was conducted in a 35- μm -wide narrowed channel because as compared to 10 μm and 15 μm particles in earlier experiments, smaller 5 μm yeast cells require stronger DEP forces for concentration. It was observed that unlike the latex particles, yeast cells were transported in the same direction as EO flow which dragged them towards the ground electrode before they were concentrated (Figure 4.9 (a)). Near high electric field regions, yeast cells were observed to form chains (Figure 4.9 (b)). The formation of such cell chains seems to be favorable to the cell concentration (Figure 4.9 (c)). The phenomenon of chain formation was also found for latex particles (Figure 4.4 (b)). It should be noted here that in the experiment for concentrating yeast cells, the total field strength was 571 V/cm that is much below 21 kV/cm at 2 MHz - the condition of causing yeast cells to lyse [151].

Figure 4.9: Concentration of yeast cells under $635\sin(\omega t)+8$ V at 5 kHz in a 600 $\mu\text{S}/\text{cm}$ NaHCO_3 solution.

4.5.7 Summary

A new electrokinetic technique using combined AC and DC electric field for concentration of particles and cells by a repulsive DEP force in a microfluidic channel has been proposed and systematically investigated. Experimental and simulation results show that in concentrating 15 μm particles, the use of the combined AC and DC field allows the total field strength to markedly decrease by 85.9% in comparison with the pure DC electric field. The investigation of experimental parameters suggests that higher buffer concentration, larger particle size and higher ratio of AC-to-DC electric fields are favorable to DEP concentration performance. The proposed technique was also shown to successfully concentrate yeast cells. The proof-of-concept experiments have proven the capability of this

new technique to be potentially useful in cell concentration in which samples are sensitive to high field strength.

Chapter 5 Manipulation and Continuous Separation of Particles and Cells by AC-Dielectrophoresis Using 3D Conducting PDMS Composite Electrodes

5.1 Introduction

As reviewed in the literature, the usual method of creating AC-DEP requiring a non-uniform electric field is through the deposition of thin-film planar electrode arrays on the channel substrate of microfluidic devices [16, 74]. However, the electric field strength generated in such kind of devices decays exponentially from the surface of the planar electrodes [168]. As a result, cells located relatively far from the electrodes will not experience the DEP force and fail to drift. To address this problem, numerous DEP devices have been developed to generate 3D electric fields or to integrate 3D electrodes in microfluidic channels using various techniques such as double planar electrode deposition on both bottom and top substrates [82, 94, 169], heavy doping of silicon [96, 170], pyrolysis of SU-8 [171], and electroplating of gold [172, 173]. However, these microfluidic devices are usually made from glass or silicon, and are subject to significant complications in their assemblage to prevent liquid leakage.

With rapid development of soft lithography techniques, PDMS is now widely used as the fabrication material of choice in microfluidic devices, owing to

several beneficial features as highlighted in Chapter 1 [174]. However, it remains difficult to implement AC-DEP in PDMS-fabricated microfluidic devices, because adhesion between metallic electrodes and PDMS is poor [175]. Manually inserting copper electrodes into a PDMS channel partially overcomes the problem, but this method is unsuitable for fabrication and also easily causes leakage problem due to usual mismatch of the thickness of copper sheet and the channel height [107, 108].

This chapter presents new development of a PDMS-fabricated microfluidic device that can utilize AC-DEP via 3D conducting PDMS composites as sidewall electrodes for manipulation and continuous separation of particles and cells. The proposed fabrication technique greatly facilitates (i) the integration of the electrodes (the conducting PDMS composites) with PDMS microstructures, and (ii) the device assembly through bonding between the PDMS channels with embedded conducting PDMS electrodes and a glass substrate, involving only oxygen plasma treatment. Furthermore, unlike planar electrodes, the sidewall conducting PDMS electrodes can generate a 3D electric field that uniformly distributes throughout the channel's height and varies along the channel's lateral direction, thereby producing stronger lateral DEP effects over the entire channel. Therefore, this feature allows not only easy observation of particles and cells in a lateral direction but also using the lateral DEP force for efficient separation of particles and cells. To demonstrate the applicability of the device, three key features are demonstrated: (i) continuous

sorting and separation of particles by size[†], (ii) characterization of the frequency-dependent DEP behaviors of submicron and micron particles[‡], yeast cells and bacterial cells, and (iii) continuous separation of live yeast and bacterial cells from interfering latex particles of similar sizes, as well as live yeast cells from dead yeast cells[£]. The studies show that high separation efficiency of 97 % can be achieved in all cases. In addition, the numerical simulation based on the Lagrangian approach is also performed to simulate the trajectory of particles under the effects of hydrodynamic and DEP force.

5.2 Experiment

5.2.1 Device fabrication

Figure 5.1 shows the soft lithography process together with a method to synthesize and integrate conducting PDMS composites into PDMS microchannels. After a 40 μm thick SU-8 layer was patterned on a silicon wafer, AZ9260 photoresist was double-coated to obtain a 40-45 μm thick layer (Figure 5.1 (a)). The wafer was heated on a hot plate at 100°C for 60 seconds (first coating) and 9 minutes (second coating). The wafer temperature was reduced gradually to prevent cracking of the AZ9260 photoresist. After UV exposure for 215 seconds (Figure 5.1 (b)), the

[†] The works related to sorting and separation of particles by size has been published in *Electrophoresis*, 2010. 31: p. 2622-2631.

[‡] The works related to characterization and separation of submicron and micron particles have been submitted to *Biomicrofluidics*.

[£] The works related to characterization and separation of latex particles and cells have been accepted to *Analytical Chemistry*.

photoresist was developed for 7.5 minutes (Figure 5.1 (c)). For such a thick AZ9260 layer, care must be taken to ensure that sufficiently long UV exposure time to crosslink the photoresist. It is noted that the patterns for conducting PDMS electrodes could have a resolution of 20 μm (better resolution can be achieved with a glass mask instead of a plastic mask, but was not necessary for the present purposes). In addition, caution should be taken for the exposure time of the UV and developing because an over-exposure could cause the patterns of AZ9260 to be slightly larger than those printed in the plastic mask by 10 μm on each side.

Figure 5.1: (a)-(g) Fabrication processes [176]: (a) spin coating AZ9260 over SU-8 patterns, (b) UV exposure, (c) developing process, (d) filling AgPDMS, (e) removal of AZ9260, (f) pouring and curing pure PDMS, and (g) device bonding by oxygen plasma treatment. (h) The final device with tubing and electrical connections for

sorting and separation by size [176]. (i) The final device consisting of large reservoirs for characterization and separation of particles and cells of similar sizes.

Conducting paste with an electrical conductivity of $\sim 2 \times 10^4$ S/m was made by mixing 1 μm silver (Ag) particles with PDMS gel matrix at a ratio of 85% w/w (above this, the paste could become too powdery). It was recently reported that adding Ag powder into PDMS can increase surface roughness of AgPDMS, as compared to PDMS, thus resulting in more hydrophobicity and higher contact angles. Depending on the ratio (0, 17, 19 and 21 vol%), the contact angles of water on AgPDMS are 109°, 119°, 127° and 131° [177]. After filling AZ9260 cavities with AgPDMS and removing excessive AgPDMS by using a blade, the device was cured at 70°C for 2 hours (Figure 5.1 (d)). AZ9260 was then dissolved from the wafer with acetone for a few minutes, which was subsequently cleaned by ethanol and DI water (Figure 5.1 (e)). It is recommended that the wafer should not be left in acetone for too long to avoid possible swelling of SU-8 masters and AgPDMS composites. Pure PDMS gel was poured on the mold and cured at 80°C for 1 hour (Figure 5.1 (f)). After the cured PDMS with embedded AgPDMS composites was peeled, the channel was punched to create inlets and outlets, and then bonded with a glass substrate using a traditional oxygen plasma treatment (80 mTorr, 85 watts and 40 seconds) as shown in Figure 5.1 (g). Finally, the bonded device was baked at 150°C for 1 hour to strengthen the bond between the AgPDMS composites and the PDMS channel.

There are two kinds of device configurations based on their function and operation. The final device of the first kind with tubing and electrical connections, shown in Figure 5.1 (h), is designed to separate particles by size and hydrodynamically transports the particles by using syringe pumps. Syringe pumps were used to inject samples and a buffer solution in the device and stainless steel needles were then used as connectors situated at inlets and outlets of the device. Since the diameter of the punched reservoirs is 0.5 mm, it is smaller than the diameter of the connectors which is 0.75 mm. Liquid in the device can be sealed effectively at a high flow rate of 10 $\mu\text{l}/\text{min}$, which is about 10 times higher than the operating flow rate in the experiments. In addition, Figure 5.1 (i) presents the final device of the second kind which is designed for DEP characterization and separation of particles and cells of similar sizes and hydrodynamically transports the samples by using gravity induced suction. The device consists of four 6 mm reservoirs to contain a sample suspension and a buffer solution. In addition, to fabricate new devices, the used wafer with SU-8 masters may be re-used; however, it is recommended to clean the wafer one time by curing and removing new PDMS to avoid any unwanted contamination.

5.2.2 Device design and separation mechanism

5.2.2.1 Design for particle separation by size

Figure 5.2 (a) presents the structure of a PDMS-based microfluidic device with sidewall AgPDMS electrodes. The device consists of a 200 μm wide and 1400 μm

long main channel with four branch channels that end up in two inlets (A and B) and two outlets (C and D), which are 1 mm in diameter. The widths of the branch channels A, B, C and D are 50 μm , 170 μm , 100 μm and 115 μm , respectively. All channels are 40 μm deep. Along one side wall of the main channel, there are four 100 μm wide AgPDMS electrodes, spaced 100 μm apart. Inlet A is for injection of particles while inlet B allows a buffer solution to focus the particles injected from inlet A. Outlet C and D are for collection of particles either sorted by applied AC voltages or separated by particle size. In Figure 5.2 (b), the mechanism of particle separation by size is illustrated. With a higher flow rate generated from branch B (compared to branch A), the device can hydrodynamically confine the particles, that are randomly transported from inlet A, into the side channel wall regions where AgPDMS electrodes produce high non-uniform electric field strength. As a result, the particles are pushed away from the AgPDMS electrodes by repulsive forces (nDEP). Using sufficiently high AC voltages, the device can produce strong DEP forces exerting on the particles to drive them into different streamlines and thus in different downstream branches. The larger particles experiencing stronger DEP forces are sorted to the upper branch D and then can be collected from outlet D. The smaller particles experiencing weaker DEP forces transport to the lower branch C and then can be collected from outlet C. The total flow rate in the main channel controls the velocity of particles and the time window for sorting and separation, while the ratio of the flow rates from branches B and A, respectively, determines the degree of hydrodynamic focusing.

Figure 5.2: (a) Schematic of a PDMS-based microfluidic device with sidewall AgPDMS electrodes. (b) Illustration of the continuous separation mechanisms for a mixture of particles of two different sizes [176].

5.2.2.2 Design for cell separation of similar sizes

A schematic of the device for continuous separation of cells from interfering particles of similar sizes is depicted in Figure 5.3 (a). In this present device, dimensions of microfluidic channels are the same as described in the previous section. However, all four reservoirs at the inlets and outlets are instead enlarged to 6 mm in diameter and each 4 mm in height, providing sufficient volumetric capacity to produce steady suction for more than 10 minutes. In addition, the present design is also employed for DEP characterization of particles and cells.

as empty dots) experience an attractive or pDEP force and hence move towards the lower branch C. To facilitate the separation of these samples experiencing different DEP forces, the outlet C should be narrow so that the device does not need high AC voltages to repel the nDEP particles towards the upper branch D. As for the pDEP cells, the width of the lower branch C is not critical as they always move near the sidewall of the channel. Furthermore, it should be noted that to generate sufficient lateral migration of particles (in the y direction) to the upper branch D, sufficiently high AC voltages are required to induce strong nDEP exerting on particles to overcome the hydrodynamic drag force within a time window set by the total flow rate in the main channel.

5.2.3 Sample preparation and experimental setup

Latex polystyrene microspheres (Duke Scientific, USA) of 5 μm , 10 μm , and 15 μm in diameter were used to perform sorting and separation by size because these sizes resemble biological cells such as yeast and red/white blood cells. 2 ml of the particle solution with a number concentration of 10^6 - 10^7 /ml was centrifuged and re-suspended with 3 ml of a 10^{-4} M NaHCO_3 solution for three times. Through a conductivity/pH meter (Fisher Scientific AR-20), the conductivity and pH of the NaHCO_3 buffer solution were measured to be 13.1 $\mu\text{S/cm}$ and 7.68, respectively. Two syringe pumps (KD Scientific) with 0.5 ml Hamilton glass syringes were used to generate two streams of flow through inlets A and B. After the channel was filled with the buffer solution thoroughly, air bubbles were carefully removed from the channel via the connectors. The prepared particle solution was continuously loaded

in inlet A while the buffer solution was continuously injected in inlet B. Two outlets were exposed to atmospheric pressure. The motion of particles was recorded at 15 frames per second (fps) using a CCD camera (Samba, EZ-140 c) mounted on an epifluorescence microscope (Zeiss, Axiostar Plus FL) under a 5× objective. A function generator (Agilent, 33250A) was connected with an amplifier (Piezo System, EPA-102) by a BNC cable to produce AC voltages which are imposed on the AgPDMS electrodes via four alligator clips as shown in Figure 5.1 (h). The AC signal was monitored by an oscilloscope (HAMEG Instruments, HM 1008).

To demonstrate DEP characterization and continuous separation of particles and cells of similar sizes, cells of *Saccharomyces cerevisiae* (baker's yeast cells) and *E. coli* DSMZ 1329 (bacterial cells), and two types of particles (Duke Scientific, USA): latex particles (2.9 μm in diameter similar to the size of *E. coli*) and fluorescent latex micro and submicron particles (5 μm in diameter similar to the size of yeast cells and 0.5 μm) were used in this study.

To prepare solutions of fluorescent 0.5 μm and 5 μm particles for DEP characterization, 0.02 ml of 0.5 μm particle solution (of the original concentration in DI water) and 0.1 ml of 5 μm particle solution (of the original concentration in DI water) were added in two different micro-centrifuge tubes containing 1 ml of a NaHCO_3 solution. The solution's conductivity ranges from 11.7 $\mu\text{S}/\text{cm}$ to 500 $\mu\text{S}/\text{cm}$. Each micro-centrifuge tube was then centrifuged at 13300 rpm for 5 minutes and re-suspended with 1 ml of the NaHCO_3 solution for three times. As for

separation, the mixture of both sizes was prepared by mixing these two particle solutions in a micro-centrifuge tube.

To prepare yeast cell samples, 50 mg of baker's yeast cells was suspended in 4 ml of DI water for 1 hour for re-activation. After washing with DI water three times, the samples were equally divided into two micro-centrifuge tubes. In the first tube, a live yeast suspension was centrifuged at 13300 rpm for 5 minutes and transferred into 2 ml of a 380 μ S/cm NaCl solution. To prepare dead yeast cells, the yeast sample suspension in the second tube was heated at 80°C in a water bath for 30 minutes. After washing three times with DI water, the heated cells were stained with 6 μ l of Propidium Iodide (PI) stain (Molecular Probes Inc, USA) for 30 minutes in the dark. Then, the dead yeast cells were washed and transferred into 2 ml of a 380 μ S/cm NaCl solution. Finally, the live and dead yeast cells were mixed prior to the experiment.

E. coli bacterial cells were grown in a flask containing 100 ml Luria-Bertani (LB) broth (Difco™, Becton Dickinson, USA) in a shaker incubator at 200 rpm and 37°C. Two 1 ml aliquots were transferred to a micro-centrifuge tube and centrifuged at 3400g for 5 minutes. The supernatant was then discarded to remove the medium. The sample was washed three times and transferred into 1 ml of a 380 μ S/cm NaCl solution. To differentiate them from other particles, the bacterial cells were stained with 1 μ l of fluorescent nucleic acid stain SYTO9 (Molecular Probes Inc, USA), for 30 minutes at 30°C in the dark, then washed three times with the 380 μ S/cm NaCl solution.

For DEP characterization of cell, live yeast cells and live bacterial cells were suspended in both DI water and a 380 $\mu\text{S}/\text{cm}$ NaCl solution to investigate the effect of buffer conductivity on frequency-dependent DEP behaviors. As for separation of live cells from interfering latex particles of similar sizes, three suspensions were prepared: (1) live yeast cells and 5 μm fluorescent latex particles in a 380 $\mu\text{S}/\text{cm}$ NaCl solution; (2) live bacterial cells stained with SYTO9 and 2.9 μm latex particles in a 380 $\mu\text{S}/\text{cm}$ NaCl solution; and (3) live yeast cells and dead yeast cells stained with PI in a 600 $\mu\text{S}/\text{cm}$ NaCl solution. It is noted that the particle solution of 2.9 μm originally in DI water was diluted with a 380 $\mu\text{S}/\text{cm}$ NaCl solution at the volumetric ratio of 1:54. Prior to each separation experiment, a 5% bovine serum albumin (BSA) solution was used to coat the microchannel for ~ 4 hours to prevent adhesion of particles and cells, then thoroughly washed with a NaCl solution to remove BSA.

5.3 Determination of separation efficiency

For experiments in which two types of cells or particles were separated, the separation efficiency was computed as

$$\eta = \left(1 - \frac{R_{Loss}}{R_A} \right) \times 100\%,$$

where R_A is the number of samples (counted per minute) flowing from inlet A (or known as the system throughput), and R_{Loss} is the number of samples (counted per minute) flowing into the wrong outlet. The throughput is obtained by multiplying

the sample velocity and the number of the samples within the traveled distance of a given time. The separation efficiency is averaged over the efficiencies at 1, 2, 3 minutes after application of AC field.

5.4 Modeling of particle motion

As illustrated in Figure 5.4, the motion of a particle suspended in a buffer solution is controlled by the repulsive nDEP force along the direction of electric field gradient away from the AgPDMS electrodes, the hydrodynamic force due to hydrodynamic flow (along the direction flow), and the frictional drag force that balances the difference between the DEP force and the hydrodynamic force, namely in the opposite direction of the sum of these two forces. Furthermore, the sum of the hydrodynamic force and the frictional drag force is known as the Stokes hydrodynamic drag force governed by Stokes' law. These forces are shown in Figure 5.4 below.

Figure 5.4: Physical forces acting on a particle moving near a pair of AgPDMS electrodes fabricated on the side wall of the device main channel [176].

The analysis of particle motion is simplified into a two-dimensional problem with the following assumptions: (i) the particle travels at the same height in the z direction, (ii) particle sedimentation is negligible as the density of particles (1.05 g/cm³) is close to that of the suspending buffer solution (1 g/cm³), (iii) Joule heating is insignificant due to the low conductivity of buffer solution (13.1 μS/cm) [148], and (iv) the interactions between particles are not considered as the particle number concentration is low [149]. In creeping flows where $Re \ll 1$, a spherical particle moving in a microchannel experiences a hydrodynamic drag force which is given by Stokes' law [32],

$$\vec{F}_{\text{hydrodynamic drag}} = \vec{F}_{\text{fluid flow}} + \vec{F}_{\text{frictional drag}} \quad (5.1)$$

$$\vec{F}_{\text{hydrodynamic drag}} = 6\pi\eta a(\vec{u}_{\text{flow}} - \vec{u}_p) \quad (5.2)$$

When a latex particle is subjected to a non-uniform electric field, it experiences a time-averaged AC-DEP force as shown in equation (2.11). In the steady state, a force balance between the hydrodynamic drag force and the AC-DEP force give a terminal velocity of the particle, expressed as

$$\vec{u}_p = \vec{u}_{\text{flow}} + \frac{c\epsilon_m a^2 \text{Re}\left[f_{CM}(\omega)\right] \nabla \vec{E}_{rms}^2}{3\eta} \quad (5.3)$$

To obtain the particle terminal velocity, the velocity field of pressure-driven flow and the electric field are needed. In a microfluidic channel, the steady state velocity field is governed by Navier-Stokes equation and the continuity equation expressed as

$$\rho(\vec{u} \cdot \nabla \vec{u}) = -\nabla p + \eta \nabla^2 \vec{u} \quad (5.4a)$$

$$\nabla \cdot \vec{u} = 0 \quad (5.4b)$$

where p is the local pressure in the channel. The electric field $\vec{E} = \nabla \phi$ produced by the AgPDMS electrodes on the side channel wall is governed by Laplace's equation as

$$\nabla^2 \phi = 0 \quad (5.5)$$

where ϕ is the electric potential in the channel. It is noted that the actual device has four branches which are about 4 mm in length and 40 μm in height. To reduce the computational cost, the top portion (in the x-y plane) of the actual channel with 500 μm long branches is considered as the domain in the simulation, illustrated in Figure 5.5. The middle plane of the actual channel is then considered as a symmetry plane as highlighted. The boundary conditions for equations (5.4) and (5.5) are summarized in Table 5.1. The boundary conditions for Navier-Stokes equation consist of the non-slip velocities on channel walls, the symmetry condition on the bottom plane, the average velocities at inlet A and B, and the atmospheric pressure at outlet C and D.

Figure 5.5: 3D structure of the microfluidic channel used in the simulation

As for Laplace's equation, the boundary conditions are set based on the facts that the electric field components perpendicular to insulating PDMS and glass walls must vanish whereas electrodes are alternatively assigned with a potential (V_{AC}) and electrically grounded. On the bottom plane, the symmetry condition is applied.

Table 5.1: Boundary conditions for Laplace's equation and Navier-Stokes equation

Governing equations	Boundary conditions			
	Channel walls	Symmetry boundary	Inlets and outlets	Electrodes
Navier-Stokes	$\vec{u}_{wall} = 0$	$\vec{u}_{wall} \cdot \vec{n} = 0$	$\vec{u}_{avg,A}, \vec{u}_{avg,B}$ and $P_C = P_D = 0$	$\vec{u}_{wall} = 0$
Laplace	$\vec{E} \cdot \vec{n} = 0$	$\vec{E} \cdot \vec{n} = 0$	$\vec{E} \cdot \vec{n} = 0$	$\phi = V_{AC}$ or $\phi = 0$

5.5 Simulation of velocity field and electric field

The numerical simulation based on the finite-element method was performed by using COMSOL MultiphysicsTM for flow velocity field and non-uniform electric field. Since all reservoirs (1 mm in diameter) are many times larger than microfluidic channels, the incoming fluid flows produced by syringe pumps can be assumed to be uniform as entering the channels. To simulate the actual situations of sorting and separation where the flow rates at inlet A and B were 0.05 $\mu\text{l}/\text{min}$ and 0.63 $\mu\text{l}/\text{min}$, respectively, the corresponding entrance velocities of inlet A and B were calculated to be 400 $\mu\text{m}/\text{s}$ and 1544 $\mu\text{m}/\text{s}$, respectively, based on the channel dimensions that the width of inlet A and B are 50 μm and 170 μm respectively with a constant 40 μm channel depth.

The 3D structure of the channel was meshed with the optimum grid sizes as indicated in Figure 5.6. The grid sizes of 2.5 μm and 0.5 μm were set for the edges of inlets and outlets and upper corners of the channel, respectively. The global grid size was varied from 40 μm , 30 μm , 20 μm , 10 μm to 7.5 μm to perform grid-size independency check. The 10 μm grid size was found to be optimal as the simulation results indicate convergence with a maximum difference of $\sim 0.2\%$ as compared to the 7.5 μm grid size.

Figure 5.6: Meshing of 3D structure of the microfluidic channel with the optimal grid sizes.

Figure 5.7 (a) presents the velocity distributions of steady-state flow in the AgPDMS electrode area on the x-y plane as indicated in Figure 5.5. The magnitude of the velocity varies across the width of the main channel. The simulation indicates the minima at channel walls due to the no-slip boundary condition and the flat velocity profile at the middle because of large difference between the channel's width and height. Similarly, as shown in Figure 5.7 (b), the simulated velocity profile in the x-z plane exhibits the minimum at the top wall (or $z=20\ \mu\text{m}$) and the maximum at the symmetry plane (or $z=0\ \mu\text{m}$). Moreover, Figure 5.7 (c) shows the simulated velocity field in the x-y plane with the hydrodynamic focusing achieved at the 12-time difference in the flow rates between inlet A and B. The effect of hydrodynamic focusing is illustrated by showing a decrease in arrows' height along the flow direction. As will be shown in Figure 5.8 (b) and (c), the gradients of the electric field exhibit quite high within such region indicated by the arrows.

Figure 5.7: Numerical simulation results: the pressure-driven velocity profile in (a) the x-y plane, (b) the x-z plane and (c) the velocity field with the hydrodynamic focusing [176].

To simulate electric field, an electric potential of 10 V was applied to the first and third electrodes (counted from left) while the second and fourth electrodes were electrically grounded. Figure 5.8 (a) and (b) show the electric field lines (lines) and the strength of DEP forces (arrows), both negative (panel a) and positive (panel b). The accumulation of electric field lines results in a marked increase in the strength of DEP forces near the edges of the AgPDMS electrodes. The electric field strength however decays near the center of the electrode and within the gap between two electrodes. The direction of DEP forces depends on the sign of the CM factor. When the CM factor is negative (nDEP; Figure 5.8 (a)), DEP forces point from the electrodes into the fluid, suggesting that cells experience repulsive forces that push them away from the electrodes. The opposite happens when the CM factor is

positive (pDEP; Figure 5.8 (b)). The gradients of the square of the electric field at various distances (e.g., 10 μm , 20 μm and 30 μm) away from the AgPDMS electrodes are plotted in Figure 5.8 (c). The gradients at the electrode edges are expectedly profound in magnitude but dramatically decay at the centre of the electrodes and the gap region between two electrodes. Therefore, the particle moving near the electrodes' edges will experience considerably strong DEP forces, leading to high separation efficiency.

Figure 5.8: Numerical simulation results for (a) negative DEP and (b) positive DEP forces generated by AgPDMS electrodes (thick black segments). Lines show the

contours of the electric field. DEP forces at the boundary are shown by arrows, of length proportional to force strength. (b) the gradients of the square of the electric field at various distances away from the AgPDMS electrodes and (c) the magnitude of hydrodynamic force and DEP force acting on a 15 μm particle at different vertical distances in the y-axis direction [176].

Furthermore, as shown in Figure 5.7 (a) and Figure 5.8 (c), the particle located at different vertical distances in the separating channel may experience different magnitudes of the hydrodynamic force and the DEP force. Figure 5.8 (d) presents the variation of these forces acting on a particle of 15 μm at different vertical distances (in the y direction) from the second edge of the first electrode. The simulation results show that at the 10 μm distance away from the electrodes, the DEP force is much stronger than the hydrodynamic force because of the low magnitudes of flow velocity and the markedly high electric field gradient. However, the DEP force becomes comparable to the hydrodynamic force at around 20 μm . Beyond 20 μm , the hydrodynamic force dominates the DEP force.

5.6 Experimental results

5.6.1 Continuous sorting of particles

In sorting experiments, the flow rates at inlet A and B were respectively set to 0.05 and 0.63 $\mu\text{l/min}$. This yields about 12-time difference in the flow rates, which caused the particles in randomly dispersed positions coming from branch A to be confined near the side channel wall where AgPDMS electrodes are embedded. The

total flow rate in the main channel was 0.68 $\mu\text{l}/\text{min}$, resulting in $Re \ll 1$. To obtain the trajectories of moving particles during continuous sorting, consecutive images captured by a CCD camera at 15 fps were superimposed as shown in Figure 5.9. Particles suspended in this creeping flow ($Re \ll 1$) could maintain their paths if no electric field was applied (Figure 5.9 (a) and (d)). Once an electric field is applied, 3D AgPDMS electrodes produce 3D-DEP forces that are uniformly distributed along the depth direction but decay along the width direction of the main channel. Whether such DEP forces can push the confined particles to deviate from their original streamlines depends on the magnitude of DEP forces, which in turn is determined by applied AC voltages. As shown in Figure 5.9 (b) and (e), under an AC voltage of 25 V at 1 MHz, the particles of 10 μm and 15 μm exhibited repulsive forces (nDEP) repelling the particles away from the electrodes, but the DEP strength was not sufficient to repel the particles into the upper branch. In comparison, the particles of 15 μm before leaving the main channel exit reached their lateral distances farther than the particles of 10 μm owing to the cubic power of size-dependent DEP force as expressed in equation (2.11). To achieve the particle sorting into the upper branch, higher voltages of 50 V and 55 V were applied for the particles of 10 μm and 15 μm , respectively, as shown in Figure 5.9 (c) and (f). As clearly seen in Figure 5.9 (a-f), the experimental results show the effect of AC voltage and particle size on the trajectories of particles.

Figure 5.9: Effect of AC voltage on the sorting of 10 μm particles at (a) 0 V, (b) 25 V and (c) 55 V, and 15 μm particles at (d) 0 V, (e) 25 V and (f) 50 V [176].

Furthermore, the trajectories of particles under the applied voltages in Figure 5.9 (b, c, e and f) also manifests that the particles could experience continuing strong DEP forces induced by the four sidewall electrodes as they were confined near the electrodes due to the effective two-flow hydrodynamic focusing. In comparison, if only two electrodes were integrated, the particles would not be able to experience continuing DEP forces from another pair of electrodes like in the case of four electrodes. Thus, the device would require a higher voltage to sort these particles. This information suggests that an integration of more electrodes (e.g., six or ten) in the device could potentially reduce the voltages especially for high throughput sorting or separation. Clearly, these experimental evidences have demonstrated that this device is able to effectively sort microparticles by size in a continuous manner no matter where the particles come from. Thus, with the

appropriate flow rates to confine the particles, the sorting is solely dependent on applied AC voltages.

To predict how the particles transport under the effect of the hydrodynamic and DEP force, the terminal velocity of the particle in equation (5.3) was computed. As the particle is assumed to travel at the same height and there is no variation of electric fields induced by the 3D electrodes along the height of the channel, the flow velocity ($\bar{u}_{flow}$) and the electric field at the middle height of the channel, computed using COMSOL MultiphysicsTM, were selected and used in equation (5.3). In addition, under the DEP assumption given in Chapter 2 that the original DEP force expression can be assumed to be valid if the scale of electric field non-uniformity is large, compared to the dimension of the particle. In the present work, the dimension of AgPDMS electrodes is of 100 μm , which are much larger than the particle sizes of about 5-15 μm . Therefore, the effect of the particle size in the electric field is considered negligible, and the correction factor should approach unity, $c=1$. This situation is different from the simulations for the H-filter (in Chapter 3) and the channel with contraction (in Chapter 4) where the corners of insulating blocks and contraction area were used to induce non-uniform electric fields, and the sizes of such corners were clearly comparable to the particle size. Similar studies presented by Cetin *et al* and Kang *et al* employed electrodes whose sizes were comparable to the particles [107, 108]. For example, Cetin *et al* employed a triangular electrode with a sharp tip to separate particles of 5 μm and 10 μm . Kang *et al* utilized an insulating hurdle to induce DEP forces at the hurdle's corners. Thus, such correction factor is important in these studies.

Comparisons between the experimental and the simulated particle trajectories under various operating AC voltages are shown in Figure 5.10 (a) and (b) for 10 μm and 15 μm particles, respectively. Generally, a good agreement for the particle trajectories is found between the experimental data and the numerical predictions. The simulations are in accordance with experiments that the particles started to deviate from the back edge of the first electrode (from the left) rather than the front edge of the first electrode; this is due to the presence of the minuscule gradient of the square of electric field at the first edge as shown in Figure 5.8 (c). In addition, both the experiments and the simulations also show that the changes of the particle trajectory are significant near the following electrodes. This scenario is also consistent with Figure 5.8 (c) showing the magnitude of the gradient of the square of the electric field.

Figure 5.10: Comparison of experimental and simulated particle trajectories under various applied voltages for (a) a 10 μm particle and (b) a 15 μm particle [176].

Also, the comparisons in Figure 5.10 (a) and (b) show that the experiments require higher voltages than the simulations in the order of about 1.8-2.5 times for

particle sorting. For example, the experiments require 25 V for sorting both the 10 μm and 15 μm particles. However, only 10 V is needed in the simulations. The discrepancy in voltages is due to the fact that the numerical model presented in this study does not consider electric conduction in AgPDMS composites. Due to low electrical conductivity of the AgPDMS composites, electric conduction can cause voltage drop across the AgPDMS composite electrodes. The measured conductivity of an AgPDMS composite sample (having dimensions of 5.2x3.5 mm² in area and 40 μm in depth) is 2×10^4 S/m which is two orders lower than pure silver powders ($\sim 10^6$ S/m) [175]. In addition, the presence of electrical capacitance of AgPDMS composites in AC circuit also plays a role in increasing the impedance of the composites, thus causing more voltage drop across the composites. However our experiments showed that the AgPDMS composite electrodes possessing a conductivity of 2×10^4 S/m were able to generate sufficient 3D-DEP forces for continuous sorting in the present device although certain voltage drop had to be compensated. Nevertheless, despite the difference in the required voltages, the present model can give good estimation for the trend showing the effects of hydrodynamic force and DEP force on both 10 and 15 μm particle trajectories in the microfluidic device.

5.6.2 Continuous separation of particles by size

To achieve continuous separation of particles by size, it is important to identify the threshold voltage required for repelling the larger particles to the upper branch D but still keeping the smaller particles to the lower branch C. Using the experimental

findings from the previous section, the device was able to successfully separate 5 μm particles from a mixture of 5 μm and 10 μm particles at 55 V with the successful rate at 87.7% for the 5 μm particles at outlet C and 100 % for the 10 μm particles at outlet D as shown in Figure 5.11 (a). These successful rates imply that 12.3% of the original 5 μm particle flowing from inlet A was mixed with 10 μm particles at outlet D but there was no 10 μm particles found at outlet C. In addition, the device was operated to separate 10 μm particles from a mixture of 10 μm and 15 μm particles at 50 V with the successful rate at 100% for both particle sizes (Figure 5.11 (b)).

As seen from the results, the successful rate in the case of the smaller sizes of particles (5 μm and 10 μm particles) was lower than that of the larger sizes (10 μm and 15 μm particles). Experimental observations revealed that with the 12-time difference in the flow rates, some 5 μm particles were insufficiently focused near the electrodes. When experienced nDEP forces, they could be repelled towards outlet D, reducing the separation efficiency. In the case of larger particles of 10 and 15 μm , all particles were found sufficiently focused near the electrodes and efficiently separated. To realize a better successful rate in the case of 5 μm and 10 μm particles, or smaller sizes, an increase of flow rate ratios becomes necessary. Moreover, as the present device consists of only four electrodes, more electrodes (e.g., six or ten electrodes) would definitely enhance the separation and thus improve the resolution in terms of particle size. Figure 5.11 (c) and (d) show a comparison between the numerical simulations and the experimental results for separation of particles by size, corresponding to the images shown in Figure 5.11

(a) and (b), respectively. The separated particles of different sizes can be collected in the reservoirs in a continuous way for further analysis.

Figure 5.11: Continuous separation of particles (a) separation of 5 μm particles from a mixture of 5 μm and 10 μm particles, (b) separation of 10 μm particles from a mixture of 10 μm and 15 μm particles, (c) comparison of experimental and simulated particle trajectories for (a), and (d) comparison of experimental and simulated particle trajectories for (b) [176].

5.6.3 Effect of flow rate

Sample throughput is one of the important factors required for a microfluidic device to operate its particle sorting or separation functions. By increasing a flow rate,

sample throughput can be enhanced and thus the process of sorting and separation can be achieved faster. To account for the increased transport velocity of samples, the required DEP forces however should be proportionally increased as well. In the experiment, the flow rates of at inlet A and B were increased to 0.1 and 0.9 $\mu\text{l}/\text{min}$, respectively. Thus, the flow rate in the main channel was increased to 1 $\mu\text{l}/\text{min}$ or approximately 1.5 times higher than the previous cases. As shown in Figure 5.12 (a), the device required a higher voltage of 55 V to produce sufficiently strong DEP forces to push the 15 μm particles into the upper branch instead of the required voltage of 50 V in the previous cases. Similarly, the numerical simulation shown in Figure 5.12 (b) indicates that corresponding to a higher flow rate, a higher voltage of 30 V is needed for the sorting of 15 μm particles (rather than previous 20 V). Moreover, a comparison of the experiment and the simulation shows that the trajectory of 15 μm particles is well predicted by the current model with a correction factor in the voltage drop across AgPDMS composites. In order to cope with the demand of high flow rates, as discussed in section 5.6.1, more electrodes should be introduced to produce additional continuing DEP forces. Together, the gaps between electrodes can be reduced to increase DEP effects for high throughput applications.

Figure 5.12: The trajectory of a 15 μm particle at a flow rate of 1 μl/min [176]

5.6.4 DEP characterization

As AgPDMS electrodes embedded along the sidewall of the microfluidic device can produce 3D electric fields, the resulting DEP forces acting on particles and cells are generated along the width and throughout the height of the channel, thus allowing easy observation of cell motion. To demonstrate this function, the device is used to characterize the frequency-dependent DEP behaviors of submicron particles, micron particles, yeast cells and bacterial cells in various medium solutions, by varying AC frequencies ranging from 10 kHz to 80 MHz.

5.6.4.1 Characterization of submicron and micron particles

Equation (2.13) suggests that the CM factor for a particle can be changed from positive to negative by adjusting the conductivity of suspending medium. Figure 5.13 (a) shows the effect of medium conductivity on the CM factor for a submicron latex particle of 0.5 μm with a surface conductance of 2.1 nS [37] in the 11.7 μS/cm

and 500 $\mu\text{S/cm}$ NaHCO_3 solutions. In the absence of electric field, experimental results confirms the prediction of the CM factor that at low medium conductivity of 11.7 $\mu\text{S/cm}$, the particles were observed to be attracted by pDEP under 20 V at 1 MHz (Figure 5.13 (b)) and repelled by nDEP at 80 MHz (Figure 5.13 (c)), with a cross-over frequency at 3.5 MHz. However, by selecting a medium with a higher conductivity (e.g., 500 $\mu\text{S/cm}$) than the particle conductivity, experiments revealed that the pDEP behavior could be suppressed and only nDEP was observed in the entire spectrum of frequencies.

Figure 5.13: (a) Effect of medium conductivity on the CM factor for 0.5 μm submicron latex particles. At 20 V, the particles suspended in a 11.7 $\mu\text{S}/\text{cm}$ NaHCO_3 solution exhibit (b) pDEP at 1 MHz and (c) nDEP at 80 MHz.

In addition, not only is the medium conductivity but also particle size critical in altering the frequency-dependent DEP behaviors as described by the surface conductivity term ($2Ks/a$) in equation (2.13). As shown in Figure 5.14 (a), the CM factor predicts that, the latex particles of 0.5 μm and 5 μm , having surface conductances of 2.1 nS [37] and 1.2 nS [38] respectively in a 80 $\mu\text{S}/\text{cm}$ NaHCO_3 solution exhibit distinct DEP behaviors for most frequencies less than 50 MHz. In line with this prediction, under an AC voltage of 20 V, the latex particles of 0.5 μm were experimentally observed to experience pDEP at low frequency region (1 MHz in Figure 5.14 (b)) and nDEP at high frequency region (60 MHz in Figure 5.14 (c)). However, with increasing particle size from 0.5 μm to 5 μm , the surface conductivity term reduces, resulting in the negative CM factor for all the frequency regions. The resulting nDEP force repels 5 μm particles from the electrodes (at 60 MHz) as shown in Figure 5.14 (d).

Figure 5.14: (a) Effect of particle size on the CM factor of $0.5 \mu\text{m}$ and $5 \mu\text{m}$ particles suspended in an $80 \mu\text{S/cm}$ NaHCO_3 solution. Under 20 V, $0.5 \mu\text{m}$ particles experience (b) pDEP at 1 MHz and (c) nDEP at 60 MHz, and (d) Under 20 V, $5 \mu\text{m}$ particles experience nDEP at 60 MHz.

5.6.4.2 Characterization of yeast cells

Figure 5.15 (a) and (d) show yeast cells suspended in a $380 \mu\text{S/cm}$ NaCl solution in the absence of an electric field and fluid flow. Imposing an AC voltage of 10 V to the first and third AgPDMS electrodes at a frequency of 10 kHz caused repulsive forces (nDEP) on the yeast cells and their movement away from the electrodes, as

shown in Figure 5.15 (b) after 90 seconds and Figure 5.15 (c) after 180 seconds. This indicates that cells are less polarized than the suspending medium (negative CM) at low frequency. In contrast, at a frequency of 10 MHz yeast cells experienced strong attractive forces (pDEP), moving towards and accumulating at the electrodes' edges (Figure 5.15 (e) and (f)). At 80 MHz, no DEP response was observed. This was expected, because at very high frequencies the difference in the complex permittivities of cells and medium (which determines the CM factor) becomes insignificant, thereby causing DEP to be vanished. Furthermore, the cross-over frequency from nDEP to pDEP was found to be ~300 kHz and the cross-over frequency from pDEP to no DEP was ~40 MHz. All these experimental observations are in good agreement with the prediction of the CM factor in Figure 2.8 (a).

Figure 5.15: DEP characterization of yeast cells in a 380 $\mu\text{S}/\text{cm}$ NaCl solution, in the absence of fluid flow. (a,d) Without an applied electric field. (b,c) 10 V at 10 kHz, showing nDEP after 90 and 180 seconds. (e,f) 10 V at 10 MHz, showing pDEP after 90 and 180 seconds. Images were obtained with a 20 $\times$ objective.

According to equation 2.16, suspending cells in a medium with sufficiently low conductivity can reverse the cell DEP behavior from nDEP to pDEP. Experiments with yeast cells suspended in DI water (2 $\mu\text{S}/\text{cm}$) confirmed this prediction: yeast cells in DI water experienced pDEP for all frequencies up to a cross-over frequency of 40 MHz, above which no motion of yeast cells was observed as DEP became too weak. This is in agreement with Figure 2.8 (a) (dashed

line) and in stark contrast to observations of yeast cells in the 380 $\mu\text{S}/\text{cm}$ NaCl solution reported above, which exhibited both nDEP and pDEP.

Interestingly, yeast cells can form chains when exposed to a non-uniform electric field (Figure 5.15 (b, c, e and f)). Chain formation occurs as a result of the dipole-dipole interactions, in which two induced dipoles attract each other when aligned with the applied field, but repel each other when aligned perpendicularly to the field [33]. This explains the striking formation of chains in the same direction as the field lines shown in Figure 5.8 (a).

5.6.4.3 Characterization of *E. coli*

E. coli exhibited similar DEP behaviors as yeast cells in the 380 $\mu\text{S}/\text{cm}$ NaCl solution. Weak pDEP was found at 400 kHz and 30 MHz, but relatively strong pDEP at 1 MHz. Figure 5.16 (a) shows bacterial cells in the NaCl solution in the absence of electric field. An application of 15.3 V at 1 MHz resulted in the accumulation of *E. coli* at the edges of electrodes, as shown by images acquired from 30 to 180 seconds after application of the field (Figure 5.16 (b-d)). Two cross-over frequencies were identified as 150 kHz (from no DEP to pDEP) and 35 MHz (from pDEP to no DEP). These DEP behaviors are in reasonably good agreement with the prediction from Figure 2.8 (b), although below 150 kHz and above 35 MHz no DEP was observed. Furthermore, *E. coli* suspended in DI water always exhibited pDEP at frequencies from 10 kHz to 30 MHz, with a cross-over frequency of 35 MHz. The chain formation of *E. coli* was also observed.

Figure 5.16: DEP characterization of *E. coli* in a 380 $\mu\text{S}/\text{cm}$ NaCl solution. (a) Before application of the electric field. (b, c, d) 15.3 V at 1 MHz frequency caused pDEP, as shown at 30, 90 and 180 seconds, respectively.

The two cross-over frequencies of *E. coli* and yeast cells suspended in the 380 $\mu\text{S}/\text{cm}$ NaCl solution were rather similar. However, at a low frequency of 10 kHz yeast cells undergo nDEP whereas *E. coli* exhibited pDEP, indicating that yeast cells are in general less conductive than *E. coli*. In general, as indicated by equations (2.17) and (2.18), different size ratios of the cell wall, membrane, cytoplasm and nucleus can alter the CM factor and thus the crossover frequencies. Moreover, as cytoplasm of both cell types have much higher electrical conductivities than the suspending medium, DEP characterization showed strong pDEP under medium-to-high frequencies (e.g., 500 kHz - 10 MHz).

5.6.5 Separation of submicron and micron particles

Unlike the separation of microparticles by size using nDEP, this section demonstrates a continuous separation of submicron and micron particles by pDEP and nDEP. As previously performed, the DEP characterization evidently confirmed the prominent effect of surface conductance when the particle size is scaled down from micrometer (5 μm) to sub-micrometer (0.5 μm). Under AC electric field frequencies ranging from 1 kHz to 60 MHz, micron particles exhibited nDEP while submicron particles experienced pDEP in an 80 $\mu\text{S/cm}$ NaHCO_3 solution. To realize particle separation of these two sizes which exhibit different DEP behaviors, the device designed for separation of similar sizes, shown in Figure 5.3, is employed to attract the pDEP submicron particles and repel the nDEP micron particles, thereby achieving separation.

Figure 5.17 (a) shows a mixture of 0.5 μm and 5 μm fluorescent latex particles which are confined near the electrodes. In the absence of electric field, the mixture flows to the lower outlet C. Under an AC voltage of 30 V at 1 MHz, the device repelled 5 μm particles by nDEP force towards the upper branch D and attracted 0.5 μm particles by pDEP near the electrodes and transported towards the lower branch C. Clear separation could be observed at 14 seconds (Figure 5.17 (b)) and even lasted for 3 minutes (Figure 5.17 (c)) after the application of AC field. The efficiency in separating 5 μm particles from the mixture of 0.5 μm and 5 μm particles is as high as 100%. For these experiments, the time window set by the

speed of the flow ($222 \mu\text{m/s}$) and the channel length ($1400 \mu\text{m}$) was ~ 6.3 seconds, thus giving a nDEP drift velocity of $\sim 8 \mu\text{m/s}$.

Figure 5.17: Experimental images for a mixture of $0.5 \mu\text{m}$ and $5 \mu\text{m}$ fluorescent latex particles near the device outlets (a) with no applied electric field. (b) Continuous separation of $5 \mu\text{m}$ from $0.5 \mu\text{m}$ particles under an AC voltage of 30 V at 1 MHz at 14 seconds and (c) at 3 minutes. Images were acquired with a $10\times$ objective.

5.6.6 Separation of yeast cells from $5 \mu\text{m}$ latex particles

Based on the prediction that yeast cells and latex particles have distinct DEP

responses at frequencies ranging from 300 kHz to 1 MHz (Figure 2.8 (a)), the microfluidic device was used to separate a mixture of yeast cells and 5 μm fluorescent latex particles suspended in a 380 $\mu\text{S}/\text{cm}$ NaCl solution. The particle size was chosen to be close to that of the cells to eliminate size-dependent effects. Separation was achieved in continuous-flow mode, where particles and cells are exposed to DEP for a limited time window, set by the speed of the flow (306 $\mu\text{m}/\text{s}$) and the channel length (1400 μm). For these experiments, that time window was about 4.6 seconds, thus giving a nDEP drift velocity of about 11 $\mu\text{m}/\text{s}$.

Figure 5.18 (a) shows yeast cells (black dots) and fluorescent latex particles (white dots) in a continuous flow, in the absence of electric field: cells and particles are confined near the channel wall with embedded AgPDMS electrodes, due to flow focusing. After an AC voltage of 31.2 V at 300 kHz was applied, clear separation was observed (Figure 5.18 (b-c)). The separation effect is shown clearly even after only 2 seconds of the application of the voltage (Fig. 8b). The continuous separation of yeast cells (throughput $\sim 780/\text{min}$) and 5 μm latex particles (throughput $\sim 1200/\text{min}$) were reliably continued for >5 minutes at the flow rate of 0.15 $\mu\text{l}/\text{min}$. The latex particles were pushed away from the electrodes by nDEP, as expected from the prediction in Figure 2.8 (a). In contrast, the yeast cells experienced weak pDEP and were attracted even further towards the electrodes (the reason yeast cells experienced weak pDEP rather than no DEP, as one would predict from Figure 2.8 (a), is that 300 kHz is close to the crossover frequency and the sign of the DEP force is thus highly sensitive to small frequency variations in this regime). As a

result, the yeast cells ended up preferentially in outlet C and the particles in outlet D (Figure 5.18 (d)), with a separation efficiency >97%.

Figure 5.18: Separation of yeast cells (dark dots) from 5 μm latex particles (white dots). (a) Without an applied electric field. (b, c) With an AC voltage of 31.2 V at 300 kHz. Clear separation can be seen at 2 seconds and 2 minutes. (d) Separation efficiency of yeast cells and 5 μm latex particles. Images were acquired with a 5× objective.

5.6.7 Separation of *E. coli* from 2.9 μm latex particles

Separation of *E. coli* and 2.9 μm latex particles in a 380 $\mu\text{S/cm}$ NaCl solution was also successfully achieved. In the absence of electric field, the stream of *E. coli* and particles was again focused near the wall (Figure 5.19 (a)). According to the predicted CM factor (Figure 2.8 (b)) the frequency of 10 MHz is most suitable to separate *E. coli* and the latex particles, because it leads to strong pDEP for bacterial cells and strong nDEP for particles. However, generation of a higher voltage (40.4 V) limited the maximum frequency attainable by the amplifier to 1 MHz. Even so, clear separation was obtained (Figure 5.19 (b) and (c)) with a throughput of 1440 particles/min and 780 cells/min at the flow rate of 0.06 $\mu\text{l/min}$, and very high separation efficiency >97% (Figure 5.19 (d)). For these experiments, the time window set by the speed of the flow (123 $\mu\text{m/s}$) and the channel length (1400 μm) was ~11.4 seconds, thus giving a nDEP drift velocity of ~4.4 $\mu\text{m/s}$. An increase of the flow rate would yield poor separation because the maximum voltage produced by the amplifier is limited at 50 V. As time progresses (Figure 5.19 (c)), some *E. coli* bacterial cells accumulate near the electrodes due to pDEP, which overcomes the hydrodynamic force on the cells at these slow flow rates. One could use higher flow rates to remove this attachment, and would then need a longer main channel to achieve the same degree of separation.

Figure 5.19: Continuous separation of *E. coli* and $2.9\ \mu\text{m}$ latex particles. The mixture of samples at (a) outlets with no applied electric field. (b) Separation of *E. coli* and latex particles when applying an AC voltage of 40.4 V at 1 MHz at 21 seconds and (c) at 6.18 minutes. (d) Separation efficiency of *E. coli* and $2.9\ \mu\text{m}$

latex particles. *E. coli* was stained with SYTO9 and was visualized using an epifluorescence microscopy with a 10x objective.

5.6.8 Separation of live and dead yeast cells

Separation of live and dead cells is one of the most important tasks in biomedical analysis. Here, the device was tested by separating live and dead yeast cells in a 600 $\mu\text{S}/\text{cm}$ NaCl solution, based on the fact that the electric properties of a cell change when the cell dies. We observed that at a frequency of 1 MHz live cells experienced pDEP, whereas dead ones exhibited strong nDEP. By imposing an AC voltage of 26.5 V at a frequency of 1 MHz, continuous separation of live (black dots) from dead (white dots) yeast cells at a flow rate of 0.087 $\mu\text{l}/\text{min}$ was successfully achieved (Figure 5.20), with a throughput of 480 cells/min for both. The separation efficiency was >97%. For these experiments, the time window set by the speed of the flow (181 $\mu\text{m}/\text{s}$) and the channel length (1400 μm) was ~ 7.7 seconds, thus giving a nDEP drift velocity of ~ 6.5 $\mu\text{m}/\text{s}$.

Figure 5.20: Separation of live and dead yeast cells was achieved with an AC voltage of 26.5 V at a frequency of 1 MHz. Dead cells were stained with Propidium Iodide.

5.7 Summary

This chapter reports a PDMS-fabricated microfluidic device that can utilize AC-DEP via 3D conducting AgPDMS composites as sidewall electrodes for manipulation and continuous separation of various samples. Compared to other existing DEP-based microfluidic devices relying on planar and 3D metallic electrodes, the present device has several advantages. First, the sidewall AgPDMS electrodes generate 3D electric field that uniformly distributes throughout the channel height and varies along the channel's lateral direction, thereby giving rise to 3D lateral DEP forces for effective and continuous separation while allowing for easy observation of the motion of samples in the channel. Second, the proposed fabrication technique greatly facilitates (i) the integration of such conducting PDMS composite electrodes with PDMS microchannels, and (ii) the device assembly

involving only oxygen plasma treatment; thus, liquid leakage can be easily prevented. Third, the proposed flow-focusing coupled with DEP provides a highly controlled and efficient separation process.

The fabricated device has been successfully demonstrated to perform several important microfluidic applications: (i) continuous sorting and separation of microparticles by size, (ii) characterization of the DEP properties in terms of pDEP, nDEP and cross-over frequencies for submicron particles, micron particles, yeast cells and *E. coli*, and (iii) continuous separation of yeast cells from similar-size latex particles (5 μm in diameter), *E. coli* from similar-size latex particles (2.9 μm in diameter) and live yeast cells from dead yeast cells. In addition to experiments, the numerical model for simulating particle motion based on the Lagrangian approach can provide good estimation to predict the trend of particle trajectories under the effects of hydrodynamic force and DEP force. These successful validation and the high separation efficiency (>97%) make this new device promising in a broad number of applications and a very good candidate for integration in more complex lab-on-chip platforms for biological and medical analysis.

Chapter 6 Conclusions and Future Studies

6.1 Contributions of this study

Following the objectives of this study, extensive and rigorous research on manipulation and continuous separation of particles and cells in PDMS-based microfluidic systems using the principle of DEP-FFF are performed through experimental investigation and numerical simulations. The study contributes new development and offers useful knowledge to microfluidics community by presenting innovative microfluidic devices, new electrokinetic techniques and microfabrication techniques for particle and cell separation technologies. To sum up, the major contributions made during this study are as follows:

1. Continuous sorting and separation of particles by size in the modified PDMS-based H-filter platform with multi-insulating blocks

The modified PDMS-based H-filter platform is developed by integrating multi-insulating blocks into the H-filter platform for manipulating and continuously separating particles by size. The integrated multi-insulating blocks not only enhance DEP forces but also focus particles in a small region of the gap between the block and the channel wall, thereby facilitating the manipulation and separation of particles. By adjusting the magnitude of DC voltages obtained from only a single channel DC power supply, the device operation is greatly simplified and the device can sort and separate microparticles by size continuously. Through experiments and

simulation based on the Lagrangian approach, the study provides insights into the effect of applied voltage and particle size on particle motion during sorting and separation. In addition to the pure DC electric field, for the first time, the use of combined AC and DC electric field is evidently discovered to reduce the threshold voltage required in separation. Further systematic studies also provide the first-hand information on the effects of the number and geometrical structures of insulating blocks on the threshold voltages as the critical guideline to obtain optimal device configurations. The optimal dimensions of insulating blocks are then used to continuously separate multiple particles by size and type at the same time, which is never successfully reported by any iDEP device. In addition, the utilization of insulating blocks is further extended to successfully trap SWCNTs for potential biosensing applications.

2. Electrokinetically-driven concentration of particles and cells by DEP using a combined AC and DC electric field

A new electrokinetic technique using iDEP generated by combined AC and DC electric field is proposed for microfluidic concentration of particles and cells. Extensive experiments validated by numerical simulation of particle trajectory (based on the Lagrangian approach) reveal striking findings that the total electric field strength required to concentrate microparticles can be reduced significantly by 85.9% as compared to the pure DC electric field. Moreover, systematic studies are carried out, and the experimental findings show that higher buffer concentration, larger particle size and higher ratio of AC-to-DC electric fields are favorable to

such iDEP concentration performance. Also, the proposed new iDEP technique is successfully demonstrated for concentrating yeast cells in a continuous manner.

3. Manipulation and continuous separation of particles and cells by AC-DEP using 3D conducting PDMS electrodes

The integration and utilization of 3D conducting PDMS composites as sidewall electrodes are proposed for the first time and successfully demonstrated to characterize DEP properties of particles and cells and to continuously separate various particles and cells in a PDMS-based microfluidic device. The developed fabrication technique greatly facilitates (i) the integration of the conducting PDMS electrodes with PDMS microchannels, and (ii) device assembly by using only oxygen plasma treatment. With these features, the device can be operated at high flow rates without liquid leakage. Moreover, unlike planar electrodes, 3D conducting PDMS electrodes produce a 3D electric field that distributes uniformly throughout the entire channel height and varies along the channel lateral direction. Thus, strong lateral DEP forces can be generated over the entire channel. Comparison of particle trajectory from experiments and simulation is in reasonable agreement. In all cases of separations, the efficiency higher than 97% is a clear indicator proving the device's capability in dealing with various samples including submicron particles, micron particles, live and dead yeast cells and bacterial cells in a continuous-flow condition.

6.2 Recommendations for future studies

Based on the results presented in this study, recommendations for future research are listed below

1. High-throughput separation and concentration

The use of combined AC and DC electric field greatly reduces the threshold voltage in separation and concentration compared with the pure DC electric field, but does not provide high throughput. Thus, in order to deal with target cells with dilute concentration, a further study will aim to achieve separation and concentration with high-throughput. One potential possibility could be to combine electrokinetics with other force fields such as hydrodynamics, optics or magnetic field.

2. New conducting polymer-based composites for 3D electrodes

3D conducting PDMS composites are clearly efficient in characterization, sorting and separation of particle and cells, but there were some drawbacks associated with voltage drop across the PDMS composites. Therefore, one challenge will be to develop new conducting polymer-based composites offering minimized voltage drop. The composite electrodes can then produce stronger DEP forces acting on cells for attaining higher throughput with lesser voltages. In addition, another study will look into a new microfluidic device using conducting polymer-based electrodes for separation of actual biosamples (e.g., blood) which often contains multiple interfering particles of different sizes and types. Nevertheless, future research will

also aim to develop a new PDMS-based device for separation, concentration and biosensing by using 3D conducting PDMS pillars or blocks constructed in the main channel.

3. Effect of particle-wall interaction on DEP

Based on the Lagrangian approach, the simulation reasonably predicts the trend of electrokinetically-driven particle trajectory under electrokinetic transports but needs a correction factor in the DEP force term so that simulation results could match with experimental results. The reason is that the current modeling does not consider the effects of finite particle size and the presence of channel wall on DEP force. Therefore, future research will explore such particle-wall interaction on DEP by formulating more comprehensive mathematical modeling with consideration of particles of arbitrary size and shape (e.g., spherical, cylindrical and elliptical) subject to non-uniform electric field in a bounded microfluidic domain. Additionally, extension of this study will also include the effect of EDL on DEP force especially for submicron particles. The study will provide better understanding on how the finite particle size and the wall boundary affecting conventional DEP force.

4. Integrated microfluidic chip towards micro-flow cytometry systems

The ultimate goal of future research will seek to develop micro-flow cytometry systems by combining microfluidic separation devices and detection systems in a single PDMS-based chip. Furthermore, such micro-flow cytometry systems must be

able to analyze a large number of samples e.g., blood cells, bacterial cells, DNA and virus within a short time. Therefore, the success in replacing the costly and bulky flow cytometry with disposable and inexpensive microfluidic chip will dramatically impact on the large-scale biomedical industries and the future of healthcare in the developing world.

Appendix A: Derivation of Time-average AC-DEP force

From Chapter 2, the instantaneous DEP force in equation (2.8) is

$$\vec{F}_{DEP}(t) = (\vec{P} \cdot \nabla) \vec{E}_{ac}(r, t) \quad (A1)$$

where $\vec{P} = 4\pi\epsilon_m a^3 \underline{f}_{CM}(\omega) \vec{E}_{ac}(r, t)$

$$\vec{F}_{DEP}(t) = (4\pi\epsilon_m a^3 \underline{f}_{CM}(\omega) \vec{E}_{ac}(r, t) \cdot \nabla) \vec{E}_{ac}(r, t) \quad (A2)$$

Since the electric field can be expressed as $\vec{E}_{ac}(r, t) = \vec{E}_{ac}(r) e^{i\omega t}$, equation (A2)

becomes

$$\vec{F}_{DEP}(t) = (4\pi\epsilon_m a^3 \underline{f}_{CM}(\omega) \vec{E}_{ac}(r) e^{i\omega t} \cdot \nabla) \vec{E}_{ac}(r) e^{i\omega t} \quad (A3)$$

Considering only the real part of the CM factor and phasor of the electric field, the DEP force is

$$\vec{F}_{DEP}(t) = 4\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega) \vec{E}_{ac}(r) e^{i\omega t}] \cdot \nabla \text{Re}[\vec{E}_{ac}(r) e^{i\omega t}] \quad (A4)$$

$$\vec{F}_{DEP}(t) = 4\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] (\text{Re}[\vec{E}_{ac}(r) e^{i\omega t}] \cdot \nabla \text{Re}[\vec{E}_{ac}(r) e^{i\omega t}]) \quad (A5)$$

Note that $\text{Re}[\vec{E}_{ac}(r) e^{i\omega t}] \cdot \nabla \text{Re}[\vec{E}_{ac}(r) e^{i\omega t}] = \frac{1}{2} \nabla (\text{Re}[\vec{E}_{ac}(r) e^{i\omega t}])^2$

$$\vec{F}_{DEP}(t) = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla (\text{Re}[\vec{E}_{ac}(r) e^{i\omega t}])^2 \quad (A6)$$

Using $\vec{E}_{ac}(\vec{r})e^{i\omega t} = \vec{E}_{ac}(\vec{r})(\cos(\omega t) + i\sin(\omega t))$, equation (A6) is reduced to

$$\vec{F}_{DEP}(t) = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla(\vec{E}_{ac}(\vec{r}) \cos(\omega t))^2 \quad (\text{A7})$$

By integrating equation (A7) over one full period 2π , a time-average AC-DEP force is given by

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} \int_0^{2\pi} \vec{F}_{DEP}(t) dt \quad (\text{A8})$$

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} \int_0^{2\pi} 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla(\vec{E}_{ac}(\vec{r}) \cos(\omega t))^2 dt \quad (\text{A9})$$

$$\langle \vec{F}_{DEP} \rangle = \epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{ac}^2(\vec{r}) \int_0^{2\pi} \cos^2(\omega t) dt \quad (\text{A10})$$

$$\langle \vec{F}_{DEP} \rangle = \pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{ac}^2(\vec{r}) \quad (\text{A11})$$

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{rms,ac}^2(\vec{r}) \quad (\text{A12})$$

Appendix B: Derivation of Time-average DC-DEP force

Since the DC electric field is not a function of time, the instantaneous DEP force in equation (A7) becomes

$$\vec{F}_{DEP}(t) = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{dc}^2(r) \quad (\text{B1})$$

Thus, by integrating equation (B1) over one full period 2π , a time-average DC-DEP force is

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} \int_0^{2\pi} 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{dc}^2(r) dt \quad (\text{B2})$$

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{dc}^2(r) \int_0^{2\pi} dt \quad (\text{B3})$$

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \vec{E}_{dc}^2(r) \quad (\text{B4})$$

Appendix C: Derivation of Time-average DC-offset, AC-DEP force

When an AC electric field is biased with a DC electric field, the total electric field is mathematically expressed as

$$\vec{E}(r, t) = \vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r})e^{i\omega t} \quad (C1)$$

Thus, equation (A7) becomes

$$\vec{F}_{DEP}(t) = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla (\text{Re}[\vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r})e^{i\omega t}])^2 \quad (C2)$$

Through the integration over one full period 2π , a time-average DC-offset, AC-DEP force is

$$\langle \vec{F}_{DEP} \rangle = \frac{1}{2\pi} \int_0^{2\pi} 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla (\vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r}) \cos(\omega t))^2 dt \quad (C3)$$

$$\langle \vec{F}_{DEP} \rangle = \epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)] \nabla \int_0^{2\pi} (\vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r}) \cos(\omega t))^2 dt \quad (C4)$$

Consider the term in the integral expression

$$\begin{aligned} & \int_0^{2\pi} (\vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r}) \cos(\omega t))^2 dt \\ &= \int_0^{2\pi} (\vec{E}_{dc}^2(\vec{r}) + 2\vec{E}_{dc}(\vec{r})\vec{E}_{ac}(\vec{r}) \cos(\omega t) + \vec{E}_{ac}^2(\vec{r}) \cos^2(\omega t)) dt \end{aligned} \quad (C5)$$

$$= \vec{E}_{dc}^2(\vec{r})(2\pi) + 2\vec{E}_{dc}(\vec{r})\vec{E}_{ac}(\vec{r})\frac{\sin(\omega t)}{\omega}\bigg|_0^{2\pi} + \frac{1}{2}(2\pi)\vec{E}_{ac}^2(\vec{r}) + \frac{1}{2}\vec{E}_{ac}^2(\vec{r})\frac{\sin(2\omega t)}{2\omega}\bigg|_0^{2\pi} \quad (C6)$$

Since $\omega = \frac{2\pi}{T} = \frac{2\pi}{2\pi} = 1$, we have

$$\int_0^{2\pi} (\vec{E}_{dc}(\vec{r}) + \vec{E}_{ac}(\vec{r})\cos(\omega t))^2 dt = 2\pi\vec{E}_{dc}^2(\vec{r}) + \pi\vec{E}_{ac}^2(\vec{r}) \quad (C7)$$

Inserting equation (C7) into equation (C4), the time-average DEP force can be expressed as

$$\langle \vec{F}_{DEP} \rangle = \pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla(2\vec{E}_{dc}^2(\vec{r}) + \vec{E}_{ac}^2(\vec{r})) \quad (C8)$$

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla\vec{E}_{dc}^2(\vec{r}) + \pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla\vec{E}_{ac}^2(\vec{r}) \quad (C9)$$

In term of root mean square, equation (C8) and (C9) respectively become

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla(\vec{E}_{dc}^2(\vec{r}) + \vec{E}_{rms,ac}^2(\vec{r})) \quad (C10)$$

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla\vec{E}_{dc}^2(\vec{r}) + 2\pi\epsilon_m a^3 \text{Re}[\underline{f}_{CM}(\omega)]\nabla\vec{E}_{rms,ac}^2(\vec{r}) \quad (C11)$$

Appendix D: Publications

JOURNAL PAPERS

1. **Lewpiriyawong, N.** and Yang, C., Dielectrophoretic Characterization and Continuous-flow Separation of Submicron and Micron Particles Using AC-Dielectrophoresis via Conducting PDMS electrodes, (submitted to *Biomicrofluidics*)
2. **Lewpiriyawong, N.**, Yang, C. and Lam, Y. C., Continuous Separation of Multiple Particles by Size and Type Using Alternating-Current Dielectrophoresis in a Modified H-filter with insulating blocks, (In preparation for submission to *Electrophoresis*).
3. **Lewpiriyawong, N.**, Yang, C. and Lam, Y. C., Electrokinetically-Driven Concentration of Particles and Cells by Dielectrophoresis Using a DC-Offset, AC Electric Field, (submitted to *Microfluidics and Nanofluidics*).
4. **Lewpiriyawong, N.**, Kandaswamy, K., Yang, C., Ivanov, V. and Stocker, R., Microfluidic Characterization and Continuous Separation of Cells and Particles Using Conducting PDMS Electrode Induced AC-Dielectrophoresis, (accepted to *Analytical Chemistry*)
5. **Lewpiriyawong, N.**, Yang, C. and Lam, Y. C., Continuous Sorting and Separation of Microparticles by Size Using AC Dielectrophoresis in a PDMS Microfluidic Device with 3D Conducting PDMS Composite Electrodes, *Electrophoresis*. **2010**, 31, 2622-2631.
6. **Lewpiriyawong, N.**, Yang, C. and Lam, Y. C., Dielectrophoretic Manipulation of Particles in a Modified Microfluidic H-filter with Multi-insulating Blocks, *Biomicrofluidics*. **2008**. [DOI: 10.1063/1.2973661]

CONFERENCE PAPERS

1. **Lewpiriyawong, N.**, Yang, C. and Lam, Y. C., Electrokinetically-Driven Concentration of Particles and Cells by Dielectrophoresis Using a dc-Offset, ac Electric Field in *Advances in Microfluidics and Nanofluidics and Asian-Pacific International Symposium on Lab on Chip*, Singapore, Jan 5-7, **2011**.
2. **Lewpiriyawong, N.**, Yang, C., Kandaswamy, K., Ivanov, V. and Stocker, R., A PDMS-Based Microfluidic Device for Characterizing and Continuously Separating Cells Using AC-DEP via 3D Conducting PDMS Composite Electrodes in *Advances in Microfluidics and Nanofluidics and Asian-Pacific International Symposium on Lab on Chip*, Singapore, Jan 5-7, **2011**.
3. **Lewpiriyawong, N.**, Yang, C., Kandaswamy, K. and Ivanov, V., Dielectrophoretic Characterization and Continuous Separation of

Micro/Submicron Particles and Cells in a PDMS-Based Microfluidic Device with Silver-PDMS Electrodes in *Particles 2010*, Florida, USA, May 22-25, **2010**.

4. **Lewpiriyawong, N.** and Yang, C., Continuous Sorting and Separation of Microparticles Using AC-Dielectrophoresis in a PDMS-Based Microfluidic Device with 3D PDMS Composite Electrodes in *Southeast Asia International Advances in Micro/Nano-Technology*, Bangkok, Thailand, March 8-11, **2010**.
5. Ivanov, V., Yang, C., Stocker, R., **Lewpiriyawong, N.** and Kandaswamy, K., Polarization and Separation of Live and Dead Cells in Electric Field in *BioElectromagnetics*, Davos, Switzerland, June 14-19, **2009**.
6. **Lewpiriyawong, N.**, Yan, D. G., Yang, C., and Lam, Y. C., Electrokinetic Control, Sorting and Fractionation of Particles Using Modified Microfluidic H-filter Structures in *International Conference on Materials for Advanced Technologies*, Singapore, July 1-6, **2007**.

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