Numerical modeling of motion and deformation of cells in microscale hydrodynamic and electric fields

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**NUMERICAL MODELING OF MOTION AND
DEFORMATION OF CELLS IN
MICROSCALE HYDRODYNAMIC AND
ELECTRIC FIELDS**

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Abstract

Cell performance is of great interest in a variety of biological processes and bioengineering applications, such as the cell motion in a manipulating biochip, and the cell deformation in a capillary. The present research work focuses on the numerical investigation of the motion and deformation of a single cell or two cells in the parabolic hydrodynamic field, nonuniform electric field, and parabolic hydrodynamic and nonuniform electric coupled field, respectively.

A two-fluid model is presented to describe the flow characteristics of cell suspending in a fluid, considering the interactions between the cell and hydrodynamic field, between the cell and electric field, and between the two cells. The shell model is adopted to describe the interaction between the cell and hydrodynamic field, identified by the membrane mechanical force, where the cell membrane is treated as an incompressible and elastic shell with a uniform thickness and allowed to undergo the stretching and bending deformation. The Maxwell stress tensor (MST) approach is used to describe the interaction between the cell and electric field, identified by the dielectrophoresis (DEP) force due to the cell polarization. The Morse potential model is employed to describe the interaction between the two cells, identified by the intercellular interaction force behaving as a weak attractive force at far distance but a strong repulsive force at near distance.

As the first academic achievement made in this thesis, the two novel numerical methods are developed to efficiently treat the two-fluid model, namely the modified particle binary level set (MPBLS) method for tracking the cell membrane, and the

modified semi-implicit method for pressure linked equations-revised (modified SIMPLER).

- The MPBLS method employs the binary level set function to distinguish different materials, and further scatters a number of Lagrangian particles on the interface to correct the interface and provide the interface information. Compared with the previous particle level set (PLS) approach, the MPBLS method is more accurate and less time-consuming, and more suitable to multiphase and interfacial flow applications with Lagrangian particles. For 3D problems however, the MPBLS method becomes tedious as usual, since the Lagrangian particles in 3D domain are not easily used to describe the information of surface-like interface.
- The modified SIMPLER algorithm introduces an improved velocity correction to speed up the convergence of momentum equations, and further integrates a fine adjustment to satisfy the continuity equation with higher accuracy. Compared with the previous SIMPLER algorithm, the modified SIMPLER algorithm can achieve higher accuracy and convergence, and be more applicable to solve the incompressible Navier-Stokes equations with the low Reynolds number.

As the second academic achievement made in this thesis, three studies are conducted by numerically solving the two-fluid model, namely the cell performance in the hydrodynamic, electric, and hydrodynamic-electric fields, respectively.

- In the hydrodynamic field, the motion and deformation of a single red blood cell (RBC) or two RBCs in a parabolic capillary flow are studied, and several parameters are discussed in detail, including the RBC position, density, deformability, intercellular interaction, etc. The simulation results reproduce the axisymmetric and asymmetric motion of RBC in the capillary flow, in which the RBC deforms into the characteristic parachute and slipper shapes, respectively. The RBC density plays a significant role in the RBC deformation, and even a slight variation of cell density will result in the different cell deformation. Therefore, the two-fluid model is more accurate than the pure-fluid model to characterize the RBC performance in a capillary flow. Besides, it is shown that the cell deformation and aggregation can interact slightly with each other due to the different intercellular distance.

- In the electric field, the motion and deformation of a single RBC or two RBCs are simulated under the condition that the electric field is nonuniform and alternating. The simulation results demonstrate that the RBC performance in the nonuniform electric field depends on the various parameters, including the RBC size, electrode potential, electric frequency, RBC permittivity and RBC conductivity. At the low frequency, the RBC is repelled away from the region with strong electric field strength if it is less conductive than the suspending fluid, and its lower part deforms narrower and narrower, i.e., the RBC exhibits the negative DEP (n-DEP). On the contrary, the RBC is attracted towards that region if it is more conductive than the suspending fluid, and its lower part deforms wider and wider, i.e., the RBC experiences the positive DEP (p-DEP). At the high frequency, the RBC experiences the n-DEP if it is less polarized than the suspending fluid, whereas the RBC exhibits the p-DEP if it is more polarized. Furthermore, if the intercellular interaction strength becomes strong enough, the motion trend of RBC may be changed. For example, the RBC exhibiting the p-DEP may move away from the region with strong electric field strength.
- In the hydrodynamic-electric field, a microfluidic-microelectric hybrid chip is numerically designed, and used to manipulate a single RBC or two RBCs. The working principle of hybrid chip is that the nonuniform AC electric field makes the RBC deviate first from the centerline of parabolic fluid flow, and then the parabolic fluid flow makes the RBC transported with the different velocities. The simulation results show that the RBCs can be separated successfully by the hybrid chip either at the low frequency according to their different conductivities, or at the high frequency according to their different permittivities. However, whichever the high frequency or low frequency is applied, a necessary condition required for successful separation is that both the n-DEP and p-DEP phenomena occur.

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List of Main Symbols

Symbol	Unit	Description	Symbol	Unit	Description
C_{mem}	F/m ²	Membrane capacitance	β	m ⁻¹	Scaling factor
$\tilde{C}_{mem}$	F/m ²	Complex membrane capacitance	ε	—	Tensile strain
d_1	m	Electrode width	ϕ	—	Binary level set function
d_2	m	Electrode gap	$\tilde{\phi}$	V	Electric potential
D_e	J	Intercellular interaction strength	κ	m ⁻¹	Membrane curvature
$\tilde{E}$	V/m	Electric field	Γ	—	Cell membrane location
E_S	N/m	Membrane elastic modulus	μ	Pa s	Viscosity
E_B	N m	Membrane bending stiffness	μ_c	Pa s	Cell viscosity
f_{dep}	N/m ²	Dielectrophoresis force strength	μ_m	Pa s	Fluid viscosity
f_{int}	N/m ²	Intercellular interaction force strength	Θ	—	Heaviside function
f_{mem}	N/m ²	Membrane mechanical force strength	ρ	kg/m ³	Density
f_{tot}	N/m ²	Total force strength	ρ_c	kg/m ³	Cell density
G_{mem}	S/m ²	Membrane conductance	ρ_m	kg/m ³	Fluid density
K_{CM}	—	Clausius-Mossotti factor	σ_c	S/m	Cell conductivity
L	m	Computational domain length	σ_{in}	S/m	Cell interior conductivity
$\mathbf{n}$	—	Unit normal direction	σ_m	S/m	Fluid conductivity
r	m	Intercellular distance	ω	rad/s	Angular frequency
r_0	m	Zero force length	ξ_c	F/m	Cell permittivity
R	m	Cell radius	ξ_{in}	F/m	Cell interior permittivity
t	s	Time	ξ_m	F/m	Fluid permittivity
$\mathbf{t}$	—	Unit tangent vector	$\tilde{\xi}_c$	F/m	Complex cell permittivity
$\mathbf{u}$	m/s	Velocity	$\tilde{\xi}_{in}$	F/m	Complex interior permittivity
V_0	V	Electrode potential	$\tilde{\xi}_m$	F/m	Complex fluid permittivity
W	m	Computational domain width	ψ	J	Morse potential function

Chapter 1

Introduction

1.1 Background and motivation

Basically, a cell is a structural and functional unit of living organism. It is often compared to the building brick of life, and determines the diversity of organisms from the root. Some organisms are unicellular, such as bacteria, consisting of a single cell. Other organisms are multicellular, for example, humans having about 10^{14} cells. Even though the virus does not have a cellular structure, it cannot grow or reproduce without the living cells. The cell is generally classified into two types: prokaryotic and eukaryotic cells. The prokaryotic cell is simple and small due to lack of a nucleus, and often found in the unicellular organisms, such as bacteria and archaea. By contrast, the eukaryotic cell is more complex with a nucleus, and much larger than the prokaryotic cell, and often encountered in multicellular organisms. Either prokaryotic or eukaryotic cells have a “skin” called the plasma membrane, protecting their interior from the outside environment. The plasma membrane consists of a lipid bilayer in which a supportive skeleton of proteins is embedded. This characteristic structure makes the cell membrane involved in a variety of cellular processes, including the cell motion and deformation [1].

Cell motion is an essential phenomenon from simple unicellular organisms to complex multicellular organisms [2, 3]. Many biological processes require the orchestrated motion of cells in particular directions to specific location, for example,

organ and tissue formation during embryonic development, wound healing, immune response of white blood cells (WBCs), and oxygen delivery of red blood cells (RBCs). Otherwise, serious consequences may be caused, such as tumor formation, cancer metastasis, vascular diseases, and so on. Understanding of cell motion helps to provide valuable knowledge in the prevention and treatment against the relevant diseases. In addition, the cell motion is often encountered in a broad range of bioengineering applications, such as the pharmaceutical production and cell manipulation. In this regard, it is also important to investigate the cell motion for guiding practical applications.

Cell deformation is another common but important phenomenon in living organisms [4]. Generally, a cell has a certain shape in the healthy state, and it may change its own shape subject to external or internal environmental stimuli. For example, in order to deliver oxygen and pick up waste carbon dioxide, mature RBCs in human deform from the normal biconcave shape into a parachute shape to pass through capillaries often narrower than RBCs themselves in diameter. However, cells may deform into abnormal shapes if they are subjected to the false stimuli, resulting in some diseases, such as the circular shape in the hereditary spherocytosis, and the elliptical shape in the macrocytic anemia. Therefore, the cell deformation is sometimes regarded as an indicator of some diseases. In addition, the miniaturization trend is of particular significance in the cell engineering applications with the development of micro and nano technology, such that the cell deformation plays an essential and non-negligible role in the miniaturized biochip.

The present research work focuses on the motion and deformation of cells in the hydrodynamic and electric fields. Much of interest in these fields is motivated by the significance of cells through capillaries in microcirculation, and the promising application of electric field in cell manipulation. Microcirculation is one of the basic characteristics of life, directly involved in the transmission of material, energy and information between the organism and surrounding environment. If the microcirculation is impaired, the physiological function of each organ may be influenced, leading to vascular diseases. For instance, the massive aggregation of RBCs blocks the blood vessels easily, severely reducing the amount of oxygen and nutrients transported, and thus may cause the thrombosis and cerebral infarction. Moreover, currently cell manipulation is of great interest in biomedical and biotechnological applications, for example, separating RBCs from patient's blood to treat polycythemia vera, and sifting

out tumor cells to improve cancer diagnosis. There are several techniques based on different types of forces for cell manipulation, such as the electric, mechanical, optical, and magnetic forces. Thereinto, the electric force is one of the most promising techniques because of its simple and cheap peripheral equipment and easy integration with microchips [5]. In brief, it is interesting and worthwhile to investigate the motion and deformation of cells in the hydrodynamic and electric fields.

1.2 Objective and scope

The objective of the present research work is to investigate numerically the motion and deformation of a single cell or two cells in the parabolic hydrodynamic field, nonuniform electric field, and parabolic hydrodynamic and nonuniform electric coupled field, respectively. The specific objectives are detailed as follows.

- Formulation of the mathematical model. A multiphysics two-fluid model is formulated to describe the cell performance in the hydrodynamic and electric fields, where the cells and suspending fluid have the different physical properties, such as density, viscosity, permittivity, and so on. In this model, we consider the interactions between the cell and fluid field, between the cell and electric field, and between the two cells. These three interactions are identified by the membrane mechanical force, dielectrophoresis force, and intercellular interaction force, respectively.
- Development of the numerical methods. In order to solve the two-fluid model, there are three main components required for the numerical treatment. The first is to track the cell membrane for adjusting different physical properties inside and outside the cells. As such, a novel front tracking method is developed to efficiently track the cell membrane, termed the modified particle binary level set (MPBLS) method. The second is to efficiently solve the governing equations of the two fluids inside and outside the cells, such that a modified semi-implicit method for pressure linked equations-revised (modified SIMPLER) algorithm is proposed. The last but not least component is to calculate the membrane mechanical force, dielectrophoresis force, and intercellular interaction force. For this purpose, several simple techniques are used, such as finite difference method, Lagrangian interpolation, and trapezoidal integration rule.

- Exploration of the cell performance. Based on the two-fluid model solved by the numerical methods, the cell performance in the hydrodynamic, electric, and hydrodynamic-electric coupled fields is investigated in detail qualitatively and quantitatively. Before that however, two preliminary studies are conducted. One is the sensitivity analysis of the mesh size in computational domain and the particle number on the cell membrane, which minimizes the effect of numerical discretization. The other is the membrane tracking in a steady Poiseuille flow, which provides a comparison with the cell performance in the hydrodynamic and electric fields.
 - *Sensitivity analysis and membrane tracking.* Taking the deformed shape of a single cell in the hydrodynamic field for example, the effects of mesh size in the computational domain and the particle number on the cell membrane are analyzed, and thus the suitable mesh size and particle number are chosen well. After that, the performance of a single cell or two cells is simulated in a steady Poiseuille flow, where the membrane mechanical force, dielectrophoresis force and intercellular interaction force are not considered. As a result, the cell performance is totally attributed to the shear force of the steady Poiseuille flow. This study is used to evaluate the accuracy of tracking membrane, and more importantly to provide comparisons with the further studies on the cell in the hydrodynamic and electric fields.
 - *Cell performance in the hydrodynamic field.* In this study, both the shear force and membrane mechanical force are included, as well as the intercellular interaction force in the case of two cells. It is predictable that the cell deforms difficultly due to the additional resistance of membrane mechanical force, and either the attraction or repulsion is found because of the intercellular interaction force. The present study improves the understanding of the contributions of the membrane mechanical force and intercellular interaction force to the cell motion and deformation.
 - *Cell performance in the electric field.* In this study, the dielectrophoretic force and membrane mechanical force are included, and the intercellular interaction force is also considered when treating the two cells. However,

the shear force of fluid flow is excluded. This study benefits to elucidate the mechanism of the cell motion and deformation caused by the DEP force.

- *Cell performance in the hydrodynamic-electric coupled field.* A microfluidic-microelectric hybrid chip is numerically designed, and applied to manipulate cells. The present study considers the membrane mechanical force, DEP force, intercellular interaction force, and the shear force of fluid flow. Therefore, it is the more comprehensive study in the present research work, and helpful for guiding practical applications related to the hydrodynamic-electric coupled fluid flow problem.

1.3 Thesis organization

The thesis is mainly composed of six chapters, outlined as follows.

Chapter 1 introduces the motivation of investigating the motion and deformation of cells in the hydrodynamic and electric fields, followed by the objective and outline of the present research work.

Chapter 2 provides a literature review of existing research on the motion and deformation of cells in the hydrodynamic field covering the simple shear flow and Poiseuille flow, in the electric field including the uniform and nonuniform fields, and in the hydrodynamic-electric coupled field focusing on the dielectrophoretic field-flow fractionation, respectively.

Chapter 3 formulates a two-fluid model to characterize the motion and deformation of cells in the hydrodynamic and electric fields, integrating a shell model for the membrane mechanical behavior, a Maxwell stress tensor model for the dielectrophoresis phenomena, and a Morse potential model for the intercellular interaction. The model nondimensionlization is also carried out in this chapter.

Chapter 4 develops the numerical methods to efficiently handle the two-fluid model. Particularly, two novel numerical methods are proposed, and they are called the modified particle binary level set (MPBLS) method to track the location and shape of cell membrane, and the modified semi-implicit method for pressure linked equations-revised (modified SIMPLER) algorithm to solve the Navier-Stokes equations. Besides,

several numerical techniques are also discussed briefly for calculating the membrane mechanical force, dielectrophoresis force and intercellular interaction force.

Chapter 5 explores the cell performance in the hydrodynamic and electric fields. In the hydrodynamic field, the motion and deformation of a single RBC or two RBCs in a parabolic capillary flow are investigated, and several parametric studies are conducted in detail. In the electric field, the motion and deformation of a single RBC or two RBCs are investigated under the condition that the electric field is nonuniform and alternating, and influencing factors are also examined. In the hydrodynamic-electric field, a microfluidic-microelectric hybrid chip is numerically designed, and applied to manipulate a single RBC or two RBCs with different permittivities and conductivities. Before investigating the cell performance in these three fields, as the preliminary studies, the mesh size in the computational domain and the particle number on the cell membrane are chosen first, and the free motion and deformation of a single RBC and two RBCs in a steady Poiseuille flow are then simulated.

Chapter 6 summarizes the present research work, and recommends the possible future work.

Besides these chapters, five appendices are arranged to explain some crucial issues in the main body of thesis. Appendix A introduces the cubic smoothing spline method used to smooth the derivatives in the calculation of membrane mechanical force. Appendix B presents four basic measures to quantitatively describe the cell motion and deformation, including the cell area, centroid, extension and orientation. Appendix C documents the weighted essentially non-oscillatory (WENO) scheme to interpolate the binary level set function with the high resolution. Appendix D explains the calculation of effective velocity of Lagrangian particles using the main idea of the particle in cell (PIC) method. Appendix E shows the simple preprocessing software developed to set the parameter values in simulations.

Chapter 2

Literature Review

This chapter concentrates on a literature review on the cell performance in the hydrodynamic field covering the simple shear flow and Poiseuille flow, in the electric field including the uniform and nonuniform fields, and in the hydrodynamic-electric coupled field focusing on the dielectrophoretic field-flow fractionation, respectively.

2.1 Cells in hydrodynamic field

2.1.1 Simple shear flow

Over the past several decades, there have been enormous efforts to explore the motion and deformation of cells in the simple shear flow. The three different modes were found, namely the steady tank-treading [6-15], unsteady tumbling [16-20], and transitional trembling [21-23] modes. In the first mode, the cell reaches a steady state with a stationary configuration and finite orientation, but its membrane rotates around the liquid inside, like tank treading as shown in Fig. 2.1(a). The second mode is an unsteady state, where the cell flips continuously, as illustrated in Fig. 2.1(b). The third mode is the transitional state between the steady and unsteady states, also known as swinging or vacillating breathing, in which the cell undergoes a periodic deformation accompanied by small orientation oscillation, as shown in Fig. 2.1(c). It was found that which mode is present depends on the shear rate of shear flow and the viscosity ratio of the inner to outer liquids [4, 24, 25]. The tank-treading mode occurs at the low shear rate and low viscosity ratio, and the tumbling mode at the low shear rate but the high viscosity ratio.

However, if the shear rate increases, the oscillation amplitude of cell decreases, and thus trembling appears.

Fig. 2.1 Three different modes of cells in the simple shear flow [4], (a) the steady tank-treading, (b) unsteady tumbling, and (c) transitional trembling modes.

Another behavior of cells in the shear flow is the cell aggregation, which has also attracted increasing attention recently due to its significance in many biological processes, such as the red blood cells (RBCs) aggregation in microcirculation, and the cancer cell aggregation during metastasis. However, the exact underlying mechanism of the cell aggregation is still ambiguous so far. Currently there are the two coexisting theoretical models, namely the bridging [26] and depletion [27] models. In the bridging model, it is assumed that the macromolecules, such as fibrinogen or dextran, can adhere onto the adjacent cell surface and bridge them together. If the bridging force exceeds the disaggregation force including the electrostatic repulsion, membrane shearing and membrane bending, the cells tend to aggregate. The depletion model proposes that the attractive force results from the polymer depletion with a gradient of the osmotic pressure near the cell surface, whereas the disaggregation force is still attributed to the electrostatic repulsion, membrane shearing and membrane bending. So far, several studies have been conducted on the aggregation and disaggregation of RBCs in the simple shear flow using the lattice Boltzmann algorithm [28, 29], Monte Carlo approach [30], finite element method [31, 32], and immersed boundary method [33], etc.

2.1.2 Poiseuille flow

In a Poiseuille flow, the asymmetric and axisymmetric behaviors of cells are exhibited and of considerable interest. Generally, the cell shape is asymmetric with respect to the

centerline of Poiseuille flow when the cell enters into a microchannel, and thus the shear force acts on the cell asymmetrically. As a result, the asymmetric behavior occurs, where the cell gradually deforms from the biconcave shape into a slipper shape, as shown in Fig. 2.2(a). Secomb and Skalak [34] studied the asymmetric behavior of a RBC in a two-dimensional capillary flow based on the lubrication theory, and found that the tank-treading mode usually accompanies therein. In order to elucidate this tank-treading mode, Sugihara-Seki and Skalak [35] further investigated the asymmetric motion of a file and two files RBCs using the finite element method, under the assumption that the RBC is regarded as a rigid cylinder post and not located at the centerline of Poiseuille flow. Over the last decade, the asymmetric behavior of cells in a Poiseuille flow has been well studied based on 2D and 3D models [36-43].

Fig. 2.2 Two different modes of cells in the Poiseuille flow [44], (a) the asymmetric and (b) axisymmetric motion.

If the microchannel is quite narrow and the flow velocity is large, however, the asymmetric behavior approaches an axisymmetric one, where the tank-treading motion is absent, and the cell deforms into a characteristic parachute shape [45], as illustrated in Fig. 2.2(b). In such a case, Secomb [45] used the lubrication method again to examine the axisymmetric motion of a RBC in narrow tubes, and obtained the characteristic parachute shape. This parachute shape guarantees that the RBC traverses through the various capillaries successfully, including the smaller capillaries compared with the undeformed RBC in diameter. Pozrikidis [44] further analyzed the axisymmetric motion of a file of RBCs, and examined the effect of cell spacing using a boundary integral method for Stokes flow without consideration of the intercellular interaction. In recent

years, an increasing interest has been observed in the numerical simulation of a single cell or a file of cells in the Poiseuille flow to analyze the influencing factor on the cell deformation [46-52]. Moreover, the aggregation of cells has also attracted more attention in the Poiseuille flow [53-56].

Although some progress has been achieved so far, detailed knowledge is still poor for the dynamic behavior of cells in a hydrodynamic field, for example, the coupled effects of cell deformation and aggregation. Besides, excessive assumptions were often proposed in the most previous studies, such as the same cell density and viscosity as the flow fluid, and the ignorance of intercellular interaction.

2.2 Cells in electric field

The electrical phenotype of a cell is the collection of its electric properties, most notably its net charge and polarizability [57]. In general, a cell has a negative surface charge that originates from the ionization of surface molecules or the adsorption of ions from the liquid solution [58]. When subject to an electric field, the cell will move due to the Coulombic force, which is well known as the electrophoresis (EP) [59]. Moreover, a cell can be easily polarized so that it also moves due to its polarization when subject to a nonuniform electric field, called the dielectrophoresis (DEP) [60]. Therefore, both the EP and DEP occur if a cell is placed in a nonuniform electric field. However, the EP is usually not considered in the most studies of DEP, because the charge of a cell is significantly small about -70 mV. Apart from that, the AC electric field is often applied in the DEP applications, which causes the EP force to switch directions every half-cycle and in turn damps out the EP.

2.2.1 Uniform electric field

2.2.1.1 Electrophoresis of charged particles

Electrophoresis is the motion of charged particles in an applied electric field, where the particles are usually treated to be rigid, and the applied electric field is uniform as well [58]. Positive charged particles move towards a negative electrode, while negative charged particles migrate towards a positive electrode, as shown in Fig. 2.3. In practice, many important biomolecules, such as proteins, DNA and RNA, nucleic acid etc., have ionizable groups, and therefore can have negative or positive charges at a specific pH.

Subject to an applied electric field, they move towards the electrode with opposite electrical polarity. Since the biomolecules have the different charge, size and shape, their migration rates are different, which is the principle of electrophoresis in separation, characterization and handling of samples.

Fig. 2.3 Schematic illustration of the electrophoresis of a negative charged particle subject to a uniform electric field, where it moves towards the anode.

Electrophoresis was first observed in 1807 by the Russian physicist Reuss [59], who noticed that clay particles dispersed in water migrate under an applied electric field. In 1909, Michaelis [59] determined the isoelectric points of various enzymes in the U-shaped tube, and first defined the electrophoresis to describe the mobility of particles in an electric field. However, the electrophoresis was not employed as an experimental technique until the introduction of a new electrophoresis apparatus based on a moving-boundary method by Tiselius in 1937 [61]. Using Tiselius apparatus, it was demonstrated for the first time that the globulin molecules could be separated into different classes, the alpha, beta, and gamma globulin. For his contribution on protein separation by electrophoresis, Tiselius was awarded a Nobel Prize in 1948. In the same year, Wieland and Fischer [62] were the first use of paper in electrophoresis to separate amino acids and peptides, which inaugurated a new era of the electrophoresis using solid materials as a support medium, such as filter paper [63], cellulose acetate [64], agarose gel [65], starch gel [66], and so on. A major breakthrough occurred with the introduction of synthetic gels from polyacrylamide in 1959 [67], which significantly improved the electrophoresis resolution. In the following three decades, polyacrylamide gel electrophoresis has become widespread in biochemical and biomolecular

applications, as one of the most efficient and inexpensive techniques. However, a notable drawback with all electrophoresis is the generation of Joule heat when electric current passing through the support medium [59]. To overcome this drawback, the capillary electrophoresis (CE) was developed by Jorgenson and Lukacs in 1981 [68-70], where thin capillaries with 20-100 μm in diameter were used. The thin capillaries can minimize the Joule heat due to the high ratio of surface area to volume of capillaries, such that very high voltages could be employed to enable high speed and efficient analysis. In recent years, the CE has become a promising analytical technique in many biomolecular applications, and various techniques have been developed [71-74], such as the capillary zone electrophoresis, capillary gel electrophoresis, capillary isoelectric focusing, capillary isotachopheresis, etc.

2.2.1.2 *Electrodeformation of neutral vesicles*

Cells can be treated not only as rigid particles at the large scale about 100 μm , but also as deformable vesicles at the small scale about 10 μm . Electrophoresis focuses on the cell motion in an applied electric field, but doesn't concern the cell deformation. To date, several studies have been carried out on the deformation of neutral cells in a uniform electric field, but almost no studies on the deformation of charged cells.

Previous experimental and theoretical studies [75-82] showed that the neutral vesicles (including cells) in a uniform AC electric field can deform into the spherical, prolate or oblate shape, depending on the frequency f and conductivity ratio γ inside and outside the vesicles, as indicated in Fig. 2.4. At the low frequency (few kHz), the vesicle deforms into the prolate shape with the longer axis oriented along the field direction at both $\gamma > 1$ and $\gamma < 1$. At the intermediate frequency (several hundred kHz), the vesicle at $\gamma > 1$ still deforms into the prolate shape, while the vesicle at $\gamma < 1$ assumes the oblate shape. At the high frequency (several MHz), however, the spherical shape is observed at both $\gamma > 1$ and $\gamma < 1$. Therefore, four characteristic transitions are exhibited for neutral vesicles in the uniform electric field [82]. The first transition occurs at the low frequency region, which is the change from the prolate to oblate shape for $\gamma < 1$ with increasing the frequency. The second transition is the change from the oblate to spherical shape for $\gamma < 1$ at the high frequency region, and the third is the prolate to spherical shape for $\gamma > 1$ at the high frequency region. All the above three

transitions are induced by changing the frequency. The last transition is the change from the prolate to oblate shape at the intermediate frequency region, but induced by increasing the conductivity outside the vesicles. Currently, the physical mechanism responsible for vesicle transitions was not fully understood, and most studies still focused on the experimental observations [77].

Fig. 2.4 Deformation of neutral vesicles with different conductivities in a uniform AC electric field with different frequencies, where the first row represents the conductivity ratio $\gamma > 1$ and the second row $\gamma < 1$ [82].

2.2.2 Nonuniform electric field: dielectrophoresis

Dielectrophoresis is the motion of particles in a nonuniform electric field, where the particles are usually treated to be rigid [83, 84]. Compared with the EP, the DEP attracted more attention in applications of cell manipulation due to its advantages over the EP in terms of specificity and cell health [57]. The DEP arises from the cell polarizability, integrating more information about the cell structure instead of the net charge only in the EP, and therefore can manipulate more types of cells. Besides, the very low electric potential is applied in the DEP, which is almost innocuous to the cell health [57]. So far, a large number of studies have been done on the DEP from theoretical, experimental and numerical points view, and several different techniques have been developed to separate, trap, and pattern particles.

2.2.2.1 Theory of dielectrophoresis

The physical basis behind the DEP phenomenon is schematically described in Fig. 2.5, where a charged particle and a neutral particle are placed in both a uniform and nonuniform electric fields. In the uniform field, the charged particle feels a net force (Coulomb force), while the neutral particle doesn't experience a net force because a pair of equal and opposite forces is balanced each other, as shown in Fig. 2.5(a). In the nonuniform field, a net force acts on the charged particle, which is easily understood. For the neutral particle however, the negative and positive parts experience the forces with different magnitudes due to the nonuniform electric field, and therefore a net force is also generated, as illustrated in Fig. 2.5(b). This force is called the DEP force, resulting from the particle polarization in a nonuniform electric field.

Fig. 2.5 Physical basis of the dielectrophoresis described by comparing the behavior of a charged particle and a neutral particle in (a) the uniform and (b) nonuniform electric fields, respectively.

So far, several analytical approaches have been developed to express the DEP force acting on a particle, including the effective moment method [85], Maxwell stress tensor (MST) approach [86], and energy method [87]. The effective moment method is most commonly used because it provides a simple analytical expression with a good physical insight into the DEP phenomenon. However, it is not suitable to evaluate the DEP force on deformable cells due to the accuracy limitation. The MST approach, as the most general approach, makes up for this defect and has a distinct advantage in numerical simulations, whereas it is also limited to some simple applications due to its

mathematical complexity. The energy approach provides similar results to the first two methods, but it is invalid when the energy conservation breaks down.

The effective moment method [85] includes the dipole and multipole moment approximations. In the dipole approximation, it is assumed that the particle is so small, compared to the characteristic length of applied electric field, that only a dipole is induced on it [88]. The DEP force $\mathbf{F}_{dep}$ acting on the particle suspending in a dielectric medium can be thus approximated as [88],

$$\mathbf{F}_{dep} \approx (\mathbf{p} \cdot \nabla) \mathbf{E} \quad (2.1)$$

where $\mathbf{E}$ is the applied electric field strength, and $\mathbf{p}$ is the effective dipole moment. For a spherical particle, the effective dipole moment is given by [89, 90]

$$\mathbf{p} = 4\pi\tilde{\xi}_m \text{Re}(K_{CM})R^3\mathbf{E} \quad (2.2)$$

where $\tilde{\xi}_m$ is the permittivity of suspending medium, $\text{Re}(\cdots)$ refers to the real part of a complex number, and R is the particle radius. The term K_{CM} is the well-known Clausius-Mossotti (CM) factor, as a measure of the effective polarizability of spherical particle,

$$K_{CM} = \frac{\tilde{\xi}_c - \tilde{\xi}_m}{\tilde{\xi}_c + 2\tilde{\xi}_m} \quad (2.3)$$

where $\tilde{\xi}_c$ and $\tilde{\xi}_m$ are the complex permittivities of spherical particle and suspending medium, respectively. Therefore, the DEP force acting on a spherical particle is expressed by the dipole moment method as [89, 90]

$$\mathbf{F}_{dep} = 2\pi R^3 \tilde{\xi}_m \text{Re}[K_{CM}] \cdot \nabla |\mathbf{E}|^2 \quad (2.4)$$

According to the above formula, it is concluded that the DEP force is a function of many parameters, including the particle size, the permittivities and conductivities of particle and suspending medium, the strength and frequency of electric field. In addition, the following conclusions can be drawn straightforwardly.

- The DEP force is exerted on a particle only when it is exposed to the nonuniform electric field ($\nabla|\mathbf{E}|^2 \neq 0$), and the particle should be relatively polarizable ($\text{Re}[K_{CM}] \neq 0$).
- The direction of DEP force is independent of the polarity of applied electric field ($|\mathbf{E}|^2 = |-\mathbf{E}|^2$), totally determined by the CM factor.
- The DEP force attracts particles to the region of strong electric field strength when $\text{Re}[K_{CM}] > 0$ (called the positive DEP or p-DEP), whereas it repels particles away from that region when $\text{Re}[K_{CM}] < 0$ (called the negative DEP or n-DEP).

One can see that the CM factor plays an important role in the DEP phenomenon, which indicates the effects of the dielectric properties of particles and suspending medium, and the electric frequency. Firstly, the real part of CM factor only varies between -0.5 to 1.0 , as plotted in Fig. 2.6, meaning that the p-DEP force can be twice as strong as the n-DEP force. Secondly, the CM factor takes the limits at the very low and high frequencies. For a spherical particle [91], the CM factor reduces from Eq. (2.3) to

$$\lim_{\omega \rightarrow 0} K_{CM}(\omega) = \frac{\sigma_c - \sigma_m}{\sigma_c + 2\sigma_m} \quad (2.5)$$

$$\lim_{\omega \rightarrow \infty} K_{CM}(\omega) = \frac{\xi_c - \xi_m}{\xi_c + 2\xi_m} \quad (2.6)$$

where ξ_c and σ_c are the permittivity and conductivity of particle, ξ_m and σ_m are the permittivity and conductivity of suspending medium, and ω is the angular frequency of electric field. Thirdly, the CM factor indicates the competition between the complex permittivities of particle and suspending medium. The suspending medium is usually treated as the homogeneous dielectric with the constant permittivity and conductivity, such that its complex permittivity is written as

$$\tilde{\xi}_m = \xi_m + \frac{\sigma_m}{i\omega} \quad (2.7)$$

where i is the imaginary number. However, there are several types of the complex permittivity of particles. For a homogeneous particle, such as plastic microspheres, its complex permittivity is easily expressed as

$$\tilde{\xi}_c = \xi_c + \frac{\sigma_c}{i\omega} \quad (2.8)$$

For a biological cell, the dielectric behavior is more complex than that of a homogeneous particle due to the existence of membrane [91]. Generally, the single shell model [92-95] is used to characterize the cell dielectric property, where the cell is modeled as a homogeneous interior enclosed by an insulated membrane. The complex permittivity of a spherical biological cell is thus approximated as [96, 97]

$$\tilde{\xi}_c = \frac{R\tilde{\xi}_{in}\tilde{C}_{mem}}{\tilde{\xi}_{in} + R\tilde{C}_{mem}} \quad (2.9)$$

under the condition that the membrane thickness is much less than the cell radius R , where $\tilde{\xi}_{in}$ and $\tilde{C}_{mem}$ are the complex permittivity of homogeneous cell interior and the complex capacitance of cell membrane,

$$\tilde{\xi}_{in} = \xi_{in} - i\sigma_{in}/\omega, \quad \tilde{C}_{mem} = C_{mem} - iG_{mem}/\omega \quad (2.10)$$

in which ξ_{in} and σ_{in} are the permittivity and conductivity of homogeneous cell interior, and C_{mem} and G_{mem} are the capacitance and conductance of cell membrane. Besides, the two-shell model [91, 95] was sometimes applied to characterize the complex permittivity of plant cells and unicells due to the presence of cell wall and membrane. Fig. 2.6 plots the real part of CM factor for the homogeneous and single-shell particles, respectively. It was shown that the single-shell particle is more complicated than the homogeneous one, and more accurate to describe the dielectric behavior of biological cells [92-95].

Although the dipole moment approximation can provide good result in some cases, its accuracy becomes worse and worse as the particle size increases [98]. This is because the high-order moments become important for the large particle except the dipole moment. In order to overcome this disadvantage, the multipole moment method with consideration of the high-order moments was first proposed by Washizu [99], and

Washizu and Jones [100, 101]. Subsequently, Schnelle et al. [98] investigated the influence of high-order moments in detail, and pointed out that the multipole approximation is more applicable for large dielectric particles. However, the multipole approximation is still inclined to treat the rigid particles so far [102-104], and very difficult to handle the deformable vesicles [105].

Fig. 2.6 Real part of the CM factor for the homogeneous and single-shell particles suspending in a medium with the permittivity $\xi_m = 80\xi_0$ and conductivity $\sigma_m = 0.005$ S/m. The homogeneous particle has the dielectric properties $\xi_c = 57\xi_0$ and $\sigma_m = 0.52$ S/m, and the single-shell particle representing a spherical RBC [106] has the dielectric properties $C_{mem} = 11.8$ mF/m², $G_{mem} = 271$ S/m², $\xi_{in} = 57 \xi_0$, $\sigma_{in} = 0.52$ S/m, and $R = 3.91$ μ m.

The Maxwell stress tensor (MST) approach is currently regarded as the most general and rigorous approach for deriving the DEP force, in which an integration of the MST $\hat{T}$ is required over the particle surface,

$$\mathbf{F}_{dep} = \int_{\Gamma} \hat{T} \cdot \mathbf{n} ds \quad (2.11)$$

where $\mathbf{n}$ is the normal direction of the particle surface Γ . Based on this method, Wang et al. [86] derived the general expression for the DEP force acting on particles with arbitrary configurations, and also demonstrated that the MST method can provide the identical expression with the effective moment method for a homogenous spherical

particle. In order to evaluate the MST method, Benselama et al. [107] compared with the dipole moment approximation using the finite element method (FEM), and a good agreement was found when the particle size is very small. Subsequently, Rosales and Lim [105] further carried out numerical comparisons with the multipole approximation, and pointed out that the MST approach has the great potential for treating deformable cells because no assumption is required for the cell shape and applied electric field. Since only a surface integration is needed, the MST approach is straightforwardly applied in numerical simulations of DEP phenomena, such as the boundary element method (BEM) [108]. Nevertheless, this method is still not widely used so far due to the mathematical complexity [86].

The energy method is based on the law of energy conservation before and after putting a particle into the suspending medium. Using this method, Schwarz [87] first provided a general expression of DEP force acting on a particle in an AC electric field. Subsequently, Sher [109], and Pohl et al. [110] applied the Schwarz's results to the spherical and cylindrical particles, and provided satisfactory results if the particle and suspending medium are assumed to be perfect loss-free dielectrics. However, if the dielectric loss becomes dominant, the energy method breaks down because the energy conservation cannot hold [110]. Consequently, the energy method is not as common as the effective moment and MST methods at present.

2.2.2.2 Application of dielectrophoresis

The DEP is mainly applied to the particle manipulation [60], such as separation, trapping, sorting and transportation of various particles including latex beads, biological cells, virus, DNA molecules, etc. Based on the different types of applied electric field, the DEP techniques used in the particle manipulation are broadly categorized into four classes [58], namely the conventional DEP (cDEP), traveling wave DEP (twDEP), moving DEP (mDEP), and insulator-based DEP (iDEP).

The cDEP technique [88] is based on different types of particles exhibiting distinct behavior ranging from the p-DEP to n-DEP in a conventional nonuniform electric field, such that the p-DEP particles move towards the electrodes but the n-DEP away from the electrodes. Based on this technique, some pioneering work [111, 112] concentrated on the separation of living and dead yeast cells using a simple wire-wire electrode chamber, but the separation efficiency was quite poor. In order to improve the

separation efficiency, various complex electrode systems emerged after 1990s, such as the castellated electrodes array [113-120], and planar interdigitated electrodes array [121-125], etc. Meanwhile, the cDEP technique is also applied to particle trapping [126-130], patterning [131-133], positioning [134, 135], and so on. Fig. 2.7 shows the different kinds of electrodes for particle separation, trapping and patterning.

Fig. 2.7 Different types of the electrode arrays, (a) the castellated electrode array for particle separation [115], (b) the planar interdigitated array for particle separation [122], (c) the quadruple electrodes for particle trapping [126], and (d) the planar interdigitated ring electrode array for particle patterning [131].

The twDEP technique was developed by Huang et al. [136], in which particles undergo the translational motion by following the electrode track in a travelling electric field, as illustrated in Fig. 2.8. Due to the presence of travelling electric field, a dipole is induced across the particle, and moves with the field peak. If the travelling wave field moves sufficiently fast, the dipole lags behind the field. Consequently, a net force is induced on the particle, and drives it travelling along the electrodes [58]. Unlike the cDEP, the twDEP doesn't require to pump fluid for delivering particles. As a pioneering

work, Huang et al. [136] studied the electrokinetic behavior of yeast cells in a travelling electric field, and derived a formulation of twDEP using the dipole moment approximation. To date, the twDEP technique has been developed further in the theoretical [137, 138] and numerical [139-142] aspects, and widely used for the particle separation [143-147], and transportation [148, 149] by fabricating various electrode systems.

Fig. 2.8 Schematic illustration of the twDEP, where a particle is levitated above the electrodes by the n-DEP, and further driven by a translational force.

The mDEP technique [150], combining the cDEP and twDEP, was developed to separate and transport cells in recent years. This technique is generated by sequentially energizing a single electrode or an array of electrodes to form a dynamic electric field that drives particles moving continuously along the electrodes. The particle fractionation is carried out by controlling the electric field frequency, while the particle transportation is achieved by sequentially energizing adjacent electrodes. At a certain electric frequency, the p-DEP particle moves toward the high electric field gradient region, but the n-DEP particle is repelled from that region, as shown in Fig. 2.9. Therefore, the particles are separated successfully, and move with the energized electrode. In the mDEP technique, there is no electric phase variation similar to the cDEP, and the particle travels along the electrodes without fluid flow similar to the twDEP [150]. Kua et al. studied the mDEP in detail, not only developing the theoretical model of mDEP [151], but also applying the mDEP technique to separate the biological cells experimentally [150, 152]. Besides, Alazzam et al. [153] further used the Fourier series to formulate the electric field distribution and the mDEP force.

Fig. 2.9 Schematic illustration of the mDEP [150], in which the n-DEP particle (hatched sphere) is always attracted towards the electrode edge, whereas the p-DEP particle (filled sphere) is repelled away from electrode edge.

The iDEP technique, also called electrodeless DEP, was initially proposed by Cummings and Singh [154]. In this technique, the nonuniform electric field is generated by building some special insulating structures in an applied DC electric field, instead of an AC electric field used in the cDEP, twDEP and mDEP techniques. As a result, the electrodes may be located remotely so as to avoid generating the fouling and gas bubble on them [58]. Currently, two structures, known as the posts [154-161] and hurdle [162-166], are most widely used to separate particles, as shown in Fig. 2.10. In the iDEP technique, the EP effect is worth paying attention to because the DC electric field is employed. It has been mentioned that the EP effect is usually neglected in the most studies of DEP when the particle charge is small and the AC electric field is applied. However, if the particle is highly charged and subjected to a DC electric field, the EP effect should be considered. Based on this understanding, several studies [154, 155,

159-161, 167, 168] were done on the combination of EP and DEP in order to more accurately explain the particle behavior in the DC electric field.

Fig. 2.10 Two typical insulating structures in the iDEP, (a) the posts for virus trapping [156], and (b) hurdle for cell separation [164].

To sum up, a great deal of work has currently been done on the DEP application. However, most of efforts focused on the manipulation of rigid particles whichever the DEP technique was used. Only few studies have so far attempted to characterize the behavior of deformable vesicles in a nonuniform electric field. For example, Bjørklund [169] examined the deformation behavior of a droplet in a nonuniform electric field with considering the surface tension only. Le et al. [108] investigated the trapping of deformable cells using the cDEP technique, based on the assumption of neglecting the bending deformation of cells. A comprehensive knowledge is still poor in the DEP application for the deformable vesicles. One possible reason lies in the fact that the chip designed in the previous studies is significantly larger than the manipulated particles in size. The other reason may be attributed to the difficulty of calculating DEP force acting on the deformable vesicles.

2.3 Cells in hydrodynamic-electric coupled field

The majority of previous studies on cells in the hydrodynamic-electric coupled field focused on the electrical field-flow fractionation (ElFFF) [170], an important continuous separation technique in microfluidic devices. In the field-flow fractionation (FFF), a field is applied to drive samples differing in mass, size, or other physical properties, into different velocity regions usually provided by a parabolic hydrodynamic flow, as illustrated in Fig. 2.11. As a result, the samples are carried through the channel at different velocities, and exit the channel after different retention times [171, 172]. In

ElFFF, the electric field is applied to drive the samples, and two kinds were developed, EP FFF [173, 174] and DEP FFF [175]. However, currently the DEP FFF has attracted more attention than the EP FFF due to the fact that the DEP FFF employs the low voltage, and also works on neutral samples [58]. The widely-used DEP FFF in bioengineering applications can be broadly classified into the conventional DEP FFF, and lateral DEP FFF according to the different layouts of electrode array. The fundamental difference between them lies in whether the sedimentation force is used to separate the samples. Compared with the lateral DEP FFF, the conventional DEP FFF employs the sedimentation force to determine the motion trends of samples except the DEP force and drag force of flow.

Fig. 2.11 Schematic illustration of the FFF, where the field is usually perpendicular to the hydrodynamic flow.

2.3.1 Conventional dielectrophoretic field-flow fractionation

In the conventional DEP FFF [176], the electrode array is placed at the bottom or top wall of microchannel, providing a nonuniform electric field along the height direction, and therefore a vertical DEP force is formed, as shown in Fig. 2.12. Subject to the vertical DEP force, particles are either attracted down or lifted up in the microchannel, and thus levitated at different balanced heights under the influence of the DEP force, drag force of hydrodynamic flow, and sedimentation force [176]. Within a parabolic hydrodynamic flow, the particles at the different heights are carried through the microchannel at the different velocities, and therefore can be separated and collected at the different time. However, since the electrical field decays exponentially with the distance away from the electrodes in the conventional DEP FFF, particles far away from the electrodes will be less controllable [177]. Besides, the conventional DEP FFF usually requires particles to exhibit the n-DEP force for balancing the downward

sedimentation force, such that it is limited to separate n-DEP particles only [58]. Nevertheless, using this technique a lot of work was still conducted on the separation of various particles, such as biological cells [175, 178-182], nanotube [183], microbeads [123, 184, 185], etc., which mainly lies in its advantage of the continuous manipulation.

Fig. 2.12 Schematic illustration of the conventional DEP FFF, (a) the 3D configuration, and (b) 2D configuration (front view of the 3D configuration). The parabolic hydrodynamic flow is applied through the microchannel, and the electrode array is placed at the microchannel bottom. Different types of cells are levitated at different equilibrium heights, and move out from the top or bottom of microchannel subject to the DEP force F_{dep} , drag force of flow F_{drag} and sedimentation force F_{sed} .

2.3.2 Lateral dielectrophoretic field-flow fractionation

Different from the conventional DEP FFF, the electrode array is vertically placed at the back or front wall of microchannel in the lateral DEP FFF [177], which generates a lateral nonuniform electric field, and thus a DEP force is formed along the width direction of microchannel, as shown in Fig. 2.13. When particles exhibit the p-DEP, they are attracted to the electrodes, i.e., the back wall of microchannel. In contrast, the particles exhibiting the n-DEP are repelled away from the electrodes to the front wall of microchannel. Since most of microfluidic devices are 2D microchannel networks, the lateral DEP FFF technique is more compatible with other on-chip analysis for continuous manipulation and parallel processing of particles [177]. Currently, the lateral

DEP FFF is attracting increasing interest, and several studies [186-190] have been performed on the separation and focusing of microparticles. More importantly, it has been applied into some microfluidic devices with multiple outputs to collect the separated particles, such as the double-output [191], three-output [192, 193], and five-output [177] devices.

Fig. 2.13 Schematic illustration of the lateral DEP FFF, (a) the 3D configuration, and (b) 2D configuration (bottom view of the 3D configuration). The parabolic hydrodynamic flow is applied through the microchannel, and the electrode array is placed at the back wall of microchannel. Different types of cells exhibit either the n-DEP or p-DEP, and move out from the front or back of microchannel subject to the DEP force F_{dep} and drag force of flow F_{drag} .

2.3.3 Remark

In summary, the previous studies on the DEP FFF, including the conventional DEP FFF and lateral DEP FFF, are not sufficient to comprehend the behavior of cells in the hydrodynamic-electric coupled field. Several deficiencies are revealed in the previous studies, described as follows.

- The deformation behavior of cells is usually not concerned, because the microchannel is significantly wider or higher than cells.

- The DEP force acting on a cell is usually calculated by the dipole moment approximation, because the cell deformation is not considered.
- The cell motion is usually decoupled from the hydrodynamic flow because the cell is very small in size. The cell motion is often governed by the Newton's second law, and has no effect on the fluid flow. Therefore, the flow properties are treated to be continuous, such that the discontinuity across the interface between the cell and hydrodynamic flow is not considered.

Chapter 3

Model Formulation

The objective of this chapter is to present a two-fluid model for analysis of the motion and deformation of cells in the hydrodynamic-electric coupled field. The 3D physical system is described first, and then simplified into a 2D problem. After that, a two-fluid model is presented to characterize the 2D problem, incorporating the governing equations of fluid and electric fields, the interaction between the cell and fluid field, the interaction between the cell and electric field, and the intercellular interaction. In the two-fluid model, the fluid field is governed by the Navier-Stokes equations, and the electric field by Maxwell's equations. The three interactions are identified by the membrane mechanical force, dielectrophoresis force and intercellular interaction force, respectively. Finally, the two-fluid model is nondimensionlized for computational convenience and error minimization.

3.1 Problem statement

The schematic diagram of 3D physical system is shown in Fig. 2.1(a), in which a fluid flow passes through the microchannel, and electrodes are laterally positioned as a periodic layout on the back wall to generate a lateral nonuniform electric field. The lateral positioning currently attracts increasing interest in microdevice design, because it is easily compatible with other devices [177]. In general, the electrode length H is significantly larger than the microchannel width W , such that the 3D system can be simplified into a 2D problem. Furthermore, in view of the periodicity of electrode

layout, the electrodes are considered only in a period with the electrode width d_1 and electrode gap d_2 in the 2D problem, as illustrated in Fig. 2.1(b).

Fig. 3.1 (a) Schematic diagram of the 3D system, where the fluid flow passes through the microchannel, and the electrodes are laterally placed as a periodic layout on the back wall. (b) Schematic diagram of the 2D computational domain for two cells in the microchannel, extracted from the bottom view of 3D system.

The simplified 2D problem consists of a fluid field, a nonuniform electric field, and two cells. In order to formulate the 2D problem, four components are necessarily required to be addressed in detail, as follows.

- Distributions of fluid and electric fields. Because there are differences of fluidic and dielectric properties between the cell and suspending fluid, the fluid and electric fields vary with the position and shape of cells. In turn, the distributions of fluid and electric fields determine the motion and deformation of cells. Therefore, governing equations are required for describing both the fluid and electric fields.
- Interaction between the cell and fluid field. A cell subject to the fluid flow moves and deforms, while its membrane in turn develops a mechanical force to resist the deformation resulting from the fluid flow. This implies that the membrane mechanical force can identify the interaction between the cell and fluid field.

- Interaction between the cell and electric field. When a cell is placed in a nonuniform electric field, it will experience a dielectrophoresis (DEP) force due to the polarization. The DEP force depends on the shape and size of cell, and also induces the motion and deformation of cell. Therefore, it associates the cell with electric field, and can describe the interaction between them.
- Interaction between the two cells. It was well known that there is an interaction between the two cells [26, 27]. They exhibit a weak attraction at far distance, but strong repulsive at near distance. This interaction can be characterized by a force, termed the intercellular interaction force.

The following subsections in this chapter will concentrate on the formulation of these four components one by one.

3.2 Governing equations

3.2.1 Fluid field

At the microscale level, it is generally accepted that the cells and fluid behave as incompressible Newtonian fluids on the assumption of the shear rate above 100 s^{-1} [194]. In the present work, the shear rate is about 250 s^{-1} . Therefore, the incompressible Navier-Stokes (N-S) equations are used to govern the cell motion and fluid flow under the assumption of neglecting the heat effect. The governing equations in the domain of either cells or fluid, including the continuity and momentum equations, can be expressed in the integral form [195],

$$\int_{\Omega_h} \nabla \cdot \mathbf{u}_h d\Omega = 0 \quad (3.1)$$

$$\int_{\Omega_h} \left(\frac{\partial \rho_h \mathbf{u}_h}{\partial t} + \nabla \cdot (\rho_h \mathbf{u}_h \mathbf{u}_h) \right) d\Omega = \int_{\partial\Omega_h} \hat{H}_h \cdot \mathbf{n}_h ds + \int_{\partial\Omega_h} \hat{T}_h \cdot \mathbf{n}_h ds \quad (3.2)$$

where the subscript h stands for either the cell (taking c) or fluid medium (taking m), $\mathbf{u}_h$ is the velocity vector, ρ_h is the density, t is the time, Ω_h is the integration domain, $\mathbf{n}_h$ is the unit normal direction of the domain surface $\partial\Omega_h$, and $\hat{H}_h$ and $\hat{T}_h$ are the hydrodynamic [195] and Maxwell stress tensors [86] given by

$$\hat{H}_h = -p_h \hat{I} + \mu_h (\nabla \mathbf{u}_h + \nabla \mathbf{u}_h^T) \quad (3.3)$$

$$\hat{T}_h = \xi_h \left(\mathbf{E}_h \mathbf{E}_h - \frac{1}{2} |\mathbf{E}_h|^2 \hat{I} \right) \quad (3.4)$$

where p_h and μ_h are the pressure and viscosity, ξ_h is the permittivity, $\mathbf{E}_h$ is the electric field strength, and $\hat{I}$ is the unit tensor.

Considering an arbitrary domain Ω containing a portion of cell and fluid, the cell membrane Γ divides it into the two subdomains Ω_c and Ω_m for the cell and fluid, respectively. By defining

$$\begin{aligned} \mathbf{u} &= \begin{cases} \mathbf{u}_c & \mathbf{x} \in \Omega_c \\ \mathbf{u}_m & \mathbf{x} \in \Omega_m \end{cases} & \rho &= \begin{cases} \rho_c & \mathbf{x} \in \Omega_c \\ \rho_m & \mathbf{x} \in \Omega_m \end{cases} \\ \hat{H} &= \begin{cases} \hat{H}_c & \mathbf{x} \in \Omega_c \\ \hat{H}_m & \mathbf{x} \in \Omega_m \end{cases} & \hat{T} &= \begin{cases} \hat{T}_c & \mathbf{x} \in \Omega_c \\ \hat{T}_m & \mathbf{x} \in \Omega_m \end{cases} \end{aligned} \quad (3.5)$$

the governing equations in the arbitrary domain Ω are expressed as

$$\int_{\Omega} \nabla \cdot \mathbf{u} d\Omega = 0 \quad (3.6)$$

$$\begin{aligned} \int_{\Omega} \left(\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) \right) d\Omega &= \int_{\partial\Omega} \hat{H} \cdot \mathbf{n} ds - \int_{\Gamma} \hat{H}_c \cdot \mathbf{n}_c ds - \int_{\Gamma} \hat{H}_m \cdot \mathbf{n}_m ds \\ &+ \int_{\partial\Omega} \hat{T} \cdot \mathbf{n} ds - \int_{\Gamma} \hat{T}_c \cdot \mathbf{n}_c ds - \int_{\Gamma} \hat{T}_m \cdot \mathbf{n}_m ds \end{aligned} \quad (3.7)$$

where $\mathbf{x}$ is the spatial vector. Applying the divergence theorem, the surface integral of hydrodynamic stress tensor (HST) in Eq. (3.7) is converted as

$$\int_{\partial\Omega} \hat{H} \cdot \mathbf{n} ds = \int_{\Omega} \nabla \cdot \hat{H} d\Omega \quad (3.8)$$

Moreover, the surface integral of Maxwell stress tensor (MST) in Eq. (3.7) is cancelled out, when the permittivities of cell and fluid are assumed constant and there is no free charge induced by the electric field,

$$\int_{\partial\Omega} \hat{T} \cdot \mathbf{n} ds = 0 \quad (3.9)$$

Considering $\mathbf{n} = \mathbf{n}_c = -\mathbf{n}_m$ on the cell membrane results in

$$\begin{aligned} -\int_{\Gamma} \hat{H}_c \cdot \mathbf{n}_c ds - \int_{\Gamma} \hat{H}_m \cdot \mathbf{n}_m ds &= \int_{\Gamma} (\hat{H}_m - \hat{H}_c) \cdot \mathbf{n} ds \\ -\int_{\Gamma} \hat{T}_c \cdot \mathbf{n}_c ds - \int_{\Gamma} \hat{T}_m \cdot \mathbf{n}_m ds &= \int_{\Gamma} (\hat{T}_m - \hat{T}_c) \cdot \mathbf{n} ds \end{aligned} \quad (3.10)$$

Along the cell membrane, the jump of HST is balanced with the membrane mechanical force strength $\mathbf{f}_{mem}$ and intercellular interaction force strength $\mathbf{f}_{int}$,

$$(\hat{H}_m - \hat{H}_c) \cdot \mathbf{n} = \mathbf{f}_{mem} + \mathbf{f}_{int} \quad (3.11)$$

However, the jump of MST is balanced with the DEP force strength $\mathbf{f}_{dep}$,

$$(\hat{T}_m - \hat{T}_c) \cdot \mathbf{n} = \mathbf{f}_{dep} \quad (3.12)$$

Combining Eqs. (3.8) – (3.12) together, the momentum equation (3.7) is simplified as

$$\int_{\Omega} \left(\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) \right) d\Omega = \int_{\Omega} \nabla \cdot \hat{H} d\Omega + \int_{\Gamma} \mathbf{f}_{tot} ds \quad (3.13)$$

where $\mathbf{f}_{tot}$ is the total force strength acting on the cell membrane only,

$$\mathbf{f}_{tot} = \mathbf{f}_{mem} + \mathbf{f}_{dep} + \mathbf{f}_{int} \quad (3.14)$$

Now we continue to convert the last surface integral in Eq. (3.13) into the volume integral by introducing a Dirac delta function δ [196, 197],

$$\int_{\Gamma} \mathbf{f}_{tot} ds = \int_{\Omega} \int_{\Gamma} \mathbf{f}_{tot} \delta(\mathbf{x} - \mathbf{x}_{mem}) ds d\Omega \quad (3.15)$$

where $\mathbf{x}_{mem}$ is the location of cell membrane, and the Dirac delta function δ is given by

$$\delta(\mathbf{x} - \mathbf{x}_{mem}) = \begin{cases} \infty & \mathbf{x} = \mathbf{x}_{mem} \\ 0 & \mathbf{x} \neq \mathbf{x}_{mem} \end{cases} \quad (3.16)$$

Therefore, the final integral form of momentum equation in (3.13) is rewritten as

$$\int_{\Omega} \left(\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) \right) d\Omega = \int_{\Omega} \nabla \cdot \hat{H} d\Omega + \int_{\Omega} \int_{\Gamma} \mathbf{f}_{tot} \delta(\mathbf{x} - \mathbf{x}_{mem}) ds d\Omega \quad (3.17)$$

Since the integral domain Ω is arbitrary, the continuity equation is expressed in the differential form as

$$\nabla \cdot \mathbf{u} = 0 \quad (3.18)$$

and the momentum equation is expressed by substituting Eq. (3.3) into Eq. (3.17)

$$\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) = -\nabla p + \nabla \cdot \left(\mu (\nabla \mathbf{u} + \nabla \mathbf{u}^T) \right) + \int_{\Gamma} \mathbf{f}_{tot} \delta(\mathbf{x} - \mathbf{x}_{mem}) ds \quad (3.19)$$

in which $\mathbf{u}$ is the velocity, p is the pressure, $\mathbf{f}_{tot}$ is the total force strength in Eq. (3.14), and $\delta(\mathbf{x} - \mathbf{x}_{mem})$ is the Dirac delta function in Eq. (3.16). In addition, the density ρ and viscosity μ are rewritten by the Heaviside function $\Theta(\mathbf{x})$

$$\rho = \rho_c + (\rho_m - \rho_c) \Theta(\mathbf{x}) \quad (3.20)$$

$$\mu = \mu_c + (\mu_m - \mu_c) \Theta(\mathbf{x}) \quad (3.21)$$

where

$$\Theta(\mathbf{x}) = \begin{cases} 0 & \mathbf{x} \in \Omega_c \\ 1 & \mathbf{x} \in \Omega_m \end{cases} \quad (3.22)$$

The boundary conditions of the governing equations (3.18) and (3.19) for the fluid field are specified as no-slip condition on the microchannel wall, outflow condition at the outlet, and velocity condition at the inlet. A parabolic velocity field is applied at the inlet, given as

$$\begin{cases} u = 4.0 u_{\max} \frac{y}{W} \left(1 - \frac{y}{W} \right) \\ v = 0 \end{cases} \quad (3.23)$$

where $u_{\max}$ is the maximum velocity, W is the width of computational domain.

3.2.2 Electric field

Although the electric field doesn't directly appear in the governing equations (3.18) and (3.19) of fluid field, it determines the DEP force strength and then affects the distribution of fluid field indirectly. In order to calculate the DEP force strength, the

electric field generated by the electrode array in Fig. 2.1(b) needs to be solved in advance. In an AC electric field, the exponential notation is often adopted, for example, the electric field strength $\tilde{\mathbf{E}}(\mathbf{x}, t)$ with the frequency ω can be expressed as

$$\tilde{\mathbf{E}}(\mathbf{x}, t) = \tilde{\mathbf{E}}_0(\mathbf{x}) e^{i\omega t} \quad (3.24)$$

where $\tilde{\mathbf{E}}_0(\mathbf{x})$ is the complex modulus. The governing equation of electric field can be derived by Maxwell's equations with neglecting magnetic effects [86],

$$\nabla \cdot \tilde{\mathbf{D}}_h = \tilde{q}_h \quad (3.25)$$

$$\nabla \cdot \tilde{\mathbf{J}}_h + \frac{\partial \tilde{q}_h}{\partial t} = 0 \quad (3.26)$$

where the subscript h stands for either the cell (taking c) or fluid (taking m), $\tilde{\mathbf{D}}_h$ is the electric flux density, $\tilde{\mathbf{J}}_h$ is the current density, and $\tilde{q}_h$ is the free charge density. Considering

$$\begin{aligned} \tilde{\mathbf{D}}_h &= \xi_h \tilde{\mathbf{E}}_h \\ \tilde{\mathbf{J}}_h &= \sigma_h \tilde{\mathbf{E}}_h \end{aligned} \quad (3.27)$$

and cancelling out $\tilde{q}_h$ in Eqs. (3.25) and (3.26), we have

$$\nabla \cdot (\sigma_h \tilde{\mathbf{E}}_h) + \frac{\partial}{\partial t} \nabla \cdot (\xi_h \tilde{\mathbf{E}}_h) = 0 \quad (3.28)$$

where ξ_h and σ_h are the permittivity and conductivity, and $\tilde{\mathbf{E}}_h$ is the electric field phasor. Using the relation

$$\tilde{\mathbf{E}}_h = \nabla \tilde{\varphi}_h \quad (3.29)$$

and assuming that the permittivity and conductivity are independent of time for both the cell and fluid, Eq. (3.28) is changed into

$$\nabla \cdot (\sigma_h + i\omega \xi_h) \nabla \tilde{\varphi}_h = 0 \quad (3.30)$$

where $\tilde{\varphi}_h$ is the electric potential. Dividing Eq. (3.30) by $i\omega$ leads to the governing equation of electric field

$$\nabla \cdot (\tilde{\xi}_h \nabla \tilde{\varphi}_h) = 0 \quad (3.31)$$

in which $\tilde{\xi}_h$ is the complex permittivity. Eq. (3.31) can be expanded as

$$\begin{aligned} \nabla \cdot (\tilde{\xi}_c \nabla \tilde{\varphi}_c) &= 0 & \mathbf{x} \in \Omega_c \\ \nabla \cdot (\tilde{\xi}_m \nabla \tilde{\varphi}_m) &= 0 & \mathbf{x} \in \Omega_m \end{aligned} \quad (3.32)$$

where $\tilde{\varphi}_c$ and $\tilde{\varphi}_m$ are the electric potential inside the cell and fluid, respectively.

The boundary conditions applied to the governing equation (3.32) of electric field are given as follows,

$$\tilde{\varphi}_h|_{\Sigma} = V_0 \quad (3.33)$$

$$\tilde{\xi}_c \frac{\partial \tilde{\varphi}_c}{\partial \mathbf{n}} \Big|_{\Gamma} = \tilde{\xi}_m \frac{\partial \tilde{\varphi}_m}{\partial \mathbf{n}} \Big|_{\Gamma} \quad (3.34)$$

where the subscripts Σ and Γ denote the electrode surface and cell membrane, and V_0 is the given electrode potential.

3.3 Membrane mechanical force

When a cell in fluid flow moves and deforms subject to the shear stress, its membrane develops a mechanical force to resist the deformation. This mechanical force reflects the interaction between the cell and fluid, and is mainly attributed to the membrane structure that consists of a thin lipid bilayer and a protein skeleton. For deriving the membrane mechanical force, the classical shell model [198] is used here, where the cell membrane is treated as a curved two-dimensional shell with finite thickness, allowed to undergo stretching strain and bending deformation.

An infinitesimal segment of the membrane from s_0 to $s_0 + ds$ is taken as the analysis object, as illustrated in Fig. 3.2. The vectorial tension $\mathbf{T}(s)$ is expressed by the in-plane tension $\tau(s)$ and transverse tension $\eta(s)$ [198],

$$\mathbf{T}(s) = \tau(s)\mathbf{t} + \eta(s)\mathbf{n} \quad (3.35)$$

where $\mathbf{t}$ and $\mathbf{n}$ are the unit tangent and normal vectors along the direction of increasing the arc length s . Hence, the membrane mechanical force strength $\mathbf{f}_{mem}$ is given by

$$\mathbf{f}_{mem} = \frac{d\mathbf{T}}{ds} = \frac{d\tau}{ds}\mathbf{t} + \tau \frac{d\mathbf{t}}{ds} + \frac{d\eta}{ds}\mathbf{n} + \eta \frac{d\mathbf{n}}{ds} \quad (3.36)$$

Fig. 3.2 Schematic diagram of an infinitesimal segment of cell membrane from s_0 to s_0+ds for analyzing the membrane mechanical force.

Since the directional derivatives of unit tangent and normal vectors are given by

$$\frac{d\mathbf{t}}{ds} = -\kappa\mathbf{n}, \quad \frac{d\mathbf{n}}{ds} = \kappa\mathbf{t} \quad (3.37)$$

and the transverse tension is expressed by the moment equilibrium in the form of bending moment m as

$$\eta = \frac{dm}{ds} \quad (3.38)$$

the membrane mechanical force strength in Eq. (3.36) is rewritten as

$$\mathbf{f}_{mem} = \left(\frac{d\tau}{ds} + \kappa \frac{dm}{ds} \right) \mathbf{t} + \left(\frac{d^2m}{ds^2} - \tau\kappa \right) \mathbf{n} \quad (3.39)$$

where κ is the membrane curvature. In practice, the cell membrane behaves as a non-linear elastic and viscoelastic rubber-like material. However, it is assumed here for simplicity that constitutive relations are linear for the shear elasticity and bending deformation, given by [198]

$$\tau = E_s \varepsilon, \quad m = E_B (\kappa - \kappa_0) \quad (3.40)$$

Several previous studies have demonstrated that the linear constitutive relation can describe the main characteristic of cell deformation, and is quite convenient for engineering applications [38, 49, 199, 200]. Therefore, the membrane mechanical force strength is finally formulated as

$$\mathbf{f}_{mem} = \left(E_s \frac{d\varepsilon}{ds} + \kappa E_B \frac{d(\kappa - \kappa_0)}{ds} \right) \mathbf{t} + \left(E_B \frac{d^2(\kappa - \kappa_0)}{ds^2} - E_s \varepsilon \kappa \right) \mathbf{n} \quad (3.41)$$

where E_s and E_B are the elastic modulus and bending stiffness, ε and κ_0 are the tensile strain and resting curvature.

3.4 Dielectrophoresis force

When a cell is placed in a nonuniform electric field, it will experience a dielectrophoresis (DEP) force due to the polarization, and thus moves and deforms. In turn, the motion and deformation of cell influence the distribution of electric field. Therefore, the DEP force identifies the interaction between the cell and electric field. It is worth mentioning that electrophoresis (EP) is neglected in the present research work due to two facts. One is that the cell charge is significantly small, and the other is that the AC electric field damps out the EP. In order to express the DEP force, Maxwell stress tensor (MST) method, as the most general and rigorous approach, is employed due to no assumption made regarding the cell shape and electric field.

According to Eq. (3.12), the DEP force strength is expressed as

$$\mathbf{f}_{dep} = (\hat{T}_m - \hat{T}_c) \cdot \mathbf{n} \quad (3.42)$$

where $\hat{T}_m$ and $\hat{T}_c$ are the Maxwell stress tensors in the fluid and cell and given by Eq. (3.4). Rewriting the MST in Eq. (3.4) using the exponential notation results in [86]

$$\hat{T}_h = \frac{1}{4} \text{Re}(\tilde{\xi}_h) \left((\tilde{\mathbf{E}}_h \tilde{\mathbf{E}}_h^* + \tilde{\mathbf{E}}_h^* \tilde{\mathbf{E}}_h) - |\tilde{\mathbf{E}}_h|^2 \hat{\mathbf{I}} \right) \quad (3.43)$$

by considering

$$\mathbf{E}_h = \text{Re}(\tilde{\mathbf{E}}_h) = \frac{1}{2}(\tilde{\mathbf{E}}_h + \tilde{\mathbf{E}}_h^*) \quad (3.44)$$

and performing time averaging, where the subscript h stands for either the cell (taking c) or fluid (taking m), and the superscript $*$ means the complex conjugate. In order to simplify Eq. (3.42), the boundary conditions are used, which are imposed on the cell membrane to ensure the charge conservation and potential continuity [86],

$$\begin{aligned} \mathbf{n} \cdot (\tilde{\xi}_c \tilde{\mathbf{E}}_c - \tilde{\xi}_m \tilde{\mathbf{E}}_m) &= 0 \\ \mathbf{n} \times (\tilde{\mathbf{E}}_c - \tilde{\mathbf{E}}_m) &= 0 \end{aligned} \quad (3.45)$$

Consequently, the DEP force strength is expressed in the term of the polarized electric field $\tilde{\mathbf{E}}_c$ inside the cell as

$$\mathbf{f}_{dep} = \frac{1}{4} \text{Re}(\tilde{\xi}_m) \left(a a^* |\tilde{\mathbf{E}}_c \cdot \mathbf{n}|^2 + \text{Re}(a) |\tilde{\mathbf{E}}_c|^2 \right) \mathbf{n} \quad (3.46)$$

in which

$$a = \frac{\tilde{\xi}_c - \tilde{\xi}_m}{\tilde{\xi}_m} \quad (3.47)$$

Particularly, if the cell and fluid are assumed as pure dielectric materials, Eq. (3.46) can be simplified as

$$\mathbf{f}_{dep} = \frac{\xi_c - \xi_m}{2} \left(\frac{\xi_c}{\xi_m} E_{c,rms,n}^2 + E_{c,rms,t}^2 \right) \mathbf{n} \quad (3.48)$$

where $E_{c,rms,n}$ and $E_{c,rms,t}$ are the normal and tangential components of root-mean-square electric field $\tilde{\mathbf{E}}_c$. This formula is identical with the result of Goossens and van Biesen for pure dielectric materials [201]. In the present research work however, both the cells and suspending medium cannot be regarded as the pure dielectric material. The complex permittivity $\tilde{\xi}_m$ of suspending medium is homogeneous and determined by Eq. (2.7), whereas the cell complex permittivity $\tilde{\xi}_c$ is approximated by the single shell model in Eq. (2.9) due to the presence of cell membrane.

Moreover, if the cell is remarkably small than the characteristic length of electric field, there is an approximate formula [110] to express the polarized electric field $\tilde{\mathbf{E}}_c$ inside the cell by the externally applied electric field $\tilde{\mathbf{E}}_{ori}$,

$$\tilde{\mathbf{E}}_c = \frac{\tilde{\xi}_m}{\tilde{\xi}_m + \Lambda(\tilde{\xi}_c - \tilde{\xi}_m)} \tilde{\mathbf{E}}_{ori} \quad (3.49)$$

where Λ is called the depolarization factor, mainly determined by the cell shape. Hence, the DEP force strength in Eq. (3.46) acting on a very small cell can be approximated as

$$\mathbf{f}_{dep} = \frac{\text{Re}(\tilde{\xi}_m)}{4} \left| \frac{\tilde{\xi}_m}{\tilde{\xi}_m + \Lambda(\tilde{\xi}_c - \tilde{\xi}_m)} \right|^2 \left(aa^* |\tilde{\mathbf{E}}_{ori} \cdot \mathbf{n}|^2 + \text{Re}(a) |\tilde{\mathbf{E}}_{ori}|^2 \right) \mathbf{n} \quad (3.50)$$

This formula requires the externally applied electric field, instead of the polarized electric field. Therefore, it is unnecessary to calculate the polarized electric field inside the cell, which is a difficult task involved with the treatment of moving and deformable boundary. However, this formula cannot provide a good approximation of the DEP force strength acting on large cells. In the present research work, Eq. (3.50) is not applicable well because the cell size is not small enough. We have conducted a comparison between the approximate electric field in Eq. (3.49) and the numerical electric field obtained by solving governing equation (3.31), and found the relative error is about 10%.

3.5 Intercellular interaction force

At a certain small distance, the interaction between two cells becomes dominant, driving cells dispersion or aggregation. For analysis of this interaction, a Morse potential model [31] was employed, in which a Morse type potential function $\psi(r)$ is used to fit the interaction energy,

$$\psi(r) = D_e [e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)}] \quad (3.51)$$

where r_0 and D_e are the zero force length and intercellular interaction strength (also called surface energy), β is the scaling factor controlling the interaction decay behavior, and r is the distance between the two opposing planar elements, as shown in Fig. 3.3.

The intercellular interaction force strength $f_{p-p}(r)$ acting on a planar element is then given by

$$f_{p-p}(r) = -\frac{d\psi}{dr} \frac{\mathbf{r}}{r} = 2\beta D_e [e^{2\beta(r_0-r)} - e^{\beta(r_0-r)}] \frac{\mathbf{r}}{r} \quad (3.52)$$

where $\mathbf{r}$ is the distance vector with magnitude of r and directed towards the opposing planar element. For example, Fig. 3.4 plots the typical variation of intercellular interaction force strength $f_{p-p}(r)$ with respect to the intercellular distance r under the conditions of $D_e = 4.5 \text{ } \mu\text{J}/\text{m}^2$, $r_0 = 0.49 \text{ } \mu\text{m}$ and $\beta = 3.84 \text{ } \mu\text{m}^{-1}$. At $r < r_0$, the intercellular interaction force strength is positive and increases quickly with decreasing r . However, at $r > r_0$ it is negative and approaches to zero slowly with increasing r . In other words, the intercellular interaction force behaves as a strong repulsive force at near distance, but a weak attractive force at far distance. The Morse potential is adopted for the sake of simplicity and realistic physical description of the intercellular interaction, but it doesn't imply any intrinsic mechanism.

Fig. 3.3 Schematic representation of the two spherical cells, where the planar elements are generated by projecting the curved elements onto the plane normal to the line $\overline{c_1c_2}$.

Eq. (3.52) describes the interaction between the two planar elements, instead of the curved elements. For characterizing the interaction between two deformable cells, it should extend Eq. (3.52) to the curved elements. Bhattacharjee et al. [202] used the Derjaguin's approximation to develop the relationship between the planar element and curved element by introducing a term of element curvature, such that the intercellular interaction force strength between the two deformable cells is derived as

$$\mathbf{f}_{int}(r) = (\mathbf{n}_{c1} \cdot \mathbf{k}_{c1})(\mathbf{n}_{c2} \cdot \mathbf{k}_{c2}) \mathbf{f}_{p-p}(r) \quad (3.53)$$

where $\mathbf{f}_{p-p}(r)$ is given by Eq. (3.52), $\mathbf{n}_{c1}$ and $\mathbf{n}_{c2}$ are the outward unit vectors normal to the two curved elements, and $\mathbf{k}_{c1}$ and $\mathbf{k}_{c2}$ represent the unit vectors parallel to the line joining the centers of two cells and directed towards each other, as illustrated in Fig. 3.3.

Fig. 3.4 Typical variation of the intercellular interaction force strength $\mathbf{f}_{p-p}(r)$ with the plane-plane distance r under the conditions of $D_e = 4.5 \text{ } \mu\text{J}/\text{m}^2$, $r_0 = 0.49 \text{ } \mu\text{m}$ and $\beta = 3.84 \text{ } \mu\text{m}^{-1}$.

3.6 Nondimensionlization

For computational convenience and error minimization, the two-fluid model is nondimensionlized step by step as follows.

Step 1: Introduce the basic characteristic quantities. In the hydrodynamic field, usually there are four independent variables, namely the length, velocity, density and viscosity. Besides, the permittivity is often treated as an independent variable in the electric field. Therefore, five basic characteristic quantities are required to measure these five independent variables respectively, listed as the microchannel width W , maximum initial velocity $u_{\max}$, fluid density ρ_m , fluid viscosity μ_m , and vacuum permittivity ξ_0 .

Tab. 3.1 Characteristic quantities used in the model nondimensionlization.

Basic quantity	Symbol	Induced quantity	Symbol
Length	W	Time	t_{ch}
Velocity	$u_{\max}$	Pressure	p_{ch}
Density	ρ_m	Elastic modulus	$E_{S,ch}$
Viscosity	μ_m	Bending stiffness	$E_{B,ch}$
Permittivity	ξ_0	Electric potential	$\tilde{\varphi}_{ch}$
—	—	Surface energy	$D_{e,ch}$

Step 2: Derive the induced characteristic quantities. According to the basic characteristic quantities, the rest characteristic quantities are derived for measuring the time, pressure, elastic modulus, bending stiffness, electric potential and intercellular interaction strength (or surface energy),

$$\begin{aligned}
 t_{ch} &= \frac{W}{u_{\max}}, \quad p_{ch} = \rho_m u_{\max}^2, \quad E_{S,ch} = \rho_m u_{\max}^2 W, \\
 E_{B,ch} &= \rho_m u_{\max}^2 W^3, \quad \tilde{\varphi}_{ch} = \sqrt{\frac{\rho_m u_{\max}^2 W^2}{\xi_0}}, \quad D_{e,ch} = \rho_m u_{\max}^2 W
 \end{aligned} \tag{3.54}$$

For easy reference, all the characteristic quantities are summarized in Tab. 3.1.

Step 3: Define the dimensionless variables. Using the characteristic quantities to measure the physical variables results in the following dimensionless variables indicated by the underline,

$$\begin{aligned}
 \underline{x} &= \frac{x}{W}, \quad \underline{u} = \frac{u}{u_{\max}}, \quad \underline{\rho} = \frac{\rho}{\rho_m}, \quad \underline{\mu} = \frac{\mu}{\mu_m}, \quad \underline{\xi}_h = \frac{\tilde{\xi}_h}{\xi_0}, \quad \underline{t} = \frac{t}{t_{ch}} \\
 \underline{p} &= \frac{p}{p_{ch}}, \quad \underline{E}_S = \frac{E_S}{E_{S,ch}}, \quad \underline{E}_B = \frac{E_B}{E_{B,ch}}, \quad \underline{s} = \frac{s}{W}, \quad \underline{\kappa} = W\kappa, \\
 \underline{\tilde{\varphi}}_h &= \frac{\tilde{\varphi}_h}{\tilde{\varphi}_{ch}}, \quad \underline{\beta} = \beta W, \quad \underline{D}_e = \frac{D_e}{D_{e,ch}}, \quad \underline{r} = \frac{r}{W}, \quad \underline{r}_0 = \frac{r_0}{W}, \quad \underline{r} = \frac{r}{W}
 \end{aligned} \tag{3.55}$$

Step 4: Substitute the dimensionless variables into the two-fluid model. As a result, the two-fluid model is rewritten in the dimensionless form as follows.

- Incompressible Navier-Stokes equations:

$$\nabla \cdot \underline{\mathbf{u}} = 0 \quad (3.56)$$

$$\frac{\partial \underline{\rho \mathbf{u}}}{\partial t} + \nabla \cdot (\underline{\rho \mathbf{u} \mathbf{u}}) = -\nabla p + \frac{1}{Re} \nabla \cdot (\underline{\mu} (\nabla \underline{\mathbf{u}} + \nabla \underline{\mathbf{u}}^T)) + \int_{\Gamma} \underline{\mathbf{f}}_{tot} \delta(\underline{\mathbf{x}} - \underline{\mathbf{x}}_{mem}) d\underline{s} \quad (3.57)$$

The dimensionless total force strength $\underline{\mathbf{f}}_{tot}$ is given by

$$\underline{\mathbf{f}}_{tot} = \underline{\mathbf{f}}_{mem} + \underline{\mathbf{f}}_{dep} + \underline{\mathbf{f}}_{int} \quad (3.58)$$

and the Reynolds number Re is

$$Re = \frac{\rho_m u_{max} W}{\mu_m} \quad (3.59)$$

- Governing equation of electric field:

$$\nabla \cdot (\underline{\tilde{\epsilon}}_h \nabla \underline{\tilde{\varphi}}_h) = 0 \quad (3.60)$$

The boundary conditions is expressed in the dimensionless form as

$$\underline{\tilde{\varphi}}_h \Big|_{\Sigma} = \underline{V}_0 \quad (3.61)$$

$$\underline{\tilde{\epsilon}}_c \frac{\partial \underline{\tilde{\varphi}}_c}{\partial \underline{\mathbf{n}}} \Big|_{\Gamma} = \underline{\tilde{\epsilon}}_m \frac{\partial \underline{\tilde{\varphi}}_m}{\partial \underline{\mathbf{n}}} \Big|_{\Gamma} \quad (3.62)$$

where the dimensionless electrode potential is given as $\underline{V}_0 = V_0 / \tilde{\varphi}_{ch}$. The dimensionless electric field strength $\underline{\tilde{\mathbf{E}}}_h$ is then given by

$$\underline{\tilde{\mathbf{E}}}_h = \nabla \underline{\tilde{\varphi}}_h \quad (3.63)$$

- Membrane mechanical force strength:

$$\underline{\mathbf{f}}_{mem} = \left(\underline{E}_S \frac{d\varepsilon}{d\underline{s}} + \underline{\kappa} \underline{E}_B \frac{d(\underline{\kappa} - \underline{\kappa}_0)}{d\underline{s}} \right) \underline{\mathbf{t}} + \left(\underline{E}_B \frac{d^2(\underline{\kappa} - \underline{\kappa}_0)}{d\underline{s}^2} - \underline{E}_S \varepsilon \underline{\kappa} \right) \underline{\mathbf{n}} \quad (3.64)$$

- Dielectrophoresis force strength:

$$\underline{f}_{dep} = \frac{1}{4} \text{Re}(\underline{\tilde{\xi}}_m) \left(\underline{a} \underline{a}^* \left| \underline{\tilde{E}}_c \cdot \underline{n} \right|^2 + \text{Re}(\underline{a}) \left| \underline{\tilde{E}}_c \right|^2 \right) \underline{n} \quad (3.65)$$

The dimensionless electric field strength $\underline{\tilde{E}}_c$ inside the cell is given by Eq. (3.63), and the dimensionless coefficient $\underline{a}$ is expressed as

$$\underline{a} = \frac{\underline{\tilde{\xi}}_c - \underline{\tilde{\xi}}_m}{\underline{\tilde{\xi}}_m} \quad (3.66)$$

- Intercellular interaction force strength:

$$\underline{f}_{int} = 2(\underline{n}_{c1} \cdot \underline{k}_{c1})(\underline{n}_{c2} \cdot \underline{k}_{c2}) \underline{\beta} \underline{D}_e [e^{2\beta(\underline{r}_0 - \underline{r})} - e^{\beta(\underline{r}_0 - \underline{r})}] \frac{\underline{r}}{\underline{r}} \quad (3.67)$$

It should be noted that the underline for dimensionless variables in the above equations will be omitted for notational convenience in the following chapters.

Chapter 4

Numerical Implementation

This chapter focuses on the development of two novel numerical methods for efficiently solving the two-fluid model presented in Chapter 3, and they are the modified particle binary level set (MPBLS) method and modified semi-implicit method for pressure linked equations-revised (modified SIMPLER). The MPBLS method as a front tracking method is proposed to capture the cell membrane. Compared with the previous particle level set (PLS) approach, it is more accurate and less time-consuming, and more powerful for multiphase and interfacial flow applications with Lagrangian particles. In addition, the modified SIMPLER algorithm is developed to solve incompressible Navier-Stokes equations. Compared with the SIMPLER algorithm, it not only speeds up the convergence of momentum equations, but also satisfies the continuity equation with higher accuracy. Therefore, the modified SIMPLER algorithm is more applicable for the two-fluid system with very low Reynolds number. Before development of these two methods, the staggered grid system is introduced first to discretize the computational domain, and the different types of forces are then calculated, including the membrane mechanical force strength, dielectrophoresis force strength, intercellular interaction force strength, and total resultant force.

4.1 Grid system

There are two major types of grids used for solving Navier-Stokes equations, namely the collocated and staggered grid arrangements, as illustrated in Fig. 4.1. The collocated grid is simple, where both the scalars and vectors are stored at the same grid points. It

was found in this grid system that the pressure oscillation often appears when discretizing the pressure gradient in momentum equations, leading to the unphysical pressure predictions, such as the checker-board pressure field [203, 204]. In the staggered grid system however, the scalars are stored at the grid points but the vectors at the location offset from the grid points. As a result, the staggered grid system does not suffer from the pressure oscillation, but it also has two obvious disadvantages. Firstly, the scalars and vectors have their respective control volumes, resulting in complex coding. Secondly, the vectors at the grid points require to be interpolated, greatly increasing the computational cost. In general, the collocated grid system is frequently adopted in compressible flow, whereas the staggered grid system is used in incompressible flow. In the present research work, the staggered grid system is employed to avoid the pressure oscillation.

Fig. 4.1 Different types of the grid arrangements, (a) the collocated grid in which both scalars and vectors are stored at the same grid points, and (b) the staggered grid where scalars are stored at the grid points (circle) but vectors at the half-mesh points (triangle).

Based on the staggered grid system, the computational domain is discretized into uniform and regular meshes, as illustrated in Fig. 4.2. The rectangle with filled circles denotes the pressure boundary, and the rectangle with triangles represents the velocity boundary. The outermost dashed rectangle refers to a layer of the artificial boundary for easily handling the boundary conditions in simulations. Therefore, the total number of meshes is $(Ix+1) \times (Jy+1)$.

Fig. 4.2 Discretization of the computational domain using the staggered grid system, where the rectangle with circles refers to the pressure boundary, the rectangle with triangles to the velocity boundary, and the outmost dashed rectangle to the arbitrary boundary.

4.2 Numerical calculation of different types of forces

In the two-fluid model, three force strengths are included to characterize the interactions between the cell and fluid field, between the cell and electric field, and between the two cells, namely the membrane mechanical force strength f_{mem} , dielectrophoresis force strength f_{dep} , and intercellular interaction force strength f_{int} , respectively. These force strengths are superimposed into a total resultant force F_{tot} acting on the cell membrane only,

$$F_{tot} = \int_{\Gamma} (f_{mem} + f_{dep} + f_{int}) \delta(\mathbf{x} - \mathbf{x}_{mem}) ds \quad (4.1)$$

In order to numerically calculate the force strengths and resultant force, the cell membrane is discretized into small segments by scattering Lagrangian particles on it. If the number of Lagrangian particles is large enough, the cell membrane is approximately regarded to be connected by a large number of straight lines. It should be noted that these Lagrangian particles are also used to correct the position and shape of cell membrane, and to provide the curvature and normal direction of cell membrane.

4.2.1 Membrane mechanical force strength

The membrane mechanical force strength is listed again as

$$\mathbf{f}_{mem} = \left(E_s \frac{d\varepsilon}{ds} + \kappa E_B \frac{d(\kappa - \kappa_0)}{ds} \right) \mathbf{t} + \left(E_B \frac{d^2(\kappa - \kappa_0)}{ds^2} - E_s \varepsilon \kappa \right) \mathbf{n} \quad (4.2)$$

In order to calculate the membrane mechanical force strength at each Lagrangian particle, the strain, curvature and their derivatives should be provided in advance.

Taking the initial state of cell membrane as the reference state, the strain of the k -th segment is given by

$$\varepsilon_{k,s} = \frac{l_k - l_{R,k}}{l_{R,k}} \quad (4.3)$$

where l_k and $l_{R,k}$ are the lengths of the k -th segment at the current and reference states, having the form

$$l_k = \sqrt{(x_{k+1}^p - x_k^p)^2 + (y_{k+1}^p - y_k^p)^2} \quad (4.4)$$

where (x_k^p, y_k^p) and (x_{k+1}^p, y_{k+1}^p) are the locations of the k -th and $(k+1)$ -th particles at the current state. Hence, the strain at the k -th particle is expressed by averaging the strains of two neighboring segments,

$$\varepsilon_{k,p} = \frac{\varepsilon_{k,s} + \varepsilon_{k-1,s}}{2} = \frac{1}{2} \left(\frac{l_k}{l_{R,k}} + \frac{l_{k-1}}{l_{R,k-1}} \right) - 1 \quad (4.5)$$

Similarly, the derivative of the strain at the k -th particle is calculated as

$$\left. \frac{d\varepsilon}{ds} \right|_{k,p} = \frac{1}{2} \left(\left. \frac{d\varepsilon}{ds} \right|_{k,s} + \left. \frac{d\varepsilon}{ds} \right|_{k-1,s} \right) = \frac{1}{2} \left(\frac{\varepsilon_{k+1,p} - \varepsilon_{k,p}}{l_k} + \frac{\varepsilon_{k,p} - \varepsilon_{k-1,p}}{l_{k-1}} \right) \quad (4.6)$$

The curvature of cell membrane is defined as

$$\kappa = \nabla \cdot \mathbf{n} = \frac{\partial n_x}{\partial x} + \frac{\partial n_y}{\partial y} \quad (4.7)$$

where $\mathbf{n} = (n_x, n_y)$ is the outward normal direction of membrane. As shown in Fig. 4.3(a), directly discretizing Eq. (4.7), the curvature $\kappa_{k,p}$ at the k -th particle is expressed as

$$\begin{aligned}\kappa_{k,p} &= \frac{\frac{n_{x,k} - n_{x,k-1}}{\frac{x_{k+1}^p + x_k^p}{2} - \frac{x_k^p + x_{k-1}^p}{2} + e} + \frac{\frac{n_{y,k} - n_{y,k-1}}{\frac{y_{k+1}^p + y_k^p}{2} - \frac{y_k^p + y_{k-1}^p}{2} + e}}{2} \\ &= 2 \left(\frac{n_{x,k} - n_{x,k-1}}{x_{k+1}^p - x_{k-1}^p + e} + \frac{n_{y,k} - n_{y,k-1}}{y_{k+1}^p - y_{k-1}^p + e} \right)\end{aligned}\quad (4.8)$$

where a very small number e is introduced to avoid denominators becoming zero, usually taken the value of 1.0×10^{-10} , and $n_{x,k}$ and $n_{y,k}$ are the components of normal direction at the k -th segment, defined as

$$\begin{aligned}n_{x,k} &= \frac{y_{k+1}^p - y_k^p}{\sqrt{(x_{k+1}^p - x_k^p)^2 + (y_{k+1}^p - y_k^p)^2} + e} \\ n_{y,k} &= \frac{x_k^p - x_{k+1}^p}{\sqrt{(x_{k+1}^p - x_k^p)^2 + (y_{k+1}^p - y_k^p)^2} + e}\end{aligned}\quad (4.9)$$

Fig. 4.3 Schematic diagram for the calculation of curvature at the k -th Lagrangian particle from the normal direction using the weighted technique, (a) $n_{x,k+1} \neq n_{x,k-1}$ and $n_{y,k+1} \neq n_{y,k-1}$, (b) $n_{x,k+1} \approx n_{x,k-1}$ and $n_{y,k+1} \neq n_{y,k-1}$, and (c) $n_{x,k+1} \neq n_{x,k-1}$ and $n_{y,k+1} \approx n_{y,k-1}$.

However, Eq. (4.8) is not accurate for the curvature in the cases of $x_{k+1}^p \approx x_{k-1}^p$ and $y_{k+1}^p \approx y_{k-1}^p$, as illustrated in Fig. 4.3(b) and (c), where the denominator is so small that the curvature is quite larger than the exact value. A weighted technique is applied for improving the accuracy, and therefore the curvature is finally derived as

$$\kappa_{k,p} = 2 \left(w_1 \frac{n_{x,k} - n_{x,k-1}}{x_{k+1}^p - x_{k-1}^p + e} + w_2 \frac{n_{y,k} - n_{y,k-1}}{y_{k+1}^p - y_{k-1}^p + e} \right) \quad (4.10)$$

where w_1 and w_2 are the weights reflecting the closeness of two neighboring normal directions, defined as

$$w_1 = \frac{b_1}{b_1 + b_2}, \quad w_2 = \frac{b_2}{b_1 + b_2} \quad (4.11)$$

in which

$$\begin{aligned} b_1 &= \left| (n_{x,k} - n_{x,k-1})(x_{k+1}^p - x_{k-1}^p) \right| \\ b_2 &= \left| (n_{y,k} - n_{y,k-1})(y_{k+1}^p - y_{k-1}^p) \right| \end{aligned} \quad (4.12)$$

Fig. 4.4 Comparisons among the exact curvature (circles), numerical curvature with weights (solid line), and numerical curvature without weights (dashed line) for (a) the biconcave shape with the maximum radius of 3.91, and (b) circular shape with the radius of 0.5.

For validation of the curvature formula in Eq. (4.10), Fig. 4.4 shows comparisons between the exact and numerical curvatures for a biconcave shape and a circular shape. It is demonstrated that the curvatures with the weights are in good agreement with the exact results, while the curvatures without the weights are about twice larger than the exact values. Furthermore, the first-order derivative of curvature at the k -th particle is given by

$$\begin{aligned} \left. \frac{d(\kappa - \kappa_R)}{ds} \right|_{k,p} &= \frac{1}{2} \left[\frac{\kappa_{k+1,p} - \kappa_{R,k+1,p} - \kappa_{k,p} + \kappa_{R,k,p}}{l_k} \right. \\ &\quad \left. + \frac{\kappa_{k,p} - \kappa_{R,k,p} - \kappa_{k-1,p} + \kappa_{R,k-1,p}}{l_{k-1}} \right] \end{aligned} \quad (4.13)$$

and the second-order derivative is

$$\left. \frac{d^2(\kappa - \kappa_R)}{ds^2} \right|_{k,p} = \frac{2}{l_{k-1} + l_k} \left[\frac{\kappa_{k+1,p} - \kappa_{R,k+1,p} - \kappa_{k,p} + \kappa_{R,k,p}}{l_k} - \frac{\kappa_{k,p} - \kappa_{R,k,p} - \kappa_{k-1,p} + \kappa_{R,k-1,p}}{l_{k-1}} \right] \quad (4.14)$$

in which $\kappa_{k,p}$ and $\kappa_{R,k,p}$ are the curvatures at k -th particle at the current and reference states, respectively.

Fig. 4.5 Oscillation caused by the numerical differentiation, (a) the curvature variation, (b) first-order derivative of curvature variation, and (c) second-order derivative of curvature variation, where the dots are for the exact results, and the solid lines for the numerical results.

It should be mentioned that considerable oscillations are caused when differentiating the strain and curvature, as shows in Fig. 4.5. In general, the numerical

errors are inevitable in the calculations of strain and curvature, such that they are greatly magnified when differentiating the strain and curvature. It is found from Fig. 4.5(c) that the second-order derivative of curvature is useless. In order to overcome this oscillation, the cubic smoothing spline technique is employed to smooth the strain, curvature and their derivatives calculated, and its theoretical formulation and numerical implementation are explained in Appendix A. Fig. 4.6 illustrates the curvature and its first- and second-order derivatives after smoothing, in which they agree well with the corresponding exact values.

Fig. 4.6 Smoothed results using the cubic smoothing spline technique, (a) the curvature variation, (b) first-order derivative of curvature variation, and (c) second-order derivative of curvature variation, where the dots are for the exact results, and the solid lines for the numerical results.

To sum up, the procedure of calculating the membrane force strength is described as follows:

- Calculate the length in Eq. (4.4) and normal direction in Eq. (4.9) of each segment;
- Calculate the strain in Eq. (4.5) and curvature in Eq. (4.10) at each Lagrangian particle, and then smooth them;
- Calculate the first-order derivative of the strain in Eq. (4.6), and the first- and second-order derivatives of curvature in Eqs. (4.13) and (4.14) at each Lagrangian particle, and then smooth them;
- Calculate the elastic membrane force strength in Eq. (4.2).

Fig. 4.7 Comparison between the analytical (solid line) and numerical (dots) results for the membrane mechanical force strength acting on a biconcave shape at a deformed state.

In order to validate the numerical method described above, the membrane mechanic force strength acting on a biconcave shape at a deformed state is calculated by the analytical method and numerical method, respectively. A comparison between them is illustrated in Fig. 4.7, where a good agreement is observed. Therefore, a conclusion is drawn that the numerical calculation of curvature, the discretization of derivatives and the smoothing technique are accurate and reliable enough.

4.2.2 Dielectrophoresis force strength

The dielectrophoresis (DEP) force strength is listed again as

$$\mathbf{f}_{dep} = \frac{1}{4} \text{Re}(\tilde{\xi}_m) \left(aa^* |\tilde{\mathbf{E}}_c \cdot \mathbf{n}|^2 + \text{Re}(a) |\tilde{\mathbf{E}}_c|^2 \right) \mathbf{n} \quad (4.15)$$

Since the coefficient a is the material constant, only the electric field strength is required to be provided for the calculation of DEP force strength at each Lagrangian particle.

Using the finite difference method, the governing equation (3.31) of electric field is discretized as

$$\begin{aligned} & \frac{1}{\Delta x^2} \left[\tilde{\xi}_{h,(i-1/2,j)} \tilde{\varphi}_{h,(i-1,j)} - \left(\tilde{\xi}_{h,(i-1/2,j)} + \tilde{\xi}_{h,(i+1/2,j)} \right) \tilde{\varphi}_{h,(i,j)} + \tilde{\xi}_{h,(i+1/2,j)} \tilde{\varphi}_{h,(i+1,j)} \right] \\ & + \frac{1}{\Delta y^2} \left[\tilde{\xi}_{h,(i,j-1/2)} \tilde{\varphi}_{h,(i,j-1)} - \left(\tilde{\xi}_{h,(i,j-1/2)} + \tilde{\xi}_{h,(i,j+1/2)} \right) \tilde{\varphi}_{h,(i,j)} + \tilde{\xi}_{h,(i,j+1/2)} \tilde{\varphi}_{h,(i,j+1)} \right] = 0 \end{aligned} \quad (4.16)$$

where Δx and Δy are the spatial steps in the x and y directions. In order to solve the above linear equations, the successive over relaxation (SOR) method is employed, and the iterative scheme is given by

$$\begin{aligned} \tilde{\varphi}_{h,(i,j)}^{n+1} = & (1 - \chi) \tilde{\varphi}_{h,(i,j)}^n + \chi c_1 \left(\tilde{\xi}_{h,(i-1/2,j)} \tilde{\varphi}_{h,(i-1,j)}^{n+1} + \tilde{\xi}_{h,(i+1/2,j)} \tilde{\varphi}_{h,(i+1,j)}^n \right) \\ & + \chi c_2 \left(\tilde{\xi}_{h,(i,j-1/2)} \tilde{\varphi}_{h,(i,j-1)}^{n+1} + \tilde{\xi}_{h,(i,j+1/2)} \tilde{\varphi}_{h,(i,j+1)}^n \right) \end{aligned} \quad (4.17)$$

where χ is the relaxation factor, n and $n+1$ are the present and next iteration steps, and c_1 and c_2 are two coefficients, defined as

$$\begin{aligned}
 c_1 &= \frac{\Delta y^2}{\Delta y^2 (\tilde{\xi}_{h,(i-1/2,j)} + \tilde{\xi}_{h,(i+1/2,j)}) + \Delta x^2 (\tilde{\xi}_{h,(i,j-1/2)} + \tilde{\xi}_{h,(i,j+1/2)})} \\
 c_2 &= \frac{\Delta x^2}{\Delta y^2 (\tilde{\xi}_{h,(i-1/2,j)} + \tilde{\xi}_{h,(i+1/2,j)}) + \Delta x^2 (\tilde{\xi}_{h,(i,j-1/2)} + \tilde{\xi}_{h,(i,j+1/2)})}
 \end{aligned} \tag{4.18}$$

Without loss of generality, it is assumed that the cell membrane is located between the meshes (i, j) and $(i+1, j)$ in the x direction, as shown in Fig. 4.8. The value $\tilde{\varphi}_{c,(i+1,j)}^n$ is unknown when calculating $\tilde{\varphi}_{c,(i,j)}^{n+1}$ via Eq. (4.17). Similarly, the value $\tilde{\varphi}_{m,(i,j)}^{n+1}$ is also unknown when calculating $\tilde{\varphi}_{m,(i+1,j)}^{n+1}$. In order to determine these unknown values, the boundary condition in Eq. (3.34) is incorporated into the iteration scheme in Eq. (4.17), and approximately discretized as

$$\tilde{\xi}_{c,(i+1/2,j)} \frac{\tilde{\varphi}_{c,(i+1,j)}^n - \tilde{\varphi}_{c,(i,j)}^n}{\Delta x} = \tilde{\xi}_{m,(i+1/2,j)} \frac{\tilde{\varphi}_{m,(i+1,j)}^n - \tilde{\varphi}_{m,(i,j)}^n}{\Delta x} \tag{4.19}$$

in which there are two unknowns $\tilde{\varphi}_{m,(i,j)}^n$ and $\tilde{\varphi}_{c,(i+1,j)}^n$. Hence, one more condition is required to determine $\tilde{\varphi}_{c,(i+1,j)}^n$. Considering the continuity of electric potential at the cell membrane Γ results in

$$\tilde{\varphi}_c|_{\Gamma} = \tilde{\varphi}_m|_{\Gamma} \tag{4.20}$$

Using the extrapolation technique, it is discretized as

$$\tilde{\varphi}_{c,(i,j)}^n + \frac{\tilde{\varphi}_{c,(i+1,j)}^n - \tilde{\varphi}_{c,(i,j)}^n}{\Delta x} h = \tilde{\varphi}_{m,(i+1,j)}^n - \frac{\tilde{\varphi}_{m,(i+1,j)}^n - \tilde{\varphi}_{m,(i,j)}^n}{\Delta x} (\Delta x - h) \tag{4.21}$$

where h is the distance between the grid (i, j) and cell membrane Γ , as shown in Fig. 4.8. Combining Eqs. (4.19) and (4.21), and cancelling out $\tilde{\varphi}_{m,(i,j)}^n$, we have

$$\tilde{\varphi}_{c,(i+1,j)}^n = \tilde{\varphi}_{c,(i,j)}^n + \frac{\Delta x}{h + \tilde{\xi}_{c,(i+1/2,j)} / \tilde{\xi}_{m,(i+1/2,j)}} (\tilde{\varphi}_{m,(i+1,j)}^n - \tilde{\varphi}_{c,(i,j)}^n) \tag{4.22}$$

Similarly, if the cell membrane is located between the meshes (i, j) and $(i, j+1)$ on the y direction, we have

$$\tilde{\varphi}_{c,(i,j+1)}^n = \tilde{\varphi}_{c,(i,j)}^n + \frac{\Delta x}{h + \tilde{\xi}_{c,(i,j+1/2)} / \tilde{\xi}_{m,(i,j+1/2)}} (\tilde{\varphi}_{m,(i,j+1)}^n - \tilde{\varphi}_{c,(i,j)}^n) \quad (4.23)$$

After the value $\tilde{\varphi}_{c,(i+1,j)}^n$ is obtained, the iteration in Eq. (4.17) will continue until achieving the convergence, such that the electric field strength $\tilde{\mathbf{E}}_{h,(i,j)}$ at each grid point can be calculated by

$$\tilde{\mathbf{E}}_{h,(i,j)} = \left[\frac{\tilde{\varphi}_{h,(i+1,j)} - \tilde{\varphi}_{h,(i-1,j)}}{2\Delta x}, \frac{\tilde{\varphi}_{h,(i,j+1)} - \tilde{\varphi}_{h,(i,j-1)}}{2\Delta y} \right] \quad (4.24)$$

Based on the electric field strength at each grid point, we use the Lagrangian interpolation to calculate the electric field strength at each Lagrangian particle. Without loss of generality, it is supposed that the k -th particle is located in the mesh (i, j) , as illustrated in Fig. 4.9(a). The electric field strength at the k -th particle is thus expressed as

$$\tilde{\mathbf{E}}_{h,k} = N_{00} \tilde{\mathbf{E}}_{h,(i,j)} + N_{10} \tilde{\mathbf{E}}_{h,(i+1,j)} + N_{11} \tilde{\mathbf{E}}_{h,(i+1,j+1)} + N_{01} \tilde{\mathbf{E}}_{h,(i,j+1)} \quad (4.25)$$

where N_{00} , N_{10} , N_{11} and N_{01} are the shape functions, defined as

$$\begin{aligned} N_{00} &= (1 - \alpha_k)(1 - \beta_k) \\ N_{10} &= \alpha_k(1 - \beta_k) \\ N_{11} &= \alpha_k\beta_k \\ N_{01} &= (1 - \alpha_k)\beta_k \end{aligned} \quad (4.26)$$

where α_k and β_k are the location of the k -th particle after the coordinate transformation, introduced as

$$\begin{aligned} \alpha_k &= (x_k^p - x_{i,j}) / \Delta x \\ \beta_k &= (y_k^p - y_{i,j}) / \Delta y \end{aligned} \quad (4.27)$$

where (x_k^p, y_k^p) and $(x_{i,j}, y_{i,j})$ are the coordinates of the k -th particle and the grid point (i, j) respectively, as illustrated in Fig. 4.9. It is found that the transformation makes the shape functions in Eq. (4.26) independent of the grid points.

Fig. 4.8 Discretization of the boundary condition for calculating the electric field, where the cell membrane is located between the meshes (i, j) and $(i+1, j)$.

In the end, the procedure of calculating the DEP force strength is summarized as follows:

- Solve the governing equation of electric field using the iteration scheme in Eq. (4.17), and then calculate the electric field strength at each grid point in Eq. (4.24);
- Calculate the electric field strength at each Lagrangian particle using the Lagrangian interpolation in Eq. (4.25);
- Calculate the DEP force strength in Eq. (4.15).

Fig. 4.9 Lagrangian interpolation for calculating the electric field strength at k -th Lagrangian particle, (a) the particle coordinate before the transformation, and (b) particle coordinate after the transformation.

Fig. 4.10 Comparisons of the DEP force acting on a cylinder calculated by the MST approach and dipole moment approximation, (a) the relationship between the DEP force and cylinder radius, and (b) the relationship between the DEP force and cylinder location.

Taking the calculation of DEP force acting on a cylinder as an example, the MST approach used in the present work is validated by comparing with the dipole moment approximation. The DEP force acting on the unit length of cylinder is expressed by the dipole moment approximation [110]

$$\mathbf{F}_{dep}^{dipole} = \pi R^2 \operatorname{Re} \left(\frac{\tilde{\xi}_c^* \tilde{\xi}_c - \tilde{\xi}_m}{\tilde{\xi}_c + \tilde{\xi}_m} \right) \nabla \left(\tilde{\mathbf{E}}_{rms} \right)^2 \quad (4.28)$$

in which R is the cylinder radius, $\tilde{E}_{rms}$ is the root-mean-square electric field strength. The DEP force acting on the cylinder is given by the MST method

$$\mathbf{F}_{dep}^{MST} = \int_{\Gamma} \mathbf{f}_{dep} ds \quad (4.29)$$

where $\mathbf{f}_{dep}$ is the DEP force strength given by Eq. (4.15). The cylinder and suspending medium are treated as pure dielectric materials with the relative permittivities of 57 and 80, respectively. Fig. 4.10 shows the comparison between the MST approach and dipole moment approximation for the DEP force acting on the cylinder. It is found from Fig. 4.10(a) that the gap between them becomes wider and wider as the cylinder radius increases. This is because the dipole moment approximation is applicable only to such small dielectric body that it can be approximated as a dipole. As $R = 4.0 \mu\text{m}$, the accuracy of dipole moment approximation is relatively poor. Considering that the cell radius is commonly about $4.0 \mu\text{m}$, the MST approach is better for calculating the DEP force acting on the cells. On the other hand, Fig. 4.10(b) illustrates the trends of DEP force with respect to the cylinder location under $R = 1.0 \mu\text{m}$. It is noted that the result using the MST approach is in good agreement with that of the dipole moment approximation at $R = 1.0 \mu\text{m}$.

4.2.3 Intercellular interaction force strength

The intercellular interaction force strength is listed again as

$$\mathbf{f}_{int}(r) = 2(\mathbf{n}_{c1} \cdot \mathbf{k}_{c1})(\mathbf{n}_{c2} \cdot \mathbf{k}_{c2})\beta D_e [e^{2\beta(r_0-r)} - e^{\beta(r_0-r)}] \frac{\mathbf{r}}{r} \quad (4.30)$$

Because the unit normal directions $\mathbf{n}_{c1}$ and $\mathbf{n}_{c2}$ of two cells have already been calculated by the Lagrangian particles on the cell membrane, there are three items in Eq. (4.30) still required to calculate the intercellular force strength, namely the two unit vectors $\mathbf{k}_{c1}$ and $\mathbf{k}_{c2}$, and the distance r between the two facing elements. These three items are related to the two facing elements which can be described by the centroids of two cells. Suppose that there is an element on one cell, and its location is denoted by (x_{c1}^e, y_{c1}^e) . The facing element on the other cell is positioned such that the line joining the two elements is parallel to that joining the centroids of two cells, whose location is denoted by (x_{c2}^e, y_{c2}^e) . Therefore, the distance between the two elements is given by

$$r = \sqrt{(x_{c2}^e - x_{c1}^e)^2 + (y_{c2}^e - y_{c1}^e)^2} \quad (4.31)$$

and the two unit vectors are

$$\begin{aligned} \mathbf{k}_{c1} &= \frac{1}{r} (x_{c2}^e - x_{c1}^e, y_{c2}^e - y_{c1}^e) \\ \mathbf{k}_{c2} &= -\mathbf{k}_{c1} \end{aligned} \quad (4.32)$$

The procedure of calculating the intercellular interaction force strength is given as follows:

- Find the centroids of two cells, described in Appendix B;
- Based on the line joining the centroids of two cells, determine the distance r between two elements in Eq. (4.31), and the two unit vectors $\mathbf{k}_{c1}$ and $\mathbf{k}_{c2}$ in Eq. (4.32);
- Calculate the intercellular interaction force strength in Eq. (4.30).

4.2.4 Total resultant force

The total resultant force is defined in Eq. (4.1), including two main parts, a Delta function and a line integral.

Firstly, the Delta function should be discretized. In the present research work, the cell membrane is considered to have a finite thickness instead of zero, such that the total resultant force should be distributed on the transition zone centering on the cell membrane. Therefore, the Delta function $\delta(\mathbf{x} - \mathbf{x}_{mem})$ in Eq. (4.1) is discretized as a smooth Delta function [196],

$$D(\mathbf{x} - \mathbf{x}_k^p) = \begin{cases} \frac{1}{4\Delta x \Delta y} \left(1 + \cos \frac{\pi |x - x_k^p|}{\Delta x} \right) \left(1 + \cos \frac{\pi |y - y_k^p|}{\Delta y} \right) & \text{if } |x - x_k^p| < \Delta x \text{ and } |y - y_k^p| < \Delta y \\ 0 & \text{otherwise} \end{cases} \quad (4.33)$$

where (x_k^p, y_k^p) is the coordinate of the k -th particle, and $\mathbf{x} = (x, y)$ is the spatial variable. It is found in Eq. (4.33) that the thickness of cell membrane is assumed as the two-mesh size, and

$$\iint_{\Omega} D(\mathbf{x} - \mathbf{x}_k^p) dx dy = 1 \quad (4.34)$$

where Ω is the whole computational domain. Fig. 4.11 shows the geometric feature of the smooth Delta function. It takes the maximum value $1/(\Delta x \Delta y)$ at $\mathbf{x} = \mathbf{x}_k^p$, and zero value outside the domain of $|x - x_k^p| < \Delta x$ and $|y - y_k^p| < \Delta y$.

Fig. 4.11 Geometric feature of the smooth Delta function at the k -th Lagrangian particle.

Secondly, considering that the membrane segment is significantly small, the line integral is discretized by the trapezoidal rule, and therefore the resultant force at the grid point (i, j) is expressed as

$$\mathbf{F}_{tot}(\mathbf{x}_{i,j}) = \sum_{k=0}^{NP-1} \frac{\mathbf{f}_{tot,k} D(\mathbf{x}_{i,j} - \mathbf{x}_k^p) + \mathbf{f}_{tot,k+1} D(\mathbf{x}_{i,j} - \mathbf{x}_{k+1}^p)}{2} l_k \quad (4.35)$$

where NP is the number of Lagrangian particles, $\mathbf{f}_{tot,k}$ is the total resultant force strength at the k -th Lagrangian particle, $\mathbf{x}_{i,j}$ is the location of grid point (i, j) , $\mathbf{x}_k^p$ is the location of k -th Lagrangian particle, and l_k is the length of k -th segment.

4.3 Development of a novel front tracking method for capturing cell membrane: A modified particle binary level set (MPBLS) method

4.3.1 Introduction

It is well known that the front tracking plays an essential role in multifluid or multiphase flow. It not only provides the interface information, such as the interface location, curvature and normal direction etc., but also distinguishes the different fluids or phases. If the interface is not tracked properly, it will result in distortion or oscillation in the vicinity of interface.

The early front tracking techniques include the cell-type methods, in which the particles or markers scattered in meshes are tracked to describe the interface location and shape, for example, the particle in cell (PIC) method [205], and the marker and cell (MAC) method [206]. The cell-type methods roughly describe the location and shape of interface only, and cannot provide the detailed information, such as the interface curvature and normal direction. However, their basic ideas are quite significant, laying a solid foundation for future development of front tracking methods. The second type of the front tracking methods is to use line segments to describe the interface. For instance, Hirt and Nichols [207] proposed a concept of volume of fluid (VOF), where the horizontal or vertical line segments are used to reconfigure the free interface. Subsequently, the oblique line segments were introduced by Youngs [208] to reconstruct the interface in a mesh, well known as the Youngs technique. Nevertheless, this technique deals with a single mesh only, and the reconstructed interface may not be consistent at the boundary of neighboring meshes. In order to overcome this disadvantage, Ashgriz and Poo [209] extended the oblique line segments to the two neighboring meshes, and developed the flux line-segment model for advection and interface reconstruction (FLAIR). Compared with the cell-type methods, the VOF methods save huge computer storage resource, and provide more detailed information of interface.

Currently, the prevalent front tracking methods are often based on a function to describe the interface. One of them is the level set (LS) approach proposed by Osher and Sethian [210], in which a function ϕ is defined, and a level set $\phi = 0$ represents the interface. It can easily obtain the detailed interface information, and deal with the splitting and merging of interfaces. The LS method therefore has been of great interest in recent years. However, it is susceptible to numerical dissipation which results in the poor volume conservation. In other words, when the level set function is advected, the sharp edges are usually smoothed out and hence volume loss occurs. In such case, the particle level set (PLS) approach is suggested by Enright et al. [211] by correcting the LS function using the massless particles with radii scattered on the both sides of interface.

In the present research work, the Lagrangian particles have already been scattered on the cell membrane when calculating the different types of force strengths. If the PLS approach is still used to track the cell membrane, another two sets of particles are required to be scattered on the both sides of cell membrane, considerably increasing the computational cost. Besides, the cell membrane described by the PLS approach may not be consistent with that described by the Lagrangian particles on it. Therefore, a modified particle binary level set (MPBLS) method is developed in the present work, in which a binary level set function is employed to distinguish the different materials, and corrected by the Lagrangian particles scattered on the cell membrane only. There is no longer need to scatter two more sets of particles on the both sides of cell membrane in the MPBLS method.

4.3.2 Particle level set (PLS) approach

4.3.2.1 Signed distance function

In the PLS approach, the interface Γ bounding the domain Ω_{in} is usually identified by the signed distance function at the initial state, defined as

$$\phi(\mathbf{x}, t)|_{t=0} = \begin{cases} -d(\mathbf{x}, \Gamma) & \mathbf{x} \in \Omega_{in} \\ 0 & \mathbf{x} \in \Gamma \\ d(\mathbf{x}, \Gamma) & \mathbf{x} \notin \Omega_{in} \end{cases} \quad (4.36)$$

where $d(\mathbf{x}, \Gamma)$ is the distance between the spatial position $\mathbf{x}$ and interface location Γ . The motion of interface is governed by the evolution equation [211],

$$\phi_t + \mathbf{u} \cdot \nabla \phi = 0 \quad \mathbf{x} \in \Gamma \quad (4.37)$$

where $\mathbf{u}$ is the velocity field. By solving the evolution equation (4.37) with the initial condition (4.36), the interface described by the LS function can be thus tracked at any time. Furthermore, the normal direction of interface is expressed as

$$\mathbf{n} = \frac{\nabla \phi}{|\nabla \phi|} \quad (4.38)$$

and the curvature is

$$\kappa = \nabla \cdot \frac{\nabla \phi}{|\nabla \phi|} \quad (4.39)$$

4.3.2.2 Correction by particles scattered on the both sides of interface

In the PLS approach, two sets of massless particles with certain radii are seeded on the both sides of interface. One set is the positive particles in the $\phi > 0$ region, and the other is the negative particles in the $\phi < 0$ region [211], as illustrated in Fig. 4.12. It is unnecessary to seed particles far from the interface for saving computational resource. Therefore, they are usually seeded in a band of $3 \max(\Delta x, \Delta y, \Delta z)$ around the interface, and have the number of four per spatial dimension in a mesh, i.e., 16 in 2D and 64 in 3D per mesh. The particles are advected with the flow using the equation

$$\frac{d\mathbf{x}^p}{dt} = \mathbf{u}_p \quad (4.40)$$

where $\mathbf{x}^p$ and $\mathbf{u}_p$ are the location and velocity of a particle.

The particles do not cross the interface except when the interface tracking fails to accurately identify the interface location. If the particles initially scattered on one side of interface are detected on the opposite side, so-called escaped particles, this indicates an error in the level set representation of interface [211]. Using the information of escaped particles, the level set function can be rebuilt for the interface correction. That

is the main idea of PLS approach. In order to identify the escaped particles, each particle has a certain radius, set as [211]

$$r_p = \begin{cases} r_{\max} & s_p \phi(\mathbf{x}^p) > r_{\max} \\ s_p \phi(\mathbf{x}^p) & r_{\min} \leq s_p \phi(\mathbf{x}^p) \leq r_{\max} \\ r_{\min} & s_p \phi(\mathbf{x}^p) < r_{\min} \end{cases} \quad (4.41)$$

with the limits of $r_{\min} = 0.1 \min(\Delta x, \Delta y, \Delta z)$ and $r_{\max} = 0.5 \min(\Delta x, \Delta y, \Delta z)$, where s_p and $\phi(\mathbf{x}^p)$ are the sign and level set value of the particle. According to Eq. (4.41), the particle boundary is positioned tangent to the interface whenever possible by adjusting the particle radius. A particle that is on the wrong side of interface by more than its radius, determined by $\phi(\mathbf{x}^p)$, is considered to have escaped. Using the escaped positive particles, the $\phi > 0$ region can be rebuilt, and the $\phi < 0$ region using the escaped negative particles. For example, taking the $\phi > 0$ region and an escaped positive particle, the level set function is corrected first as

$$\phi^+ = \max_{\forall p \in S^+}(\phi_p, \phi^+) \quad (4.42)$$

where S^+ is the set of escaped positive particles, ϕ^+ is the maximum value between ϕ_p and ϕ , and ϕ_p is the local level set function centered at each particle, defined as

$$\phi_p(\mathbf{x}) = s_p (r_p - |\mathbf{x} - \mathbf{x}^p|) \quad (4.43)$$

in which s_p is the particle sign. Similarly, the level set function at the $\phi < 0$ region is corrected as

$$\phi^- = \min_{\forall p \in S^-}(\phi_p, \phi^-) \quad (4.44)$$

where S^- is the set of escaped negative particles. Since ϕ^+ and ϕ^- may not agree due to numerical error, merging them by taking their minimum into a single level set function results in

$$\phi^{new} = \begin{cases} \phi^+ & |\phi^+| \leq |\phi^-| \\ \phi^- & |\phi^+| > |\phi^-| \end{cases} \quad (4.45)$$

Fig. 4.12 Schematic illustration of the escaped particles used to correct the interface in the PLS approach.

4.3.3 Modified particle binary level set (MPBLS) method

4.3.3.1 Binary level set function

The signed distance function is usually used in the PLS approach for describing the interface information, including the curvature and normal direction. However, in the present research work, the interface information has already been provided by the Lagrangian particles on the interface, for example, the normal direction and curvature given by Eqs. (4.9) and (4.10), respectively. In the MPBLS method therefore, it is only required to distinguish the different materials, which is satisfied by the binary level set function, defined as

$$\phi(\mathbf{x}, t)|_{t=0} = \begin{cases} -0.5 & x \in \Omega_{in} \\ 0 & x \in \Gamma \\ 0.5 & x \notin \Omega_{in} \end{cases} \quad (4.46)$$

The motion of interface is also governed by the evolution equation,

$$\phi_t + \mathbf{u} \cdot \nabla \phi = 0 \quad \mathbf{x} \in \Gamma \quad (4.47)$$

where $\mathbf{u}$ is the velocity field. Compared with the signed distance function, the binary level set function is quite easy for numerical implementation. However, it is a discontinuous function, such that the high-order numerical scheme is required to solve Eq. (4.47).

Using the conservative difference scheme to discretize the spatial term in Eq. (4.47) leads to

$$\left(\frac{\partial \phi}{\partial t} \right)_{i,j} = - \frac{(u\phi)_{i+1/2,j} - (u\phi)_{i-1/2,j}}{\Delta x} - \frac{(v\phi)_{i,j+1/2} - (v\phi)_{i,j-1/2}}{\Delta y} \quad (4.48)$$

Considering the upwind property for stability, the each convective term is further split into two parts,

$$\begin{aligned} \left(\frac{\partial \phi}{\partial t} \right)_{i,j} = & - \frac{((u\phi)_{i+1/2,j}^+ + (u\phi)_{i+1/2,j}^-) - ((u\phi)_{i-1/2,j}^+ + (u\phi)_{i-1/2,j}^-)}{\Delta x} \\ & - \frac{((v\phi)_{i,j+1/2}^+ + (v\phi)_{i,j+1/2}^-) - ((v\phi)_{i,j-1/2}^+ + (v\phi)_{i,j-1/2}^-)}{\Delta y} \end{aligned} \quad (4.49)$$

in which $u_{i+1/2,j}^\pm$ and $v_{i,j+1/2}^\pm$ are given by

$$\begin{aligned} u_{i+1/2,j}^\pm &= \frac{u_{i+1/2,j} \pm |u_{i+1/2,j}|}{2} \\ v_{i,j+1/2}^\pm &= \frac{v_{i,j+1/2} \pm |v_{i,j+1/2}|}{2} \end{aligned} \quad (4.50)$$

In the staggered grid system, the velocities $u_{i+1/2,j}$ and $v_{i,j+1/2}$ are known, and thus don't need to be interpolated. To obtain the solution in Eq. (4.49), there are still two issues to be solved. One is how to decide the accurate values of $\phi_{i+1/2,j}^\pm$ and $\phi_{i,j+1/2}^\pm$ using $\phi_{i,j}$, and the other is how to perform the time discretization $\partial \phi / \partial t$. For the first issue, the fifth-order weighted essentially non-oscillatory (WENO) scheme [212] is employed to achieve a high-resolution tracking of interface. The detailed formulation is presented in Appendix C. For the second issue, the third-order total variation diminishing (TVD)

Runge-Kutta method [213] is used to keep consistency with the accuracy of spatial discretization. For the sake of notational simplicity, we define

$$R_{i,j}(\phi) = -\frac{\left((u\phi)_{i+1/2,j}^+ + (u\phi)_{i+1/2,j}^-\right) - \left((u\phi)_{i-1/2,j}^+ + (u\phi)_{i-1/2,j}^-\right)}{\Delta x} - \frac{\left((v\phi)_{i,j+1/2}^+ + (v\phi)_{i,j+1/2}^-\right) - \left((v\phi)_{i,j-1/2}^+ + (v\phi)_{i,j-1/2}^-\right)}{\Delta y} \quad (4.51)$$

and then Eq.(4.49) is rewritten as

$$\left(\frac{\partial \phi}{\partial t}\right)_{i,j} = R_{i,j}(\phi) \quad (4.52)$$

The third-order TVD Runge-Kutta method is expressed for the time discretization as,

$$\begin{cases} \phi_{i,j}^{(1)} = \phi_{i,j}^n + \Delta t R(\phi_{i,j}^n) \\ \phi_{i,j}^{(2)} = \frac{3}{4}\phi_{i,j}^n + \frac{1}{4}\phi_{i,j}^{(1)} + \frac{1}{4}\Delta t R(\phi_{i,j}^{(1)}) \\ \phi_{i,j}^{n+1} = \frac{1}{3}\phi_{i,j}^n + \frac{2}{3}\phi_{i,j}^{(2)} + \frac{2}{3}\Delta t R(\phi_{i,j}^{(2)}) \end{cases} \quad (4.53)$$

where $\phi_{i,j}^n$ is the value of binary level set function at the n time step, $\phi_{i,j}^{(1)}$ and $\phi_{i,j}^{(2)}$ are the intermediate values, and Δt is the time step.

4.3.3.2 Correction by Lagrange particles scattered on the interface

Different from the PLS approach, the MPBLS method employs one set of Lagrangian particles scattered on the interface, instead of two sets of particles scattered on the both sides of interface. Apart from that, the particles in the PLS approach are used to correct the interface only. However, the particles in the MPBLS method have several purposes summarized as, (a) correcting the interface, (b) providing the interface information, (c) calculating the membrane mechanical force strength, (d) calculating the dielectrophoresis force strength, (e) calculating the intercellular interaction force strength, (f) calculating the total resultant force. Finally, the number of particles in the MPBLS method depends on the interface length, and is usually taken as two per spatial step length, significantly smaller than that in the PLS approach. If the particle density of the interface is less than the grid density of the computational domain, the interface

resolution described by the Lagrangian particles is lower than that described by the domain grids, such that the Lagrangian particles cannot be used for the interface correction. On the contrary, if the particle density is much larger than the grid density, the interface distorts and folds easily due to the overlapped particles. Considering the computational cost, moreover, we prefer two particles per spatial step length, which is quite good for the MPBLS method.

Similar to the PLS approach, the motion of particles in the MPBLS method is also governed by

$$\frac{d\mathbf{x}^p}{dt} = \mathbf{u}_p \quad (4.54)$$

The particle velocity $\mathbf{u}_p$ is obtained here by the area weighted average in the particle in cell (PIC) method [205], and explained in Appendix D. After calculating the particle velocity, Eq. (4.54) is also discretized by the third-order TVD Runge-Kutta method [213] as

$$\begin{cases} \mathbf{x}^{p,(1)} = \mathbf{x}^{p,n} + \Delta t \mathbf{u}_p^n \\ \mathbf{x}^{p,(2)} = \frac{3}{4} \mathbf{x}^{p,n} + \frac{1}{4} \mathbf{x}^{p,(1)} + \frac{1}{4} \Delta t \mathbf{u}_p(\mathbf{x}^{p,(1)}) \\ \mathbf{x}^{p,n+1} = \frac{1}{3} \mathbf{x}^{p,n} + \frac{2}{3} \mathbf{x}^{p,(2)} + \frac{2}{3} \Delta t \mathbf{u}_p(\mathbf{x}^{p,(2)}) \end{cases} \quad (4.55)$$

where $\mathbf{x}^{p,n}$ and $\mathbf{u}_p^n$ are the location and velocity of particle at the n time step, and $\mathbf{x}^{p,(1)}$ and $\mathbf{x}^{p,(2)}$ are the intermediate values.

Now we turn our attention to the interface correction by the Lagrangian particles scattered on the interface. First of all, it should be identified under what condition the binary level set function ϕ needs to be corrected. Since the interface becomes wide and smooth due to the numerical dissipation, the binary level set function is accurate when taking value ± 0.5 . However, it needs to be improved when ranging from -0.5 to 0.5 . Secondly, it should be determined how to modify the binary level set function ranging from -0.5 to 0.5 . Assuming $|\phi_{i,j}| < 0.5$, we use a cross with the center of grid point (i, j) to correct it, as illustrated in Fig. 4.13. According to the position between the cross horizontal line and the interface described by the Lagrangian particles, a desired

value of the binary level set function can be determined at the grid point (i, j) , denoted by $\phi_{i,j}^x$. Similarly, a desired value $\phi_{i,j}^y$ can also be determined on the cross vertical line. Since $\phi_{i,j}^x$ and $\phi_{i,j}^y$ may not agree, taking their minimum as a single binary level set value at the grid point (i, j) results in

$$\phi_{i,j}^{new} = \begin{cases} \phi_{i,j}^x & |\phi_{i,j}^x| \leq |\phi_{i,j}^y| \\ \phi_{i,j}^y & |\phi_{i,j}^x| > |\phi_{i,j}^y| \end{cases} \quad (4.56)$$

Fig. 4.13 Schematic illustration of the cross used to correct the interface in the MPBLS method.

The present problem becomes how to calculate the two desired values of $\phi_{i,j}^x$ and $\phi_{i,j}^y$. For example, take the calculation of $\phi_{i,j}^x$ and the horizontal line of cross. There are six cases regarding the position between the cross horizontal line and the interface described by the Lagrangian particles, as plotted in Fig. 4.14. Each of them is explained as follows.

Case 1: No intersection. The desired value is easily expressed as

$$\phi_{i,j}^x = s_{i,j} \times 0.5 \quad (4.57)$$

where $s_{i,j}$ represents the sign of binary level set function at the grid point (i, j) . It is negative if the grid point (i, j) is located inside the interface, or it is positive.

Case 2: A single intersection at the left cross arm. Because the thickness of cell membrane is assumed as the two-mesh size when calculating the total resultant force, the binary level set function takes the value $s_{i+1,j} \times 0.5$ at the grid point $(i+1, j)$. Hence, the desired value at the point (i, j) is approximated by the linear interpolation as

$$\phi_{i,j}^x = s_{i+1,j} \times 0.5(i-k) \quad (4.58)$$

where k is the grid location of intersection point.

Case 3: A single intersection at the point (i, j) . This means that the interface exactly passes through the grid point (i, j) so that the desired value is given by

$$\phi_{i,j}^x = 0 \quad (4.59)$$

Case 4: A single intersection at the right cross arm. This is similar to Case 2, and the desired value is expressed as

$$\phi_{i,j}^x = s_{i-1,j} \times 0.5(k-i) \quad (4.60)$$

Case 5: Two intersections at the two cross arms. It is assumed that a linear relationship exists between the desired value ϕ^x and the variable η of intersection location. Hence, the expression at the left arm is given by

$$\phi_{(\eta,j),L}^x = a(\eta - k_1) \quad (4.61)$$

where $\phi_{(\eta,j),L}^x$ is the desired value at the left arm, a the unknown coefficient, and k_1 the location of left intersection. The similar formula at the right arm is given by

$$\phi_{(\eta,j),R}^x = b(k_2 - \eta) \quad (4.62)$$

in which $\phi_{(\eta,j),R}^x$ is the desired value on the right arm, b the unknown coefficient, and k_2 the location of right intersection. Considering the continuity of desired value at the grid point (i, j) , a constraint condition is required as

$$\phi_{(i,j),L}^x = \phi_{(i,j),R}^x \quad (4.63)$$

Moreover, since the maximum thickness of interface is the two-mesh size, we have

$$\left| \phi_{(i,j),L}^x - \phi_{(i-1,j),L}^x \right| + \left| \phi_{(i+1,j),R}^x - \phi_{(i,j),R}^x \right| = 1 \quad (4.64)$$

The two unknown coefficients are then worked out as

$$|a| = \frac{k_2 - i}{k_2 - k_1}, \quad |b| = \frac{i - k_1}{k_2 - k_1} \quad (4.65)$$

Therefore, the desired value is finally expressed as

$$\phi_{i,j}^x = s_{i,j} \times \frac{(i - k_1)(k_2 - i)}{k_2 - k_1} \quad (4.66)$$

where k_1 and k_2 are the locations of left and right intersections.

Case 6: Two or more intersections on a cross arm. This case often occurs at sharp corners of interface, involved with the resolution of tracking interface. A common approach is to treat all the intersection points as one, such that this case in fact is one of the above five cases. In addition, one can try to avoid the two or more intersections on a cross arm by refining computational domain. However, it is difficult to exactly deal with this case, even if the meshes become remarkably fine or the adaptive meshes are employed.

By the same way, the desired value $\phi_{i,j}^y$ of binary level set function at the vertical line of cross can also be calculated.

Fig. 4.14 Schematic illustration of the six cases for determining $\phi_{i,j}^x$.

4.3.3.3 Computational procedure

So far, the basic idea of the MPBLS method has been presented. In order to gain a global understanding of this method, a brief flowchart is provided, as shown in Fig. 4.15.

Fig. 4.15 Flowchart of the MPBLS method.

4.3.4 Comparisons between PLS approach and MPBLS method

4.3.4.1 Zalesak's disk in rotating flow

Consider a Zalesak's disk in constant rotating flow, which can identify the dissipation error of a front tracking method. The computational domain is taken as $\pi \times \pi$. The Zalesak's disk is initially centered at $(\pi/2, \pi/2)$ with a radius of 0.4π , a slot width of 0.1π , and a slot length of 0.4π . The constant velocity of rotating flow is given by

$$\begin{aligned} u &= -\pi(y - \pi/2) \\ v &= \pi(x - \pi/2) \end{aligned} \quad (4.67)$$

The spatial step is taken as $\Delta x = \Delta y = \pi/100$, and the time step is $\Delta t = 0.001$, such that the disk completes one revolution every 2000 steps, i.e., the time interval of two. After the disk completes four revolutions anticlockwise at $t = 8$, the velocity direction is reversed until the disk continues rotating another four revolutions clockwise at $t = 16$.

Fig. 4.16 Comparison between the PLS (solid line) and MPBLS solutions (dots) by the Zalesak's disk in rotating flow, where the Zalesak's disk rotates anticlockwise four revolutions before $t = 8$, and then rotates clockwise another four revolutions until $t = 16$.

Fig. 4.16 illustrate the comparison between the PLS and MPBLS methods, in which the Zalesak's disk rotates anticlockwise four revolutions at $t = 2, 4, 6$ and 8 , and then rotates clockwise another four revolutions at $t = 10, 12, 14$ and 16 . It is found that the PLS approach suffers from a slight area loss in the sharp corners, while the MPBLS

method almost keeps the original sharp corners. Tab. 4.1 compares the CPU time and area loss (or gain) at the different revolutions. The CPU time used by the MPBLS method is almost 10 times less than that by the PLS approach, which mainly contributes to the treatment of particles. In the PLS approach, there are about 100,000 particles used, while only 360 particles is employed in the MPBLS method. Besides, the area loss in the MPBLS method is also remarkably less than that in the PLS. Therefore, the proposed MPBLS method is more efficient and accurate than the previous PLS approach.

Tab. 4.1 Comparisons of the CPU time and area loss between the PLS and MPBLS methods for the Zalesak's disk in rotating flow.

Method	CPU time (s)	Area loss (%)							
		$t = 2$	$t = 4$	$t = 6$	$t = 8$	$t = 10$	$t = 12$	$t = 14$	$t = 16$
PLS	13761	-0.270	-0.274	-0.275	-0.271	-0.265	-0.254	-0.249	-0.245
MPBLS	1368	-0.001	0.006	0.005	0.004	0.001	0.005	0.0002	-0.004

4.3.4.2 Square plate in single vortex flow

The Zalesak's disk in rotating flow is a good indicator of dissipation error for a front tracking method, but it does not test the ability to resolve thin filaments on the mesh scale, often occurring stretching and tearing flows. Consider a square plate in single vortex flow, which can exhibit the interface stretching. The computational domain is still taken as $\pi \times \pi$, and the velocity of single vortex flow is given by

$$\begin{aligned} u &= -\pi \sin(x) \cos(y) \\ v &= \pi \cos(x) \sin(y) \end{aligned} \quad (4.68)$$

The square plate is initially centered at $(\pi/2, \pi/4)$ with sides of length 0.4π . The spatial time steps are also taken as $\Delta x = \Delta y = \pi/100$ and $\Delta t = 0.001$, respectively. After $t = 8$ the velocity direction is reversed, such that the square plate at $t = 16$ should be identical to that at $t = 0$ theoretically.

Fig. 4.17 shows the comparison between the PLS and MPBLS methods, where the square plate is stretched clockwise before $t = 8$, and the maximum stretching is reached at $t = 8$. After $t = 8$, the stretched plate goes back anticlockwise because the flow

direction is reversed. At $t = 16$, it should completely return to the original square plate. Moreover, the frames at $t = 2, 4$ and 6 should be identical to the frames $t = 14, 12$ and 10 , respectively. In Fig. 4.17, the MPBLS method can track thinner filaments, and thus maintain the area very well, whereas the PLS approach makes the thin filaments broken and disappeared, leading to the area loss. Tab. 4.2 compares the CPU time and area loss of the PLS and MPBLS methods. The MPBLS method is almost 10 times faster in the CPU time, and also almost 5 times less in the area loss than the PLS approach. The particle number is still 360 in the MPBLS method, but 150,000 or so in the PLS approach.

Fig. 4.17 Comparison between the PLS solution (solid line) and MPBLS solution (dots) by the square plate in single vortex flow, where the vortex direction is clockwise before $t = 8$, and then becomes anticlockwise until $t = 16$.

Tab. 4.2 Comparisons of the CPU time and area loss between the PLS and MPBLS methods for the square plate in single vortex flow.

Method	CPU time (s)	Area loss (%)							
		$t = 2$	$t = 4$	$t = 6$	$t = 8$	$t = 10$	$t = 12$	$t = 14$	$t = 16$
PLS	16162	-0.142	0.204	0.027	-0.246	0.102	0.151	-0.125	-0.295
MPBLS	1697	0.014	0.046	0.032	0.045	0.039	0.031	0.058	0.059

4.3.4.3 Remarks

In summary, there are two main differences between the previous PLS approach and present MPBLS method. One is the level set function, and the other is the particles. The MPBLS method uses the binary level set function only to distinguish different materials, while the PLS approach employs the signed distance function to both distinguish different materials and calculate the interfacial information. Compared with the signed distance function, the binary level set function is easy for the numerical implementation, although it is not a continuous function. Besides, the particles are seeded on the interface in the MPBLS method for multi-purposes, including the interface correction, and calculation of interfacial information. In the PLS approach however, they are seeded on the both sides of interface only to correct the interface. Therefore, the particle number in the MPBLS method is significantly smaller than that in the PLS approach.

Based on the above two testing cases, it is concluded that the MPBLS method is more accurate and less time-consuming than the PLS approach. It is more suitable to multiphase and interfacial flow with Lagrangian particles. For 3D problems however, the MPBLS method becomes tedious unlike the PLS approach, since the particles in 3D domain difficultly correct the surface-like interface and determine its normal direction.

4.4 Development of a novel algorithm for solving incompressible Navier-Stokes equations: A modified semi-implicit method for pressure linked equations revised (modified SIMPLER)

4.4.1 Introduction

There are two kinds of numerical methods widely used to solve incompressible Navier-Stokes equations, the vorticity-stream function and primitive variables methods. No

matter which kind of methods is used, the main challenge is how to obtain the distribution of pressure field because there is no direct equation for it. However, this challenge doesn't exist in compressible Navier-Stokes equations due to the presence of density in the continuity equation. In the vorticity-stream function method, the incompressible Navier-Stokes equations are transformed into the so-called vorticity-stream function equations, and a Poisson equation is introduced to govern the pressure field. Due to lack of a simple stream function in 3D cases, this method is frequently applied to 2D problems.

In the primitive variables method, the incompressible Navier-Stokes equations are expressed in terms of the pressure and velocity, and don't need to be transformed. Three techniques are usually employed to calculate the pressure field, (a) solving the Poisson equation of pressure, (b) introducing the artificial compressibility into the continuity equation, and (c) applying the pressure correction. The first technique is based on the Poisson equation of pressure, and the typical algorithms include the marker and cell (MAC) [206, 214], and the projection method [215]. The second technique changes the incompressible Navier-Stokes equations into compressible Navier-Stokes equations by introducing a time-dependent pressure term (called artificial compressibility) in the continuity equation, such that high-resolution methods in compressible problems can be applied to incompressible problems. Following this idea, several studies [216-219] have been done recently. The third technique is based on a pressure correction, which is most widely used to find the solutions of incompressible Navier-Stokes equations over the past two decades. The famous algorithm is the semi-implicit method for pressure linked equations (SIMPLE) [220], proposed by Patankar and Spalding in 1972. Afterwards, several variants of the SIMPLE algorithm were developed to improve the accuracy of SIMPLE algorithm, such as SIMPLE-revised (SIMPLER) [221], SIMPLE-consistent (SIMPLEC) [222], etc. The major advantage of SIMPLE-type algorithms is their good stability due to the semi-implicit scheme. They are quite stable even if the time step is large. Another advantage of SIMPLE-types algorithms is that the numerical solutions are easily obtained due to the iterative approach. However, the SIMPLE-type algorithms converge slowly when the Reynolds number becomes very small.

In the present work, the Reynolds number is very small about 0.001. Hence, we develop a modified semi-implicit method for pressure linked equations-revised (modified SIMPLER) to speed up the convergence of momentum equations by

introducing an improved velocity correction, and to satisfy the continuity equation with the higher accuracy by introducing a fine adjustment of velocity and pressure.

4.4.2 SIMPLER algorithm

The main idea of SIMPLER algorithm is briefly described as follows [221],

- Obtain a fairly accurate pressure field using the initial velocity field;
- Calculate the current velocity field by the momentum equations;
- Correct the current velocity field by the continuity equation;
- Take the corrected velocity field as initial velocity field, and repeat the above steps until the convergences of continuity and momentum equations are achieved.

4.4.2.1 Discrete momentum equations

Taking the x -momentum equation as an example, the discretization of momentum equations at the point $(i+1/2, j)$ is explained in detail. A staggered control volume with the center $(i+1/2, j)$ is shown in Fig. 4.18(a), where the small letter e refers to the center, and the capital letters E , W , N and S indicate the faces of control volume. Integrating the x -momentum equation over this control volume yields [221]

$$a_e u_{i+1/2, j} = a_E u_{i+3/2, j} + a_W u_{i-1/2, j} + a_N u_{i+1/2, j+1} + a_S u_{i+1/2, j-1} - \Delta y (p_{i+1, j} - p_{i, j}) + b_x \quad (4.69)$$

in which a_e , a_E , a_W , a_N and a_S are the coefficients representing the convection and diffusion influences, given by

$$\begin{aligned} a_E &= D_E A(|P_{\Delta E}|) + \max(-f_E, 0) \\ a_W &= D_W A(|P_{\Delta W}|) + \max(f_W, 0) \\ a_N &= D_N A(|P_{\Delta N}|) + \max(-f_N, 0) \\ a_S &= D_S A(|P_{\Delta S}|) + \max(f_S, 0) \\ a_e &= a_E + a_W + a_N + a_S + \rho_{i+1/2, j} \frac{\Delta x \Delta y}{\Delta t} \end{aligned} \quad (4.70)$$

where D , f and P_Δ are the conductance, flux rate and mesh Peclet number, defined in Tab. 4.3. Note that there is a simple relationship among them, expressed as

$$P_{\Delta} = \frac{f}{D} \quad (4.71)$$

The term of b_x in Eq. (4.69) is the source term, given by

$$b_x = (\rho u)_{i+1/2,j}^n \frac{\Delta x \Delta y}{\Delta t} + \Delta x \Delta y F_{i+1/2,j}^x \quad (4.72)$$

where $(\rho u)_{i+1/2,j}^n$ refers to the known value at the n time step, $F_{i+1/2,j}^x$ is the x component of total resultant force. The term of $A(|P_{\Delta}|)$ in Eq. (4.70) is a function of the Peclet number P_{Δ} , having several schemes [223], such as the upwind scheme, exponential scheme, power-law scheme, etc. In general, the power-law scheme is recommended, given by

$$A(|P_{\Delta}|) = \max\left(0, (1.0 - 0.1|P_{\Delta}|)^5\right) \quad (4.73)$$

For the sake of notational simplicity, a compact form of Eq. (4.69) is represented as

$$a_e u_{i+1/2,j} = \sum a_{nb} u_{nb} - \Delta y (p_{i+1,j} - p_{i,j}) + b_x \quad (4.74)$$

in which a_{nb} and u_{nb} indicate the coefficient and x -component velocity at the four faces of control volume.

Fig. 4.18 Control volumes of (a) the x -momentum equation at the point $(i+1/2, j)$, and (b) the y -momentum equation at the point $(i, j+1/2)$.

Similarly, based on the control volume with the center $(i, j+1/2)$, as illustrated in Fig. 4.18(b), the y -momentum equation is discretized as

$$a_n v_{i,j+1/2} = a_E v_{i+1,j+1/2} + a_S v_{i-1,j+1/2} + a_N v_{i,j+3/2} + a_S v_{i,j-1/2} - \Delta x (p_{i,j+1} - p_{i,j}) + b_y \quad (4.75)$$

where

$$\begin{aligned} a_E &= D_E A(|P_{\Delta E}|) + \max(-f_E, 0) \\ a_W &= D_W A(|P_{\Delta W}|) + \max(f_W, 0) \\ a_N &= D_N A(|P_{\Delta N}|) + \max(-f_N, 0) \\ a_S &= D_S A(|P_{\Delta S}|) + \max(f_S, 0) \\ a_n &= a_E + a_W + a_N + a_S + \rho_{i,j+1/2} \frac{\Delta x \Delta y}{\Delta t} \\ b_y &= (\rho v)_{i,j+1/2}^n \frac{\Delta x \Delta y}{\Delta t} + \Delta x \Delta y F_{i,j+1/2}^y \end{aligned} \quad (4.76)$$

The function $A(|P_{\Delta}|)$ still takes the power-law scheme of Eq. (4.73). The conductance D , flux rate f and mesh Peclet number P_{Δ} in Eq. (4.76) are given in Tab. 4.4. A compact form of Eq. (4.75) is expressed as

$$a_n v_{i,j+1/2} = \sum a_{nb} v_{nb} - \Delta x (p_{i,j+1} - p_{i,j}) + b_y \quad (4.77)$$

in which a_{nb} and v_{nb} indicate the coefficient and y-component velocity at the four faces of control volume.

Tab. 4.3 Conductance, flux and mesh Peclet number in the x direction in the SIMPLER algorithm [223].

Volume face	Conductance (D)	Flux (f)	Peclet number (P_{Δ})
East	$\frac{\mu_{i+1,j}}{Re} \frac{\Delta y}{\Delta x}$	$(\rho u)_{i+1,j} \Delta y$	$Re \left(\frac{\rho u \Delta x}{\mu} \right)_{i+1,j}$
West	$\frac{\mu_{i,j}}{Re} \frac{\Delta y}{\Delta x}$	$(\rho u)_{i,j} \Delta y$	$Re \left(\frac{\rho u \Delta x}{\mu} \right)_{i,j}$
North	$\frac{\mu_{i+1/2,j+1/2}}{Re} \frac{\Delta x}{\Delta y}$	$(\rho v)_{i+1/2,j+1/2} \Delta x$	$Re \left(\frac{\rho v \Delta y}{\mu} \right)_{i+1/2,j+1/2}$
South	$\frac{\mu_{i+1/2,j-1/2}}{Re} \frac{\Delta x}{\Delta y}$	$(\rho v)_{i+1/2,j-1/2} \Delta x$	$Re \left(\frac{\rho v \Delta y}{\mu} \right)_{i+1/2,j-1/2}$

Tab. 4.4 Conductance, flux and mesh Peclet number in the y direction in the SIMPLER algorithm [223].

Volume face	Conductance (D)	Flux (f)	Peclet number (P_Δ)
East	$\frac{\mu_{i+1/2,j+1/2}}{Re} \frac{\Delta y}{\Delta x}$	$(\rho u)_{i+1/2,j+1/2} \Delta y$	$Re \left(\frac{\rho u \Delta x}{\mu} \right)_{i+1/2,j+1/2}$
West	$\frac{\mu_{i-1/2,j+1/2}}{Re} \frac{\Delta y}{\Delta x}$	$(\rho u)_{i-1/2,j+1/2} \Delta y$	$Re \left(\frac{\rho u \Delta x}{\mu} \right)_{i-1/2,j+1/2}$
North	$\frac{\mu_{i,j+1}}{Re} \frac{\Delta x}{\Delta y}$	$(\rho v)_{i,j+1} \Delta x$	$Re \left(\frac{\rho v \Delta y}{\mu} \right)_{i,j+1}$
South	$\frac{\mu_{i,j}}{Re} \frac{\Delta x}{\Delta y}$	$(\rho v)_{i,j} \Delta x$	$Re \left(\frac{\rho v \Delta y}{\mu} \right)_{i,j}$

In order to calculate fairly accurate pressure field, the pseudovelocitity field is defined as

$$\tilde{u}_{i+1/2,j} = \frac{\sum a_{nb} u_{nb} + b_x}{a_e}, \quad \tilde{v}_{i,j+1/2} = \frac{\sum a_{nb} v_{nb} + b_y}{a_n} \quad (4.78)$$

Eqs. (4.74) and (4.77) are rearranged as

$$u_{i+1/2,j} = \tilde{u}_{i+1/2,j} - d_e (p_{i+1,j} - p_{i,j}) \quad (4.79)$$

$$v_{i,j+1/2} = \tilde{v}_{i,j+1/2} - d_n (p_{i,j+1} - p_{i,j}) \quad (4.80)$$

where

$$d_e = \frac{\Delta y}{a_e}, \quad d_n = \frac{\Delta x}{a_n} \quad (4.81)$$

Combining Eqs. (4.79) and (4.80) with the continuity equation, the pressure equation is derived as

$$a_w p_{i,j} = a_e p_{i+1,j} + a_w p_{i-1,j} + a_n p_{i,j+1} + a_s p_{i,j-1} + b \quad (4.82)$$

where a_e , a_w , a_n , a_s and a_w are given by

$$\begin{aligned}
a_e &= \rho_{i+1/2,j} d_e \Delta y, & a_w &= \rho_{i-1/2,j} d_w \Delta y \\
a_n &= \rho_{i,j+1/2} d_n \Delta x, & a_s &= \rho_{i,j-1/2} d_s \Delta x \\
a_w &= a_e + a_w + a_n + a_s
\end{aligned} \tag{4.83}$$

and b is given by

$$b = -\left[(\rho \tilde{u})_{i+1/2,j} - (\rho \tilde{u})_{i-1/2,j}\right] \Delta y - \left[(\rho \tilde{v})_{i,j+1/2} - (\rho \tilde{v})_{i,j-1/2}\right] \Delta x \tag{4.84}$$

Using the initial velocity field (u_0, v_0) , the pseudovelocity field $(\tilde{u}, \tilde{v})$ can be obtained by Eq. (4.78), and then the pressure field is calculated by solving Eq. (4.82), denoted by p^* . After that, the current velocity field (u^*, v^*) is calculated by the discrete momentum equations (4.74) and (4.77). Generally, the current velocity field is not accurate enough, and therefore needs to be corrected.

4.4.2.2 Velocity correction equations

Assume that the pressure field p^* is modified by adding a pressure correction term p' as

$$p^{**} = p^* + p' \tag{4.85}$$

and thus the corresponding velocity field is corrected as

$$\begin{aligned}
u^{**} &= u^* + u' \\
v^{**} &= v^* + v'
\end{aligned} \tag{4.86}$$

where u' and v' are the velocity corrections on the x and y directions. Combining Eqs. (4.74) and (4.77) with the continuity equation, and cancelling out u^{**} , v^{**} , u^* and v^* , we obtain the velocity correction equation, expressed as

$$\begin{aligned}
a_e u'_{i+1/2,j} &= \sum a_{nb} u'_{nb} - \Delta y (p'_{i+1,j} - p'_{i,j}) \\
a_n v'_{i,j+1/2} &= \sum a_{nb} v'_{nb} - \Delta x (p'_{i,j+1} - p'_{i,j})
\end{aligned} \tag{4.87}$$

Note that the velocity corrections $u'_{i+1/2,j}$ and $v'_{i,j+1/2}$ consist of two parts. One is the velocity corrections $\sum a_{nb} u'_{nb}$ and $\sum a_{nb} v'_{nb}$ at the neighboring points, and the other is the gradient of pressure correction $(p'_{i+1,j} - p'_{i,j})$ and $(p'_{i,j+1} - p'_{i,j})$. In the SIMPLER

algorithm, the first part is dropped, since the second part has a direct effect on the final results. As a result, the velocity correction equation is finally given by

$$\begin{aligned} u'_{i+1/2,j} &= -\Delta y/a_e (p'_{i+1,j} - p'_{i,j}) \\ v'_{i,j+1/2} &= -\Delta x/a_n (p'_{i,j+1} - p'_{i,j}) \end{aligned} \quad (4.88)$$

The velocity field is then corrected as

$$\begin{aligned} u^{**}_{i+1/2,j} &= u^*_{i+1/2,j} + u'_{i+1/2,j} \\ v^{**}_{i,j+1/2} &= v^*_{i,j+1/2} + v'_{i,j+1/2} \end{aligned} \quad (4.89)$$

Because the pressure field calculated by the pseudovelocity is fairly accurate, it is not corrected in the SIMPLER algorithm.

By taking the corrected velocity (u^{**}, v^{**}) as an initial velocity field (u_0, v_0) , one can repeat to calculate the pseudovelocity $(\tilde{u}, \tilde{v})$, pressure p^* , current velocity (u^*, v^*) , and velocity correction (u', v') until the convergences of continuity and momentum equations are achieved.

4.4.3 Modified SIMPLER algorithm

4.4.3.1 Improvement of velocity correction

In the SIMPLER algorithm, it is assumed that the influence of velocity correction at the neighboring points is negligible when simplifying the velocity correction in Eq. (4.87), resulting in inconsistency among the coefficients a_e , a_n and a_{nb} , and the convergence of momentum equations is thus slowed down. In order to overcome this disadvantage, the technique in the SIMPLEC algorithm is coupled into the modified SIMPLER algorithm to handle the velocity corrections. Subtracting the terms $\sum a_{nb} u'_{i+1/2,j}$ and $\sum a_{nb} v'_{i,j+1/2}$ from the both sides in Eq. (4.87), we have

$$\begin{aligned} (a_e - \sum a_{nb}) u'_{i+1/2,j} &= \sum a_{nb} (u'_{nb} - u'_{i+1/2,j}) - \Delta y (p'_{i+1,j} - p'_{i,j}) \\ (a_n - \sum a_{nb}) v'_{i,j+1/2} &= \sum a_{nb} (v'_{nb} - v'_{i,j+1/2}) - \Delta x (p'_{i,j+1} - p'_{i,j}) \end{aligned} \quad (4.90)$$

Since $u'_{i+1/2,j}$ and u'_{nb} ($v'_{i,j+1/2}$ and v_{nb}) have the same order of magnitude, the effect of $\sum a_{nb} (u'_{nb} - u'_{i+1/2,j})$ ($\sum a_{nb} (v'_{nb} - v'_{i,j+1/2})$) is much less than that of $\sum a_{nb} u'_{nb}$ ($\sum a_{nb} v'_{nb}$).

Hence, dropping the terms $\sum a_{nb}(u'_{nb} - u'_{i+1/2,j})$ and $\sum a_{nb}(v'_{nb} - v'_{i,j+1/2})$ results in the velocity correction equation in the modified SIMPLER algorithm,

$$\begin{aligned} u'_{i+1/2,j} &= -\frac{\Delta y}{a_e - \sum a_{nb}}(p'_{i+1,j} - p'_{i,j}) \\ v'_{i,j+1/2} &= -\frac{\Delta x}{a_n - \sum a_{nb}}(p'_{i,j+1} - p'_{i,j}) \end{aligned} \quad (4.91)$$

4.4.3.2 Fine adjustment of velocity and pressure fields

Although the SIMPLER algorithm can provided good results in some cases, it is often slow to converge when the Reynolds number is very small. The main reason may be attributed to the errors of velocity and pressure during the iteration, which directly influence the accuracy of discretizing the viscous term. When the Reynolds number is very small, the error resulting from the discretization of viscous term is remarkably magnified, and thus leads to the slow convergence. Therefore, in the modified SIMPLER algorithm, we further improve the obtained velocity and pressure fields by introducing a fine adjustment, which can satisfy the continuity equation with the higher accuracy. The divergence of velocity with the density at the point (i, j) is defined as

$$W_{i,j} = \frac{(\rho u)_{i+1/2,j} - (\rho u)_{i-1/2,j}}{\Delta x} + \frac{(\rho v)_{i,j+1/2} - (\rho v)_{i,j-1/2}}{\Delta y} \quad (4.92)$$

and thus the discrete continuity equation is expressed as

$$W_{i,j} = 0 \quad (4.93)$$

If the velocity divergence in a mesh is less than zero ($W_{i,j} < 0$), meaning that the fluid continues to flow into the mesh, the pressure should be increased to restrict the inflow of fluid. On the contrary, the pressure should be decreased to prevent the outflow of fluid when the divergence is larger than zero ($W_{i,j} > 0$). It is supposed that the slight pressure correction is denoted by δp , and the pressure field is thus corrected as

$$p_{i,j} = p_{i,j}^* + \delta p \quad (4.94)$$

The corresponding velocity field is corrected by [224]

$$\begin{aligned}
u_{i+1/2,j} &= u_{i+1/2,j}^{**} + \frac{\delta p \Delta t}{\Delta x} \\
u_{i-1/2,j} &= u_{i-1/2,j}^{**} - \frac{\delta p \Delta t}{\Delta x} \\
v_{i,j+1/2} &= v_{i,j+1/2}^{**} + \frac{\delta p \Delta t}{\Delta y} \\
v_{i,j-1/2} &= v_{i,j-1/2}^{**} - \frac{\delta p \Delta t}{\Delta y}
\end{aligned} \tag{4.95}$$

in which Δt is the time step, and Δx and Δy are the spatial steps of x and y directions. For satisfying the discrete continuity equation (4.93), the pressure correction δp is derived as

$$\delta p = \frac{-W_{i,j}}{2\Delta t \left(\frac{1}{\Delta x^2} + \frac{1}{\Delta y^2} \right)} \tag{4.96}$$

The fine adjustment is an iterative process, since the pressure in neighboring meshes will be affected when correcting the pressure in a mesh. In order to speed up the convergence, an over-relaxation factor χ is introduced in Eq. (4.96), usually taking the optimum value of 1.8, and therefore the pressure correction is expressed with the over-relaxation factor,

$$\delta p = \frac{-\chi W_{i,j}}{2\Delta t \left(\frac{1}{\Delta x^2} + \frac{1}{\Delta y^2} \right)} \tag{4.97}$$

4.4.3.3 Computational procedure

So far, the modified SIMPLER algorithm has been developed. The computational procedure of this algorithm is described as follows:

- Calculate the relatively accurate pressure field by Eq. (4.82) using the initial velocity field ;
- Using the pressure field, calculate the current velocity field by the momentum equations (4.74) and (4.77);
- Correct the current velocity field by the velocity correction equation (4.91);

- Further improve the corrected velocity and pressure by the fine adjustment in Eqs. (4.94) and (4.95);
- Take the improved velocity field as initial velocity field, and repeat the above steps until the convergences of continuity and momentum equations are achieved.

For better understanding the computational procedure, a brief flowchart of this algorithm is illustrated in Fig. 4.19.

Fig. 4.19 Flowchart of the modified SIMPLER algorithm.

4.4.4 Comparisons between SIMPLER and modified SIMPLER algorithms

4.4.4.1 Poiseuille flow with different Reynolds number

Consider a Poiseuille flow with different Reynolds numbers to test the ability of the modified SIMPLER algorithm. The incompressible Navier-Stokes equations governing the Poiseuille flow are derived from Eqs. (3.56) and (3.57) as

$$\nabla \cdot \mathbf{u} = 0 \quad (4.98)$$

$$\frac{\partial \rho \mathbf{u}}{\partial t} + \nabla \cdot (\rho \mathbf{u} \mathbf{u}) = -\nabla p + \frac{1}{Re} \Delta \mathbf{u} \quad (4.99)$$

with the boundary conditions

$$\begin{aligned} p_{inlet} &= p_0 \\ p_{outlet} &= 0 \end{aligned} \quad (4.100)$$

The exact velocity of the Poiseuille flow is given by

$$\begin{aligned} u &= \frac{p_0}{2L} Re(W - y)y \\ v &= 0 \end{aligned} \quad (4.101)$$

where L and W are the length and width of computational domain, taken as $50 \times 10 \mu\text{m}^2$. The spatial step is $\Delta x = \Delta y = 0.02$, and the time step is $\Delta t = 0.0005$.

Fig. 4.20 Comparison between the SIMPLER solution (squares) and modified SIMPLER solution (circles) for the velocity profile of Poiseuille flow with $Re = 0.001$ at the same iterations of 5,000, where the theoretical solution (solid line) is also plotted for comparison.

Fig. 4.20 shows the comparison among the SIMPLER solution, modified SIMPLER solution and theoretical result for the velocity profile of Poiseuille flow at $Re = 0.001$, where the number of iterations is fixed at 5,000. It is found that both the SIMPLER and modified SIMPLER solutions exhibit a parabolic characteristic, and are

in good agreement with the exact result. Tab. 4.5 lists their errors at the same iterations of 5,000 under the different Reynolds number. At the same iterations, the modified SIMPLER algorithm has the smaller error than the SIMPLER algorithm, indicating that the convergence is speeded up.

Tab. 4.5 Comparison between the SIMPLER and modified SIMPLER algorithms by solving the Poiseuille flow with different Reynolds number at the same iterations of 5,000.

Method	SIMPLER	Modified SIMPLER
$Re = 1$	0.015	0.005
Error (%) $Re = 10^{-3}$	0.130	0.044
$Re = 10^{-6}$	0.481	0.107

4.4.4.2 Remarks

In summary, the modified SIMPLER algorithm includes two distinct techniques, compared with the SIMPLER algorithm. One is the introduction of an improved velocity correction, which speeds up the convergence of momentum equations. The other technique is the integration of a fine adjustment, which makes the continuity equation satisfied with the higher accuracy.

According to the above tests, it is concluded that the modified SIMPLER algorithm is more accurate and convergent than the SIMPLER algorithm. It is more suitable to solve the incompressible Navier-Stokes equations with low Reynolds number.

4.5 Computational flowchart

Fig. 4.21 briefly illustrates the flowchart of the whole simulation process. The MPBLS method includes the three steps, namely *Solve binary level set equation*, *Calculate locations of particles*, and *Correct binary level set function*. The modified SIMPLER algorithm is used in the step of *Update flow field*, which consumes the majority of computational resources in the whole process.

Fig. 4.21 Flowchart of the whole simulation process.

Chapter 5

Results and Discussions

The main purpose of this chapter is to simulate and analyze the motion and deformation of a single cell or two cells in the hydrodynamic, electric, and hydrodynamic-electric coupled fields, respectively, based on the two-fluid model presented in Chapter 3 and the numerical techniques developed in Chapter 4. Before that, the two preliminary studies are performed first. They include the sensitivity analyses of mesh size in the computational domain and particle number on the cell membrane, and the tracking of the cell membrane in a steady Poiseuille flow.

5.1 Preliminary studies

5.1.1 Introduction

It is well known that the mesh size in the computational domain and particle number on the cell membrane have a significant effect on the simulation results. In order to obtain more accurate results in the following simulations, the sensitivity analyses of the mesh size and particle number are performed first by taking the deformation of a biconcave red blood cell (RBC) in an unsteady Poiseuille flow for example. After that, the cell membrane is tracked by simulating the free motion and deformation of a single RBC or two RBCs in a steady Poiseuille flow, where the membrane mechanical, dielectrophoresis (DEP) and intercellular interaction forces are not considered. Hence, the cell performance is attributed only to the shear force of the steady Poiseuille flow. This study will not only provide comparisons with the further studies, but also evaluate

the accuracy of the modified particle binary level set (MPBLS) method for tracking the cell membrane in the Poiseuille flow.

The RBC shape is expressed as [225]

$$\begin{cases} x_c = x_0 + R \cos \theta (c_0 + c_1 \sin^2 \theta + c_2 \sin^4 \theta) \\ y_c = y_0 + R \sin \theta \end{cases} \quad (5.1)$$

where (x_0, y_0) is the RBC center, R is the radius typically taken $3.91 \mu\text{m}$, θ is the polar angle variable, and c_0 , c_1 and c_2 are the three coefficients with the values [225]

$$(c_0, c_1, c_2) = (0.1035, 1.0013, -0.5614) \quad (5.2)$$

The initial velocity profile of Poiseuille flow is given by

$$\begin{cases} u = 4.0u_{\max} \frac{y}{W} \left(1 - \frac{y}{W}\right) \\ v = 0 \end{cases} \quad (5.3)$$

where $u_{\max}$ is the maximum velocity, W is the width of computational domain.

5.1.2 Sensitivity analysis

5.1.2.1 Mesh size in the computational domain

The computational domain is here set as $50 \times 10 \mu\text{m}^2$, and the maximum velocity $u_{\max}$ in Eq. (5.3) is taken as 1.25 mm/s . Five different mesh sizes in the computational domain are generated to conduct the mesh sensitivity analysis, namely $M_1(200 \times 40)$, $M_2(300 \times 60)$, $M_3(400 \times 80)$, $M_4(500 \times 100)$ and $M_5(600 \times 120)$, corresponding to 4, 6, 8, 10 and 12 meshes/ μm . Fig. 5.1 shows the effect of mesh size on the shape of deformed RBC at $t = 32 \text{ ms}$. When the mesh is relatively coarse (M_1 , M_2 and M_3), there is an obvious difference in the location and shape of deformed RBC. However, the RBC shape becomes more stable, and doesn't have significant changes when the mesh is finer (M_4 and M_5). Considering both the computational accuracy and time consumption, therefore, the simulation results reported in the present work are based on the mesh density of 10 meshes/ μm .

Fig. 5.1 Effect of mesh size on the deformed RBC shape at $t = 32$ ms.

5.1.2.2 Particle number on the cell membrane

The computational domain is also set as $50 \times 10 \text{ } \mu\text{m}^2$, and the maximum velocity $u_{\max}$ in Eq. (5.3) is still taken as 1.25 mm/s. Five different particle numbers on the cell membrane are used to analyze the particle sensitivity, namely $P_1(90)$, $P_2(180)$, $P_3(270)$, $P_4(360)$ and $P_5(450)$. The location and shape of deformed RBC at $t = 32$ ms are illustrated in Fig. 5.2. The simulation accuracy is not good enough if the membrane is divided into 90 or 180 segments, whereas the stable shape is observed in the last three cases $P_3(270)$, $P_4(360)$ and $P_5(450)$. Therefore, the simulation results reported in the present work are based on the particle number $P_4(360)$ considering the large deformation and time consumption.

Fig. 5.2 Effect of particle number on the deformed RBC shape at $t = 32$ ms.

5.1.3 Tracking of cell membrane

5.1.3.1 A single cell

The computational domain is taken as $50 \times 10 \mu\text{m}^2$, and the maximum velocity of the steady Poiseuille flow is set to $u_{\max} = 1.0 \text{ mm/s}$ in Eq. (5.3). The total time domain is set to 70 ms. The direction of Poiseuille flow is rightward before 35 ms, and then reversed. Fig. 5.3 shows the RBC shape in the steady Poiseuille flow at different time. Before $t = 35 \text{ ms}$, the RBC is stretched gradually due to the shear force of Poiseuille flow, and the stretching reaches the maximum at $t = 35 \text{ ms}$. Subsequently, the velocity direction is reversed so that the deformed RBC starts shrinking until it goes back to the initial position at $t = 70 \text{ ms}$. Theoretically, the RBC shapes at $t = 0, 10$ and 20 ms should be identical to those at $t = 70, 60$ and 50 ms , respectively. The present simulation generates a very small area error of 0.014% between the RBC shapes at $t = 0$ and 70 ms , demonstrating the high accuracy of the MPBLS method. In addition, it is asserted that the deformation of RBC should be smaller when considering the membrane mechanical force, because the RBC membrane can resist the RBC deformation in a hydrodynamic field.

Fig. 5.3 Free motion and deformation of a single RBC in a steady Poiseuille flow at different time.

5.1.3.2 Two cells

The computational domain is taken as $100 \times 20 \mu\text{m}^2$, and the maximum velocity of the steady Poiseuille flow is set to $u_{\max} = 0.5 \text{ mm/s}$ in Eq. (5.3). The total time domain is set to 320 ms. The direction of Poiseuille flow is rightward before 160 ms, and then reversed. Fig. 5.4 shows the RBC shapes in the steady Poiseuille flow at different time.

Before $t = 160$ ms, the RBCs are stretched gradually, and the stretching reaches the maximum at $t = 160$ ms. Subsequently, the velocity direction is reversed, and thus the deformed RBC starts shrinking until it goes back to the initial position at $t = 320$ ms. Compared with the case of a single RBC, the maximum stretching of two RBCs is smaller since the flow velocity is lower. The area error is 0.009% between the RBC shapes at $t = 0$ and 320 ms, smaller than that in the case of a single RBC due to the smaller stretching.

Fig. 5.4 Free motion and deformation of two RBCs in a steady Poiseuille flow at different time.

5.2 Motion and deformation of cells in the hydrodynamic field

5.2.1 Introduction

Although a considerable progress has been achieved on the cell performance in the hydrodynamic field so far, the detailed knowledge is still required, for example, the coupled effect of cell deformation and aggregation. Moreover, the excessive assumptions were often made in the most previous studies, such as the same cell density and viscosity as the fluid flow [46], and the ignorance of intercellular interaction in the case of multiple cells [44]. In the present study, the cell performance in the hydrodynamic field are investigated by simulating the motion and deformation of a single RBC or two RBCs in a capillary flow, in which the membrane mechanical force and shear force of Poiseuille flow are considered. The intercellular interaction force is also considered in the case of two RBCs. Fig. 5.5 schematically illustrated the computational domains of a single RBC or two RBCs in a capillary flow, here

characterized as a Poiseuille flow. The RBC shape and parabolic velocity profile are defined in Eqs. (5.1) and (5.3), respectively.

Fig. 5.5 Computational domains of (a) a single RBC and (b) two RBCs in a parabolic hydrodynamic field.

Tab. 5.1 Typical values of several physical quantities in numerical simulations of a single RBC in the parabolic hydrodynamic field.

Definition	Symbol	Value
Computational domain length	L	50 μm
Computational domain width	W	10 μm
Maximum flow velocity	$u_{\max}$	1.25 mm/s
Fluid density	ρ_m	$1.0 \times 10^3 \text{ kg/m}^3$
RBC density	ρ_c	$1.125 \times 10^3 \text{ kg/m}^3$
Fluid viscosity	μ_m	$1.0 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC viscosity	μ_c	$1.3 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC radius	R	3.91 μm [225]
Membrane shear modulus	E_s	$6 \times 10^{-3} \text{ dyn/cm}$ [198]
Membrane bending stiffness	E_B	$1.8 \times 10^{-12} \text{ dyn} \cdot \text{cm}$ [198]

5.2.2 A single cell in a capillary flow

In this subsection, comprehensive parametric studies are conducted to provide a complete understanding of a single cell in a capillary flow. According to the sensitivity analysis, the mesh size and particle number are taken as 500×100 and 360 for discretizing the computational domain and cell membrane, respectively. Tab. 5.1 lists typical values of several physical quantities for numerical simulations. Note that the density and viscosity of RBC are different with those of suspending fluid.

5.2.2.1 Validation

Previous studies [45, 47, 226] showed that the RBC in the Poiseuille flow deforms gradually from the biconcave shape to a parachute one, depending on the RBC velocity. In the present validation, the RBC deformation at the different velocities are analyzed and compared with the previous studies.

Fig. 5.6 Comparisons of (a) the steady-state RBC shape with the numerical predications of Secomb [45] and Hosseini et al. [47], and (b) the deformation index with the experimental observations of Tsukada et al. [226].

Fig. 5.6(a) shows the comparison with the two numerical results [45, 47] reported previously, where a steady-state RBC shape is simulated at the velocity of 0.1 mm/s. It is found that the steady-state RBC shape exhibits a characteristic parachute shape, convex in the front and concave at the rear. This parachute shape guarantees that the RBC successfully traverses through the capillary with the diameter smaller than that of undeformed RBC in microcirculation. A good agreement is observed with the results of Secomb [45] and Hosseini et al. [47], except a slight difference of RBC area attributed to the mechanical model of RBC membrane [47]. Furthermore, a quantitative comparison with the experimental data [226] is also carried out, where the relationship between the RBC velocity and deformation index (defined as the ratio of the length to width of RBC) is examined, as demonstrated in Fig. 5.6(b). An increasing trend of deformation index is found, and agrees with the exponential fitting line of experimental data of Tsukada et al. [226].

5.2.2.2 Effect of cell shape

Fig. 5.7 shows the motion and deformation of a single RBC with different initial shapes in a capillary flow. With increasing time, the RBC is gradually stretched along the x direction, but shrinks along the y direction, such that it gradually deforms into a parachute shape, convex in the front and concave at the rear. However, the circular, prolate and oblate RBCs deform much less concave at the rear and more convex in the front than the biconcave RBC. In addition, compared with the results without considering the membrane mechanical force illustrated in Fig. 5.3, the RBC deformation in a capillary flow is remarkably smaller due to the resistance of membrane mechanical force.

In order to conduct a quantitative analysis, two measures [227] are introduced to evaluate the RBC deformation and direction, namely the extension (EX) and orientation (OR). Extension is a measure how much the deformed shape differs from a circle. It takes a minimum value of zero for the circular shape, and increases without limit as the shape becomes less compact. Orientation is used to evaluate the direction of deformed shape, defined as the angle of the long axis of shape measured positive anticlockwise from the vertical axis ranging from $-\pi/2$ to $\pi/2$. The detailed formulae for the two definitions can be obtained in Appendix B. Fig. 5.8 plots the variations of the extension and orientation of the single RBC with the different initial shapes during the

deformation. For the circular RBC, the extension increases from zero to a nonzero constant value, indicating that the RBC shape changes from the initial circular shape to a steady non-circular shape. Its orientation jumps suddenly from 0 to $\pi/2$, meaning the long axis of deformed shape changes rapidly from the y axis to x axis. The extension of prolate RBC decreases first and then increases, because of the conversion between the long and short axes of deformed RBC. At the beginning, the long axis of RBC becomes gradually short, and approaches to the short axis, resulting in the decreasing extension. Subsequently, the conversion between the short and long axes occurs, and the initial short axis keeps on lengthening, leading to the increasing extension. It is found from Fig. 5.8(b) that this conversion happens at about $t=3.5$ ms, because the orientation changes from 0 to $-\pi/2$ at that moment. For the biconcave RBC, the extension decreases gradually, i.e., it becomes rounder and rounder, and thus its orientation gradually approaches to $\pi/2$ from 0. The extension of oblate RBC increases with the time, similar to that of circular RBC, and the orientation keeps a constant $\pi/2$, meaning that its long axis is along the x axis all the time, as illustrated in Fig. 5.7(d).

Fig. 5.7 Motion and deformation of a single RBC with different initial shapes, (a) the circular, (b) prolate, (c) biconcave, and (d) oblate shapes in a capillary flow.

Fig. 5.8 Variations of (a) the extension and (b) orientation of the single RBC with the different initial shapes in the capillary flow.

5.2.2.3 Effect of cell radius

Fig. 5.9 displays the motion and deformation of a single RBC with different radii, 4.5, 3.91, 3.0 and 2.0 μm in a capillary flow. The RBC moves faster and deforms rounder with decreasing the radius. This is because the small RBC is more centralized in the microchannel, and experiences a smaller shear force. The distribution of shear force in the microchannel is expressed as

$$\tau_{shear} = 4.0\mu u_{\max} \left(\frac{1}{W} - \frac{2y}{W^2} \right) \quad (5.4)$$

According to this formula, the shear force decreases from the lower and upper boundaries to the centerline of microchannel, and it is equal to zero on the centerline.

The variations of extension and orientation of the single RBC with the different radii are illustrated in Fig. 5.10. At the initial state, both the measures are identical due to the geometric similarity. With increasing time, the extension of each RBC decreases. The decreasing speed is faster for the smaller RBC, meaning that the smaller RBC is more apt to be a circle. In addition, at the beginning the orientation of each RBC is equal to zero, indicating that the long axis of initial RBC is along the y axis. Subsequently, the larger RBC changes the long axis along the x axis more quickly, i.e., its orientation reaches $\pi/2$ earlier. For the RBC with the radius of 2.0 μm , the orientation almost keeps a constant, and therefore the long axis never changes.

Fig. 5.9 Motion and deformation of a single RBC with different initial radii in a capillary flow, (a) $R = 4.5 \mu\text{m}$, (b) $R = 3.91 \mu\text{m}$, (c) $R = 3.0 \mu\text{m}$, and (d) $R = 2.0 \mu\text{m}$.

Fig. 5.10 Variations of (a) the extension and (b) orientation of the single RBC with the different initial radii in the capillary flow.

5.2.2.4 Effect of cell position

Fig. 5.11 depicts the motion and deformation of a single RBC at different initial positions in a capillary flow, whose centre is placed at $(10 \mu\text{m}, 4.5 \mu\text{m})$, $(10 \mu\text{m}, 5 \mu\text{m})$, and $(10 \mu\text{m}, 5.5 \mu\text{m})$, respectively. When the RBC center deviates slightly, the motion and deformation of RBC are quite different. If the RBC is symmetric with respect to the centerline of Poiseuille flow at the initial state, it experiences a symmetric motion with a

characteristic parachute shape, as illustrated in Fig. 5.11(b). However, an asymmetric motion occurs if the RBC is not symmetric with the centerline, where the slipper shape is assumed, as shown in Fig. 5.11(a) and (c). In the asymmetric motion, the RBC membrane rotates around the RBC interior, well known as the tank-treading motion [37]. When the RBC center is not located at the centerline of Poiseuille flow, its upper and lower parts undergo the unbalanced velocity and shear force, thus leading to the asymmetric motion and tank-treading mode.

Fig. 5.12 illustrates the variations of extension and orientation of the single RBC at the different initial positions. The extension of RBC at $(10 \mu\text{m}, 4.5 \mu\text{m})$ is almost identical to that of the RBC at $(10 \mu\text{m}, 5.5 \mu\text{m})$, because the deviation values of RBC center are the same, resulting in the same similarity to a circle. However, the orientation is opposite in these two cases. The RBC tilts clockwise when its centre is below the centerline of Poiseuille flow, or tilts anticlockwise, as shown in Fig. 5.11. The extension of RBC at $(10 \mu\text{m}, 5 \mu\text{m})$ is smallest, and the orientation increases gradually from 0 to $\pi/2$. Hence, the RBC at $(10 \mu\text{m}, 5 \mu\text{m})$ becomes roundest, and its long axis changes gradually from the y axis to x axis.

Fig. 5.11 Motion and deformation of a single RBC at different initial positions in a capillary flow, whose center (x_0, y_0) is initially located at (a) $(10 \mu\text{m}, 4.5 \mu\text{m})$, (b) $(10 \mu\text{m}, 5 \mu\text{m})$, and (c) $(10 \mu\text{m}, 5.5 \mu\text{m})$, and the filled point refers to the motion of a fixed representative particle on the cell membrane.

Fig. 5.12 Variations of (a) the extension and (b) orientation of the single RBC at the different initial positions in the capillary flow.

5.2.2.5 Effect of cell deformability

Fig. 5.13 illustrates the motion and deformation of a single RBC with different elastic moduli and bending stiffnesses in a capillary flow, namely $0.006\ \text{dyn/cm}$ and $1.8 \times 10^{-12}\ \text{dyn cm}$, $0.03\ \text{dyn/cm}$ and $9 \times 10^{-12}\ \text{dyn cm}$, and $0.12\ \text{dyn/cm}$ and $3.6 \times 10^{-11}\ \text{dyn cm}$. As expected, the resistance to the RBC deformation becomes stronger and stronger with increasing the elastic modulus and bending stiffness, and therefore the corresponding RBC deformation decreases. In turn, the decreasing deformation results in the decreasing membrane mechanical force strength, as shown in Fig. 5.14. At the RBC tails, the curvature of cell membrane is large so that the bending deformation is dominant. With increasing the elastic modulus and bending stiffness, the RBC tail deforms more smoothly, and therefore the membrane mechanical force decreases at the RBC tails. At the central part of RBC, the curvature of cell membrane is small, and the RBC is stretched obviously, and thus the stretching deformation is dominant. With increasing the elastic modulus and bending stiffness, the central part of RBC is less convex, and the stretching deformation decreases. Therefore, the membrane mechanical force strength also decreases at the central part of RBC.

Fig. 5.15 plots the variations of extension and orientation of the single RBC with the different elastic moduli and bending stiffnesses. The RBC extension changes smaller and smaller from the initial value with increasing the elastic moduli and bending stiffnesses, because the RBC is decreasingly stretched along the x direction. The orientation of RBC with the small elastic modulus and bending stiffness of 0.006

dyn/cm and 1.8×10^{-12} dyn cm gradually increases from 0 to $\pi/2$, meaning that the long axis of RBC changes from the y axis to x axis. However, the orientation of RBC with the large elastic modulus and bending stiffness in the last two cases keeps a constant of zero, indicating that the long axis of RBC is always along the y direction during the deformation.

Fig. 5.13 Motion and deformation of a single RBC with different elastic shear moduli and bending stiffnesses in a capillary flow, (a) $E_S = 0.006$ dyn/cm and $E_B = 1.8 \times 10^{-12}$ dyn cm, (b) $E_S = 0.03$ dyn/cm and $E_B = 9.0 \times 10^{-12}$ dyn cm, and (c) $E_S = 0.12$ dyn/cm and $E_B = 3.6 \times 10^{-11}$ dyn cm.

Fig. 5.14 Distribution of the membrane mechanical force strength acting on the single RBC with the different elastic shear moduli and bending stiffnesses at $t = 32$ ms, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.15 Variations of (a) the extension and (b) orientation of the single RBC with the different elastic moduli and bending stiffnesses in the capillary flow, in which $E_S = 0.006$ dyn/cm and $E_B = 1.8 \times 10^{-12}$ dyn cm.

5.2.2.6 Effect of cell density

Many studies [46, 49] reported previously on the cell behavior in a capillary flow were based on the assumptions of the same cell density and viscosity as the fluid. In fact, the cell density has a significant effect. Extremely, the cell is split like a gas bubble if its density is small enough. On the other extreme side, the RBC is not deformable like a rigid body, if its density is sufficiently large. Fig. 5.16 shows the motion and deformation of a single RBC with different densities, 950, 1000, 1125 and 1250 kg/m³ in a capillary flow. The RBC is increasingly stretched with decreasing the RBC density, and thus the central part of RBC becomes thinner and thinner. When the RBC density is 950 kg/m³, a slight decrease from the fluid density, the RBC is stretched severely, and almost cracked. On the contrary, the central part of RBC is stretched weakly and quite thick when the RBC density is 1250 kg/m³. Therefore, the slight change of RBC density can result in the remarkable difference of RBC deformation.

The variations of extension and orientation of the single RBC with the different densities are plotted in Fig. 5.17. Since the RBC with a larger density is more similar to a circle, its extension decreases with increasing the density. In addition, the orientation changes from 0 to $\pi/2$ earlier as the RBC density becomes larger. For the cases of the density 950 and 1000 kg/m³, it never changes, meaning that the RBC keeps its long axis along the y axis.

Fig. 5.16 Motion and deformation of a single RBC with different densities in a capillary flow, (a) $\rho_c = 950 \text{ kg/m}^3$, (b) $\rho_c = 1000 \text{ kg/m}^3$, (c) $\rho_c = 1125 \text{ kg/m}^3$, and (d) $\rho_c = 1250 \text{ kg/m}^3$.

Fig. 5.17 Variations of (a) the extension and (b) orientation of the single RBC with the different densities in the capillary flow.

5.2.2.7 Effect of cell viscosity

Fig. 5.18 illustrates the motion and deformation of a single RBC with different viscosities, 0.5, 1.0, 1.3 and 2.0 mPa s in a capillary flow. It is observed that the RBC deformation is quite similar except a slight difference in these four cases. Therefore, the

RBC viscosity has a minor effect on the RBC performance in a capillary flow. The RBC tails are increasingly stretched with increasing its viscosity, while the central part of RBC becomes narrow to keep the area conservation.

Fig. 5.18 Motion and deformation of a single RBC with different viscosities in a capillary flow, (a) $\mu_c = 0.5$ mPa s, (b) $\mu_c = 1.0$ mPa s, (c) $\mu_c = 1.3$ mPa s, and (d) $\mu_c = 2.0$ mPa s.

Fig. 5.19 Variations of (a) the extension and (b) orientation of the single RBC with the different viscosities in the capillary flow.

Fig. 5.19 plots the extension and orientation of the single RBC with the different viscosities, where only a subtle difference is observed. With increasing the RBC

viscosity, the extension also increases slightly, indicating that the RBC is stretched more obviously. The orientation changes from 0 to $\pi/2$ later for the large RBC viscosity, meaning that the RBC changes its long axis later.

5.2.2.8 Effect of flow velocity

It is well known that the initial velocity has a major effect on the motion and deformation of RBC, because there is a direct relationship between the shear force and velocity field [45, 47, 226]. In order to clarify this effect, the motion and deformation of a single RBC are simulated subject to four maximum initial velocities, 0.5, 1.25, 2.0 and 3.0 mm/s, as shown in Fig. 5.20. With increasing the maximum initial velocity, the RBC tails are stretched more and more severely, because the shear force is proportional to the flow velocity via Eq. (5.4). However, the central part becomes increasingly narrow to keep the area conservation.

Fig. 5.20 Motion and deformation of a single RBC subject to different initial velocities in a capillary flow, (a) $u_{\max} = 0.5$ mm/s, (b) $u_{\max} = 1.25$ mm/s, (c) $u_{\max} = 2.0$ mm/s, and (d) $u_{\max} = 3.0$ mm/s.

Fig. 5.21 illustrates the variations of extension and orientation of the single RBC subject to the different initial velocities. The larger the initial velocity is, the larger the extension will be, indicating the RBC becomes less similar to a circular shape.

Moreover, the orientation changes earlier from 0 to $\pi/2$, i.e., the RBC changes its long axis from the y axis to x axis earlier. The shear force increases as the initial velocity increases so that the RBC is stretched largely along the x direction. Therefore, the RBC looks more protruded, and changes earlier its long axis from the y axis to x axis.

Fig. 5.21 Variations of (a) the extension and (b) orientation of the single RBC subject to the different initial velocities in the capillary flow.

Tab. 5.2 Typical values of additional physical quantities in numerical simulations of two RBCs in the parabolic hydrodynamic field.

Definition	Symbol	Value
Computational domain length	L	100 μm
Computational domain width	W	20 μm
Maximum flow velocity	$u_{\max}$	1.0 mm/s
Intercellular interaction strength	D_e	1.0 μJ [28]
Scaling factor	β	3.84 μm^{-1} [28]
Zero force distance	r_0	0.49 μm [28]

5.2.3 Two cells in a capillary flow

In this subsection, the motion, deformation and aggregation of two RBCs are simulated with considering the intercellular interaction, and four main parameters are examined, namely the initial RBC shape, initial RBC position, RBC deformability, and intercellular interaction strength. The computational domain and cell membrane are

discretized into 1000×200 meshes and 360 segments, respectively. The two RBCs have the same physical properties, and typical values of additional physical quantities are listed in Tab. 5.2, except Tab. 5.1.

5.2.3.1 Validation

The RBC aggregation in static blood plasma is simulated and compared with the results published previously by Wang et al. [33]. Fig. 5.22 shows the comparison of the equilibrium configuration of RBC rouleaux with different intercellular strengths D_e , 0.0, 0.1, and $4.5 \mu\text{J}/\text{m}^2$.

The simulation domain is defined as $20 \times 20 \mu\text{m}^2$, and the initial distance is taken as $3.1 \mu\text{m}$ between the centers of two RBCs, as shown in Fig. 5.22(a). In order to follow the same conditions of Wang et al. [33], it is assumed that the RBC has the same density and viscosity as the blood plasma. The zero force distance is picked as $r_0 = 0.49 \mu\text{m}$, and the scaling factor as $\beta = 80 \mu\text{m}^{-1}$. As time elapses, the RBCs move towards each other until an equilibrium state is reached, where the intercellular interaction force strength and membrane mechanical force strength are balanced each other. The equilibrium shapes of RBCs are concave at the low interaction strength as illustrated in Fig. 5.22(b), but convex at the high interaction strength as shown in Fig. 5.22(c), which is in a good agreement with the results of Wang et al. [33].

Fig. 5.22 Comparisons of the equilibrium configuration of RBC rouleaux in static blood plasma with different intercellular interaction strengths, (a) $D_e = 0.0 \mu\text{J}/\text{m}^2$, (b) $D_e = 0.1 \mu\text{J}/\text{m}^2$, and (c) $D_e = 4.5 \mu\text{J}/\text{m}^2$, between the present simulations (solid line) and the results (dots) of Wang et al. [33].

5.2.3.2 Effect of cell shape

Fig. 5.23 illustrates the motion and deformation of two RBCs with different initial shapes in a capillary flow, namely biconcave, elliptical and circular shapes. At the initial state, the minimum distance between the two RBCs is fixed to $2.0\ \mu\text{m}$ so that an attractive trend will be present in all three cases. The two RBCs with the biconcave shape gradually deform into a well-known parachute shape at $t = 80\ \text{ms}$. However, the RBCs with the elliptical and circular shapes less deform into a parachute shape. The different performance is totally attributed to the volumetric ratio of RBCs to whole fluid in a capillary, called the hematocrit (Hct). As well known, the shape and size of RBC largely determine the RBC hematocrit. Here the RBCs with the biconcave, elliptical and circular shapes correspond to hematocrit of 0.0136, 0.0351 and 0.0361, respectively. At the low Hct, the RBCs are distributed dispersedly in the capillary so that the fluid flow has the chance to develop after passing the RBCs. The shear force of flow influences the RBCs dominantly, and thus the RBCs easily deform into a parachute shape. As the Hct increases however, the fluid flow is more blocked by the RBCs and hard to develop. Consequently, the RBCs are less deformed into a parachute shape. These simulation results are in good agreement with the previous studies [51, 52], and easily observed in multiple RBCs in a capillary.

Fig. 5.23 Motion and deformation of two RBCs with different initial shapes, (a) the biconcave, (b) elliptical, and (c) circular shapes in a capillary flow.

Fig. 5.24 illustrates the variations of extensions and orientations of the two RBCs with the different initial shapes. For the RBCs with the biconcave shape, the extensions decrease, meaning that they are stretched along the x direction. However, they do not convert their long axes from the y axis to x axis, because the orientations almost keep the zero constant. For the left RBC with the elliptical shape, the extension decreases first and then increases. This is because its long axis is converted subject to the capillary flow, and thus its orientation changes from 0 to $\pi/2$. For the right RBC with the elliptical shape however, the extension decreases all the time, and the orientation keeps a zero constant, indicating that it maintains its long axis. For the RBCs with the circular shape, the extensions increase and the orientations jump from 0 to $\pi/2$ at the beginning, meaning that they are stretched along the x direction. However, the orientation of the right circular RBC decreases subsequently because of the squeeze of left RBC.

Fig. 5.24 Variations of the extensions of (a) the left and (b) right RBCs, and the orientations of (c) the left and (d) right RBCs with the different initial shapes in the capillary flow.

Fig. 5.25 shows the distributions of membrane mechanical force strength and intercellular interaction force strength acting on the RBC membranes with the biconcave shape at $t = 80$ ms. The membrane mechanical force strength is larger in the vicinity of RBC tails because of the dominant bending deformation. At the central parts of RBCs however, it is quite small since the stretching and bending deformation is not large. The intercellular interaction force strength assumes an attractive trend, as shown in Fig. 5.25 (b), and thus these two RBCs attract each other.

Fig. 5.25 Distributions of (a) the membrane mechanical force strength and (b) intercellular interaction force strength acting on the RBC membranes with the biconcave shape at $t = 80$ ms, in which the arrow indicates the direction and magnitude of force strength.

5.2.3.3 Effect of cell position

Generally, RBCs enter a capillary from an arbitrary position at the inlet, and therefore the effect of initial position of two RBCs is examined here, as shown in Fig. 5.26. At the initial state, the minimum distance between the two RBCs is fixed to $2.0 \mu\text{m}$. It is observed that the RBC deformation is significantly different, when the RBCs enter the capillary from the different positions. The RBCs with the vertical layout deform into a parachute shape, whereas a tadpole-like shape is exhibited for the RBCs with the horizontal layout. In the oblique and stagger layouts however, the RBCs deform into the slapper shape. This deformation behavior is attributed to the different shear force of capillary flow acting on the different parts of RBC. For example, in the oblique and stagger layouts, each of them is not symmetric with respect to the centerline of microchannel, such that the different parts of RBC are subject to the asymmetric shear

force. Therefore, the RBCs undergo the asymmetric motion, and exhibit the characteristic slipper shapes. In addition, the attraction between two RBCs is also different. The RBCs seem far away from each other if they are positioned horizontally and staggerly. However, they attract gradually each other in the vertical and oblique layouts. This is because the distance between two RBCs is different, thus leading to the different intercellular interaction force.

Fig. 5.26 Motion and deformation of two RBCs at different initial positions, (a) the vertical, (b) horizontal, (c) oblique, and (c) stagger layouts in a capillary flow.

Fig. 5.27 plots the intercellular interaction force acting on a RBC denoted by solid line in Fig. 5.26, defined by integrating the intercellular interaction force strength f_{int} over the cell membrane Γ ,

$$\mathbf{F}_{int} = \int_{\Gamma} \mathbf{f}_{int} ds \quad (5.5)$$

It is found that its x component is largest for the RBCs with the vertical layout, whereas its y component is largest for the RBCs with the oblique layout. Therefore, in these two layouts, the attraction between the two RBCs is strongest, and the intercellular distance is smallest. It should be noted that the intercellular interaction force acting on

the other RBC denoted by dotted line in Fig. 5.26 is opposite to that acting on the previous RBC.

Fig. 5.27 Variations of (a) the x component and (b) y component of intercellular interaction force acting on the RBC denoted by solid line in Fig. 5.26 with the different initial positions.

Fig. 5.28 illustrates the variations of extensions and orientations of the two RBCs at the different initial positions. In the vertical layout, the left RBC has the smaller extension than the right RBC, meaning that the left RBC becomes more round. Except in the vertical layout however, both the RBCs have the similar extension but opposite orientation in the horizontal, oblique and stagger layouts respectively, because the symmetric deformation of RBCs is found in Fig. 5.26. Moreover, the RBCs in the vertical and horizontal layouts maintain their initial long axes during the deformation in that their orientations almost don't change. In the oblique layout, the extensions of both RBCs decrease first, then increase and finally decrease, meaning that the two RBCs shrink first, are then stretched and finally shrink, as shown in Fig. 5.26(c). The orientation of the RBC denoted by solid line in Fig. 5.26(c) changes from $\pi/4$ to $-\pi/4$, indicating that the RBC rotates anticlockwise $\pi/2$. On the contrary, the other RBC denoted by dotted line in Fig. 5.26(c) rotates clockwise $\pi/2$, and its orientation changes from $-\pi/4$ to $\pi/4$. In the stagger layout, the RBC extensions increase first and then decrease, and therefore both the RBCs are stretched first and then shrink. The RBCs rotate clockwise and anticlockwise from the y axis respectively, and the corresponding orientations change from 0 to $\pi/2$ and $-\pi/2$.

Fig. 5.28 Variations of the extensions of (a) the left and (b) right RBCs, and the orientations of (c) the left and (d) right RBCs at the different initial positions in the capillary flow.

5.2.3.4 Effect of cell deformability

Fig. 5.29 illustrates the motion and deformation of two RBCs with different elastic moduli and bending stiffnesses in a capillary flow, namely 0.006 dyn/cm and $1.8 \times 10^{-12} \text{ dyn cm}$, 0.03 dyn/cm and $9 \times 10^{-12} \text{ dyn cm}$, and 0.12 dyn/cm and $3.6 \times 10^{-11} \text{ dyn cm}$. At the low elastic modulus and bending stiffness, the RBC membranes weakly resist to the deformation so that the RBCs easily deform into a parachute shape, as shown in Fig. 5.29(a). At the high elastic modulus and bending stiffness, the RBCs is more rigid, and thus more difficult to deform, as illustrated in Fig. 5.29(c). In other words, the deformed RBCs are more and more similar to the initial RBCs with increasing the elastic modulus and bending stiffness.

Fig. 5.29 Motion and deformation of two RBCs with different elastic moduli and bending stiffnesses in a capillary flow, (a) $E_S = 0.006$ dyn/cm and $E_B = 1.8 \times 10^{-12}$ dyn cm, (b) $E_S = 0.03$ dyn/cm and $E_B = 9.0 \times 10^{-12}$ dyn cm, and (c) $E_S = 0.12$ dyn/cm and $E_B = 3.6 \times 10^{-11}$ dyn cm.

Fig. 5.30 Variations of (a) the average magnitude of membrane mechanical force strength, and (b) the x component of intercellular interaction force acting on the two RBCs with the different elastic moduli and bending stiffnesses, in which $E_S = 0.006$ dyn/cm and $E_B = 1.8 \times 10^{-12}$ dyn cm.

Fig. 5.30 plots the variations of the average magnitude of membrane mechanical force strength, and the x component of intercellular interaction force defined in Eq. (5.5) acting on the RBCs. The average magnitude of membrane mechanical force strength is defined as

$$|\bar{f}_{mem}| = \frac{\int_{\Gamma} \sqrt{|f_{mem}|^2} ds}{S} \quad (5.6)$$

where S is the total peripheral length of RBC membrane. With increasing the elastic modulus and bending stiffness, the average magnitude of membrane mechanical force strength decreases. This is because the membrane resistance to the RBC deformation becomes increasingly strong, and thus the RBC deformation is smaller and smaller. Meanwhile, the distance between the RBCs increases, such that the intercellular interaction force decreases with increasing the elastic modulus and bending stiffness, as illustrated shown in Fig. 5.30(b). In other words, the RBC aggregation also decreases if the RBC deformation decreases. In addition, since the left and right RBCs in each case have the similar deformation, their average magnitude of membrane mechanical force strengths are quite similar, and their intercellular interaction forces are reversed directly.

Fig. 5.31 Variations of the extensions of (a) the left and (b) right RBCs, and the orientations of (c) the left and (d) right RBCs with the different elastic moduli and bending stiffnesses in the capillary flow.

Fig. 5.31 illustrates the variations of extensions and orientations of the two RBCs with the different elastic moduli and bending stiffnesses. The extensions of both the RBCs change smaller and smaller from the initial values with increasing the elastic moduli and bending stiffnesses due to the decreasing RBC deformation. The extension of left RBC is similar to that of right RBC in each case, meaning that the deformations of both the RBCs are quite similar, as shown in Fig. 5.29. In addition, the orientations of the left and right RBCs decrease slightly with increasing the modulus and bending stiffness, indicating that the RBCs at the low modulus and bending stiffness are stretched more obviously along the x direction. However, all the orientations almost keep a constant of zero, i.e., all the RBCs don't change their long axes from the y axis to x axis.

5.2.3.5 Effect of intercellular interaction strength

Fig. 5.32 shows the motion and deformation of two RBCs with different intercellular interaction strengths D_e , 0.0, 1.0 and 5.0 $\mu\text{J}/\text{m}^2$ in a capillary flow. It is found that the deformations of RBCs are quite similar at the three intercellular strengths. However, the minimum distance between the two RBCs at $t = 80$ ms decreases slightly from 1.0, 0.65 to 0.45 μm , with increasing the intercellular strength from 0.0, 1.0 to 5.0 $\mu\text{J}/\text{m}^2$. This is because the intercellular attractive force increases with increasing the intercellular strength, as plotted in Fig. 5.33. At the intercellular interaction strength $D_e = 0.0$ $\mu\text{J}/\text{m}^2$, the intercellular interaction force is not considered, and thus the motion and deformation of RBCs result from the shear force and membrane mechanical force. At $D_e = 1.0$ $\mu\text{J}/\text{m}^2$, only the weak attractive force appears, which shortens the distance between the two RBCs. At $D_e = 5.0$ $\mu\text{J}/\text{m}^2$ however, the repulsive force is observed at about $t = 80$ ms due to the small intercellular distance, except the attractive force, as shown in Fig. 5.33. Subject to the repulsive force, the two RBCs start being squeezed oppositely and deformed more compactly, as illustrated in Fig. 5.32(c). Therefore, it can be concluded that the intercellular interaction force has an indirect effect on the membrane mechanical force via the RBC deformation. Although the effect is very slight, an increasing trend of the membrane mechanical force strength is found with increasing the intercellular interaction strength, as plotted in Fig. 5.33(a). In other words, the RBC deformation also increases if the RBC aggregation increases.

Fig. 5.32 Motion and deformation of two RBCs with different intercellular interaction strengths in a capillary flow, (a) $D_e = 0.0 \mu\text{J}/\text{m}^2$, (b) $D_e = 1.0 \mu\text{J}/\text{m}^2$, and (c) $D_e = 5.0 \mu\text{J}/\text{m}^2$.

Fig. 5.33 Variations of (a) the average magnitude of membrane mechanical force strength, and (b) the x component of intercellular interaction force acting on the two RBCs with the different intercellular interaction strengths.

Fig. 5.34 illustrates the variations of extensions and orientations of the two RBCs with the different intercellular interaction strengths. Because all the RBCs experience the similar deformations, they exhibit the similar decreasing extension and almost zero constant orientation. In other words, all the RBCs are stretched along the x direction, and keep their long axes along the y axis. However, a slight difference occurs on the

extension of the right RBC at $D_e = 5.0 \mu\text{J}/\text{m}^2$ during the time interval from 60 to 80 ms, as plotted in Fig. 5.34(b), which results from the intercellular interaction force. At about $t = 60$ ms, the intercellular attractive force reaches the maximum, and then decreases with decreasing the intercellular distance until the repulsive force appears about $t = 80$ ms, as plotted in Fig. 5.33(b). Therefore, during the time interval from 60 to 80 ms, the intercellular distance decreases gradually, such that the right RBC is squeezed along the y direction, and thus its extension exhibits an increasing trend.

Fig. 5.34 Variations of the extensions of (a) the left and (b) right RBCs, and the orientations of (c) the left and (d) right RBCs with the different elastic moduli and bending stiffnesses in the capillary flow.

5.3 Motion and deformation of cells in the electric field

5.3.1 Introduction

To date, very few studies have focused on the deformation behavior of cells in the nonuniform AC electric field, which is a quite challenging topic involved with the DEP

phenomenon of deformable cells. Therefore, the present study numerically simulates the motion and deformation of a single RBC or two RBCs in a nonuniform AC electric field, where both the DEP force and membrane mechanical force are included. Moreover, the intercellular interaction force is also considered in the case of two RBCs. Fig. 5.35 illustrated the computational domains of a single RBC or two RBCs in the nonuniform electric field, which is generated by the electrodes in a period only. The RBC shape is given by Eq. (5.1), and the uniform velocity is employed so that the shear force of fluid flow is absent here.

Fig. 5.35 Computational domains of (a) a single RBC and (b) two RBCs in a nonuniform electric field.

5.3.2 A single cell in a nonuniform AC electric field

It was demonstrated that the motion of a rigid cell in a nonuniform electric field depends on various factors, including the geometrical and dielectric properties of cell, and frequency and strength of electric field [85]. In this subsection, these factors are also examined to analyze their effects on the motion of a deformable cell. According to the sensitivity analysis, the mesh size in the computational domain and particle number on the cell membrane are taken as 500×100 and 360, respectively. Tab. 5.3 lists typical values of several physical quantities for numerical simulations.

Tab. 5.3 Typical values of several physical quantities in numerical simulations of a single RBC in the nonuniform electric field.

Definition	Symbol	Value
Computational domain length	L	50 μm
Computational domain width	W	10 μm
Uniform flow velocity	u_{uni}	1.25 mm/s
Fluid density	ρ_m	$1.0 \times 10^3 \text{ kg/m}^3$
RBC density	ρ_c	$1.125 \times 10^3 \text{ kg/m}^3$
Fluid viscosity	μ_m	$1.0 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC viscosity	μ_c	$1.3 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC radius	R	3.91 μm [225]
Membrane shear modulus	E_s	$6 \times 10^{-3} \text{ dyn/cm}$ [198]
Membrane bending stiffness	E_B	$1.8 \times 10^{-12} \text{ dyn} \cdot \text{cm}$ [198]
Electrode width	d_1	12.5 μm
Electrode gap	d_2	12.5 μm
Fluid permittivity	ξ_m	$80 \xi_0$
Fluid conductivity	σ_m	0.8 mS/m
RBC interior permittivity	ξ_{in}	$57 \xi_0$ [106]
RBC interior conductivity	σ_{in}	0.52 S/m [106]
RBC membrane capacitance	C_{mem}	11.8 mF/m ² [106]
RBC membrane conductance	G_{mem}	271 S/m ² [106]
Electrode potential	V_0	1.0 V
Electric frequency	f	10^9 Hz

5.3.2.1 Validation

The validation of a single cell in a nonuniform AC electric field is carried out by comparing with the numerical predictions published previously by Cao et al. [125], where the levitation height of latex beads was measured as a function of the electrode potential. The electrodes were situated at the microchannel bottom with consideration of the gravity. Subject to the nonuniform electric field, the DEP force is generated, and

levitates the latex bead to a certain height to balance with the gravity. The latex bead was assumed as a homogeneous dielectric with the constant permittivity and conductivity. The parameter values reported by Cao et al. [125] for the permittivity and conductivity of latex beads and suspending fluid, $\xi_c = 2.55\xi_0$ (ξ_0 the vacuum permittivity), $\sigma_c = 0.2$ mS/m, $\xi_m = 78\xi_0$, $\sigma_m = 10$ mS/m, are also used in the present validation without modification. At the electric frequency $f = 10^6$ Hz, the levitation heights of the latex beads with the radii of 0.5 and 2.0 μm are plotted in Fig. 5.36, and compared with the numerical results of Cao et al. [125]. Here a good agreement between them is achieved, although the deformation of latex beads is considered in the present validation. This is because the radii of latex beads are significantly smaller than the microchannel width of 80 μm , and thus the deformation of latex beads is negligibly small.

Fig. 5.36 Comparison of the levitation heights of latex beads with the radii of 0.5 and 2.0 μm predicted in the present validation and reported by Cao et al. [125].

5.3.2.2 Effect of cell size

Fig. 5.37 shows the motion and deformation of a single RBC with different radii, 4.5, 3.91, 3.0 and 2.0 μm in a nonuniform AC electric field. With decreasing the RBC radius, the RBC deformation becomes smaller and smaller, and even the deformation of RBC with the radius 2.0 μm is almost negligible. Therefore, the dielectric body is often treated as a rigid one in most studies of the DEP phenomenon, when its size is very small compared with the characteristic length. This is explained by the fact that the DEP force acting on a dielectric body is proportional to its volume, as plotted in Fig. 5.38,

where the DEP force is defined by integrating the DEP force strength f_{dep} over the cell membrane Γ ,

$$\mathbf{F}_{dep} = \int_{\Gamma} \mathbf{f}_{dep} ds \quad (5.7)$$

Fig. 5.37 Motion and deformation of a single RBC with different initial radii in a nonuniform electric field, (a) $R = 4.5 \mu\text{m}$, (b) $R = 3.91 \mu\text{m}$, (c) $R = 3.0 \mu\text{m}$, and (d) $R = 2.0 \mu\text{m}$.

Fig. 5.39 illustrates the distribution of DEP force strength acting on the RBC with different radii at $t = 40 \text{ ms}$. It is found that the DEP force strength is directed inward, and stronger at the lower part of RBC due to the closeness to the electrodes. As one result, the lower part of RBC is squeezed and becomes narrow with increasing time, while the upper part becomes wide to keep the area conservation, especially for the RBC with the radii of 4.5 and $3.91 \mu\text{m}$. As the other result, the DEP force is directed upward or away from the region of strong electric field, i.e., its y component is larger than zero, such that the RBC moves up as shown in Fig. 5.37. The RBC becomes more and more round subject to this inward DEP force strength with increasing the radius, and therefore its extension decreases, as plotted in Fig. 5.40(a). The RBC with the different radii has the similar orientation, as plotted in Fig. 5.40(b), because it tilts the similar angle in the four cases.

Fig. 5.38 Variation of the magnitude of DEP force acting on the single RBC with the different radii in the nonuniform electric field.

Fig. 5.39 Distribution of the DEP force strength acting on the single RBC with the different radii at $t = 40$ ms, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.40 Variations of (a) the extension and (b) orientation of the single RBC with the different initial radii in the nonuniform electric field.

5.3.2.3 Effect of electrode potential

Fig. 5.41 shows the motion and deformation of a single RBC subject to the excitation of different electrode potentials, 0.5, 1.0, 2.0 and -1.0 V. With increasing the electrode potential, the RBC moves further away from the electrodes, and deforms more obviously. At $V_0 = 0.5$ V the RBC almost exhibits a translational motion without the shape deformation, whereas at $V_0 = 2.0$ V it deforms and ascends remarkably. More interestingly, the motion and deformation of RBC at $V_0 = 1.0$ V are identical to those at $V_0 = -1.0$ V, meaning that the RBC performance is independent of the polarity of applied electric field. All these behaviors are explained well by the relationship between the DEP force and electrode potential, as plotted in Fig. 5.42. With increasing the electrode potential, the DEP force increases so that the RBC deforms and ascends more remarkably at the high electrode potential. At $V_0 = 0.5$ V, the DEP force is close to zero, and therefore the RBC almost has no deformation and upward motion. At $V_0 = 2.0$ V however, the DEP force is relatively large, and thus the RBC deforms and ascends remarkably. In turn, since the RBC ascends further away from the electrodes, the DEP force decreases with increasing time. In addition, the DEP force doesn't change when the polarity of applied electric field strength is reversed, such that the RBC at $V_0 = 1.0$ V exhibits the same performance at $V_0 = -1.0$ V. In other words, the DEP force is independent of the polarity of applied electric field, which is a well-known characteristic of DEP.

Fig. 5.41 Motion and deformation of a single RBC subject to different electrode potentials in a nonuniform electric field, (a) $V_0 = 0.5$ V, (b) $V_0 = 1.0$ V, (c) $V_0 = 2.0$ V, and (d) $V_0 = -1.0$ V.

Fig. 5.42 Variation of the magnitude of DEP force acting on the single RBC subject to the different electrode potentials in the nonuniform electric field.

Fig. 5.43 illustrates the variations of extension and orientation of the single RBC under the different electrode potentials in the nonuniform electric field. At $V_0 = 0.5$ V, since the RBC almost undergoes a translational motion, its extension and orientation almost remain the initial values. At $V_0 = 2.0$ V however, the extension of RBC decreases first and then increases, indicating the RBC shrinks first and is then stretched. The orientation changes from 0 to $-\pi/2$, meaning that the long axis of RBC rotates clockwise from the y axis to x axis. At $V_0 = 1.0$ and -1.0 V, the corresponding extension and orientation of RBC are identical each other, which quantitatively confirms that the motion and deformation of RBC is independent of the polarity of electric field.

Fig. 5.43 Variations of (a) the extension and (b) orientation of the single RBC subject to the different electrode potentials in the nonuniform electric field.

5.3.2.4 Effect of electric frequency

According to the dipole moment theory [89], the motion of a rigid dielectric body is independent of the electric frequency in the regions of low and high frequencies, respectively. In order to examine whether this conclusion still holds for a deformable dielectric body, the motion and deformation of a single RBC are investigated at the low and high electric frequencies 10^2 , 10^3 , 10^9 and 10^{10} Hz, as shown in Fig. 5.44. The similar behaviors are observed at the low ($f = 10^2$ and 10^3 Hz) and high ($f = 10^9$ and 10^{10} Hz) frequencies, respectively. Fig. 5.45 plots the magnitude of DEP force at each frequency, where the similar DEP force is also found in the regions of low and high frequencies, respectively. Therefore, for the deformable dielectric body, the motion and

deformation are also independent of the electric frequency in the regions of low and high frequencies, respectively.

Fig. 5.44 Motion and deformation of a single RBC at low and high electric frequencies in a nonuniform electric field, (a) $f = 10^2$ Hz, (b) $f = 10^3$ Hz, (c) $f = 10^9$ Hz, and (d) $f = 10^{10}$ Hz.

At the low frequencies ($f = 10^2$ and 10^3 Hz), the RBC goes down with increasing time, i.e., it is attracted towards the region of strong electric field strength, as shown in Fig. 5.44(a) and (b). That is well known as the positive DEP (p-DEP), in which the DEP force strength is directed outward and larger at the lower part of RBC, as illustrated in Fig. 5.46. As a result, the lower part of RBC becomes wider and wider, and the upper part becomes narrower and narrower to keep the area conservation. Moreover, the y component of DEP force is negative, as plotted in Fig. 5.45(b), thereby attracting the RBC towards the electrodes. On the contrary, the RBC is repelled away from the region of strong electric field strength at the high frequencies ($f = 10^9$ and 10^{10} Hz), called the negative DEP (n-DEP). In the n-DEP, the DEP force strength is directed inward, and also larger at the lower part of RBC, as illustrated in Fig. 5.46. Therefore, the lower part of RBC becomes narrower and narrower, and the y component of DEP force is positive as plotted in Fig. 5.45(b), repelling the RBC away from the electrodes.

Fig. 5.45 Variation of the magnitude of DEP force acting on the single RBC at the low and high electric frequencies in the nonuniform electric field.

Fig. 5.46 Distribution of the DEP force strength acting on the single RBC at the low and high electric frequencies, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.47 illustrates the extension and orientation of the single RBC at the low and high frequencies in the nonuniform electric field. It is found that they are quite similar at the low ($f = 10^2$ and 10^3 Hz) and high ($f = 10^9$ and 10^{10} Hz) frequencies, respectively. Furthermore, the variation of extension at the high frequencies is slightly larger than that at the lower frequencies, meaning that the RBC deforms more obviously at the high frequencies. The orientations are positive at the low frequencies, but negative at the high frequencies. In other words, the RBC tilts anticlockwise in the p-DEP at the low frequencies, but clockwise in the n-DEP at the high frequencies, as shown in Fig. 5.44.

Fig. 5.47 Variations of (a) the extension and (b) orientation of the single RBC at the low and high electric frequencies in the nonuniform electric field.

5.3.2.5 Effect of cell permittivity

For a rigid dielectric body, it was showed by the dipole moment theory that the DEP force only depends on the permittivities of the fluid and dielectric body at the high frequency, and the Clausius-Mossotti (CM) factor at the high frequency is approximated as [91],

$$\lim_{\omega \rightarrow \infty} K_{CM}(\omega) = \frac{\xi_c - \xi_m}{\xi_c + 2\xi_m} \quad (5.8)$$

Therefore, the effect of permittivity of a deformable RBC is here examined at the high frequency 10^9 Hz. Fig. 5.48 shows the motion and deformation of a single RBC with different interior permittivities, $57\xi_0$, $81.2\xi_0$ and $105\xi_0$, corresponding to the RBC permittivities $56\xi_0$, $80\xi_0$ and $103\xi_0$ obtained by the single shell model in Eq. (2.9). Note that the fluid permittivity is taken as $80\xi_0$. The RBC moves up when its permittivity is smaller than that of the fluid, whereas it goes down if its permittivity is larger. In other words, the RBC with the smaller permittivity experiences the n-DEP, while the RBC with the larger permittivity exhibits the p-DEP. However, the RBC having the same permittivity as the fluid undergoes a transitional motion only due to the uniform flow. The motion behavior of RBC is easily explained by the distribution of DEP force strength, as illustrated in Fig. 5.49. For the RBC with the smaller permittivity, the DEP force strength is directed inward and larger at the lower part of RBC, such that the DEP force is directed away from the electrodes and induces the RBC moving up.

Similarly, the RBC with the larger permittivity goes down subject to the outward DEP force strength. However, there is almost no DEP force acting on the RBC with the same permittivity as the fluid, and thus the RBC undergoes a transitional motion only. In addition, it should be noted that the lower part of RBC deforms narrower and narrower in the n-DEP, but wider and wider in the p-DEP due to the distribution of DEP force strength. The motion behavior of the deformable RBC is also roughly explained by the dipole moment theory for the rigid dielectric body. According to Eq. (5.8), if the RBC has the smaller permittivity than the fluid, the CM factor is negative, meaning that the n-DEP occurs. On the contrary, if the RBC has the larger permittivity than fluid, the CM factor is positive and the p-DEP happens. If the RBC has the same permittivity as the fluid, the CM factor is equal to zero and there is no DEP force.

Fig. 5.48 Motion and deformation of a single RBC with different interior permittivities in a nonuniform electric field, (a) $\zeta_{in} = 57\zeta_0$, (b) $\zeta_{in} = 81.2\zeta_0$, and (c) $\zeta_{in} = 105\zeta_0$.

Fig. 5.50 plots the extension and orientation of the single RBC with the different permittivities in the nonuniform electric field. Since the RBC with the same permittivity as the fluid has no deformation, its extension and orientation keeps the constant initial values. The extension of RBC in the n-DEP is smaller than that in the p-DEP, indicating that the RBC in the n-DEP shrinks more obviously, as shown in Fig. 5.48. Moreover, the orientation is negative in the n-DEP for the RBC with the smaller permittivity, but positive in the p-DEP for the RBC with the larger permittivity. In other words, the RBC in n-DEP tilts clockwise, but anticlockwise in the p-DEP.

Fig. 5.49 Distribution of the DEP force strength acting on the single RBC with the different interior permittivities, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.50 Variations of (a) the extension and (b) orientation of the single RBC with the different interior permittivities in the nonuniform electric field.

5.3.2.6 Effect of cell conductivity

According to the dipole moment theory, the DEP force at the low frequency depends on the conductivities of the fluid and dielectric body, and the Clausius-Mossotti (CM) factor is approximated as [91],

$$\lim_{\omega \rightarrow 0} K_{CM}(\omega) = \frac{\sigma_c - \sigma_m}{\sigma_c + 2\sigma_m} \quad (5.9)$$

Here the effect of the RBC conductivity is investigated at the low frequency 10^3 Hz. Fig. 5.51 shows the motion and deformation of a single RBC with different interior conductivities, 1.0, 3.1 and 520 mS/m, corresponding to the RBC conductivities 0.5, 0.8 and 1.1 mS/m obtained by the single shell model in Eq. (2.9). Note that the fluid conductivity is taken as 0.8 mS/m. When the RBC conductivity is smaller than that of the fluid, the RBC moves up, i.e., the n-DEP occurs. On the contrary, the RBC goes down and exhibits the p-DEP when its conductivity is larger. However, the RBC undergoes a transitional motion due to the uniform flow when its conductivity is equal to that of fluid. This behavior is also in good agreement with the dipole moment theory. According to Eq. (5.9), if the RBC has the smaller conductivity, the CM factor is negative, and thus the n-DEP occurs. On the contrary, the p-DEP is exhibited on the RBC with the larger conductivity. If the RBC has the same conductivity, the CM factor is zero, and there is no effect of the nonuniform electric field. Fig. 5.52 illustrates the distribution of DEP force strength, in which the n-DEP force strength is directed inward but the p-DEP force strength is outward. For both the n-DEP and p-DEP, their force strengths are larger at the lower part due to the closeness to the electrodes, such that the y components of DEP forces are upward and downward, respectively. Therefore, the n-DEP RBC moves up and has narrow lower part, while the p-DEP RBC goes down and has wide lower part.

Fig. 5.51 Motion and deformation of a single RBC with different interior conductivities in a nonuniform electric field, (a) $\sigma_{in} = 1.0$ mS/m, (b) $\sigma_{in} = 3.1$ mS/m, and (c) $\sigma_{in} = 0.52$ S/m.

Fig. 5.52 Distribution of the DEP force strength acting on the single RBC with the different interior conductivities, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.53 plots the extension and orientation of the single RBC with the different conductivities in the nonuniform electric field. When the RBC has the same conductivity as the fluid, its extension and orientation almost keep the initial constants due to no deformation. In the n-DEP, the RBC has the smaller extension and the negative orientation, compared with the RBC in the p-DEP. Hence, the RBC in the n-DEP shrinks more obviously, and tilts clockwise, as shown in Fig. 5.51. However, the RBC in the p-DEP shrinks slightly, and tilts anticlockwise.

Fig. 5.53 Variations of (a) the extension and (b) orientation of the single RBC with the different interior conductivities in the nonuniform electric field.

5.3.3 Two cells in a nonuniform AC electric field

In the subsection 5.3.2, various factors influencing the motion and deformation of a single RBC haven been examined. It is concluded that the RBC conductivity plays a dominant role at the low frequency, but the RBC permittivity at the high frequency. Based on this conclusion, the motion and deformation of two RBCs at the low and high frequencies are simulated and discussed in this subsection, where the RBCs have the given dielectric properties. Besides, the effect of intercellular interaction strength is also analyzed in this subsection. The mesh size and particle number are taken as 1000×200 and 360 for discretizing the computational domain and RBC membrane, respectively. Tab. 5.4 lists typical values of additional physical quantities for numerical simulations, and the others can be found in Tab. 5.3.

Tab. 5.4 Typical values of additional physical quantities in numerical simulations of two RBCs in the nonuniform electric field.

Definition	Symbol	Value
Computational domain length	L	100 μm
Computational domain width	W	20 μm
Uniform flow velocity	u_{uni}	0.5 mm/s
Intercellular interaction strength	D_e	1.0 μJ [28]
Scaling factor	β	3.84 μm^{-1} [28]
Zero force distance	r_0	0.49 μm [28]
Electrode width	d_1	40 μm
Electrode gap	d_2	10 μm
Fluid permittivity	ξ_m	80 ξ_0
Fluid conductivity	σ_m	1.0 mS/m
RBC interior permittivity	RBC I $\xi_{in,1}$	60 ξ_0
	RBC II $\xi_{in,2}$	85 ξ_0
RBC interior conductivity	RBC I $\sigma_{in,1}$	1.0 mS/m
	RBC II $\sigma_{in,2}$	30 mS/m
Electrode potential	V_0	2.0 V

5.3.3.1 Validation

It was demonstrated that a vesicle can deform into the prolate, oblate and circular shapes subject to a uniform AC electric field, depending on the electric frequency f and conductivity ratio γ inside and outside the vesicle [75-82]. Here the deformation behavior of a vesicle is also simulated in a uniform AC electric field, and compared with the experimental results of Aranda et al. [78]. In the presence of uniform AC electric field, an internal electric force is generated and induces the vesicle deformation. In order to resist the deformation, the vesicle develops a membrane mechanical force, such that a steady state is reached without shape change.

Fig. 5.54 Comparisons between the numerical predictions in the present validation and the experimental results of Aranda et al. [78] for (a) the frequency-dependent relative deformation, in which γ is the conductivity ratio inside and outside the vesicle, and (b) the morphological shapes of vesicles under $\gamma = 0.5$.

Fig. 5.54(a) shows the comparison of frequency-dependent relative deformation between the numerical predictions and the experimental measurements [78], where the relative deformation is defined as the ratio of the deformed vesicle axis along the electric field direction to the radius of initial circular vesicle. The slight discrepancies between them may be attributed to the effects of capacitance and conductance of cell membrane. For both the conductivity ratios $\gamma = 4.5$ and 0.5 , the relative deformation is larger than 1.0 at the low frequency ($f < 10^4$ Hz), meaning that the vesicle becomes prolate shape. At the high frequency ($f > 10^7$ Hz) however, it approaches to 1.0 , indicating that the vesicle becomes spherical shape. In the intermediate frequency region, the vesicle again assumes the prolate shape for $\gamma = 4.5$, but the oblate shape for $\gamma = 0.5$ because the relative deformation is less than 1.0 . Fig. 5.54(b) illustrates these morphological shapes of vesicle at $\gamma = 0.5$ only, which are also consistent with the experimental observations [78].

5.3.3.2 Dynamic behavior of cells at low frequency

Fig. 5.55 illustrates the motion and deformation of two RBCs with different interior conductivities at the low frequency 10^3 Hz. For convenience, the left RBC is denoted as RBC I, and the right RBC as RBC II. Their dielectric properties are listed in Tab. 5.4, where the corresponding RBC conductivities are 0.52 and 1.1 mS/m, obtained by the single shell model in Eq. (2.9), and the fluid conductivity is 1.0 mS/m. The most obvious behavior is that the RBC I moves up while the RBC II goes down. In other words, the RBC I experiences the n-DEP and is repelled away from the region of strong electric field strength, whereas the RBC II exhibits the p-DEP and is attracted to that region. These simulation results are consistent with the experimental phenomena for the rigid particles [192], and can be explained by the distribution of DEP force strength, as shown in Fig. 5.56. For the RBC I, the DEP force strength is directed inward, and stronger at the lower part of the cell. As a result, the DEP force is directed away from the region of strong electric field, and thus repels the RBC I move up. However, the DEP force strength acting on the RBC II is radially outward, and also larger at the lower part of the cell, such that the DEP force is directed to the region of strong electric field and the RBC II thus goes down.

Fig. 5.55 Motion and deformation of two RBCs with different conductivities at the low frequency $f = 10^3$ Hz, in which the dashed lines with symbols show the centroid trajectories.

Fig. 5.56 Distribution of the DEP force strength acting on the two RBCs at $t = 60$ ms at the low frequency, where the arrow indicates the direction and magnitude of force strength.

Apart from the RBC motion, the RBC deformation also should be paid special attention to. It is broadly found that the lower part of RBC I becomes narrow, while the lower part of RBC II becomes wider. This also results from the distribution of DEP force strength, as shown in Fig. 5.56. For the RBC I, the DEP force is directed inward so that the lower part force is squeezed narrower and narrower. For the RBC II however, the DEP force is outward, which stretches the lower part wider and wider. For quantitatively describing the RBC deformation, Fig. 5.57 plots the extensions and orientations of the two RBCs at the low frequency. The extensions of RBCs exhibit an alternate increasing and decreasing pattern, which is mainly attributed to the competition between the DEP force strength and membrane mechanical force strength. When the RBC is stretched severely due to the DEP force strength, the membrane

mechanical force strength resists increasingly to the large deformation, making the RBC shrink. Hence, a decreasing trend is observed on the extension of RBC. Once the RBC deformation becomes small due to the membrane resistance, the membrane mechanical force then decreases, such that the DEP force is dominant again, and stretches the RBC again. As a result, the extension of RBC increases again. The orientations of RBCs are different. For the RBC I, its orientation decreases from 0 to $-\pi/2$, meaning that the RBC I rotates clockwise $\pi/2$. However, the orientation of the RBC II almost keeps a constant of zero, indicating the RBC II maintains its long axis along the y axis.

Fig. 5.57 Variations of (a) the extensions and (b) orientations of the two RBCs at the low frequency in the nonuniform electric field.

5.3.3.3 Dynamic behavior of cells at high frequency

Fig. 5.58 illustrates the motion and deformation of two RBCs with different interior permittivities at the high frequency 10^9 Hz. The corresponding RBC permittivities are $59.3\xi_0$ and $83.6\xi_0$, obtained by the single shell model in Eq. (2.9), and the fluid permittivity is $80\xi_0$. The RBC I with the smaller permittivity exhibits the n-DEP and moves up, whereas the RBC II with the larger permittivity experiences the p-DEP and goes down. Apart from that, the lower part of RBC I becomes narrow, but the lower part of RBC II wide. In order to explain the behavior of cell motion and deformation, Fig. 5.59 shows the distribution of DEP force strength acting on the two RBCs at $t = 60$ ms. The DEP force strength is larger at the lower parts of both RBCs. It is directed inward for the RBC I experiencing the n-DEP but outward for the RBC II experiencing the p-

DEP. This distribution of DEP force strength is quite similar to that at the low frequency.

Fig. 5.58 Motion and deformation of two RBCs with different permittivities at the high frequency $f = 10^9$ Hz, in which the dashed lines with symbols show the centroid trajectories.

Fig. 5.60 plots the extensions and orientations of the two RBCs at the high frequency in the nonuniform electric field. The extensions of both the RBCs also exhibit an alternate increasing and decreasing pattern due to the competition between the DEP force strength and membrane mechanical force strength, similar to those at the low frequency. The orientation of RBC I gradually decreases from 0 to $-\pi/2$, meaning that the RBC I rotates clockwise. However, the orientation of RBC II almost keeps a constant of zero, indicating the RBC II maintains its long axis along the y axis.

Fig. 5.59 Distribution of the DEP force strength acting on the two RBCs at $t = 60$ ms at the high frequency, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.60 Variations of (a) the extensions and (b) orientations of the two RBCs at the high frequency in the nonuniform electric field.

5.3.3.4 Effect of intercellular interaction strength

Fig. 5.61 describes the motion and deformation of two RBCs with different intercellular interaction strengths D_e , 1.0, 10 and 15 $\mu\text{J}/\text{m}^2$ at the high frequency 10^9 Hz. Both the RBCs have the similar motion and deformation at $D_e = 1.0$ and 10 $\mu\text{J}/\text{m}^2$, respectively. The RBC I moves up and exhibits the n-DEP, while the RBC II goes down and experiences the p-DEP. At $D_e = 15$ $\mu\text{J}/\text{m}^2$ however, the RBC II goes down first and then rises up, although it still experiences the p-DEP.

Fig. 5.61 Motion and deformation of two RBCs at the high frequency under different intercellular interaction strengths, (a) $D_e = 1.0$ $\mu\text{J}/\text{m}^2$, (b) $D_e = 10$ $\mu\text{J}/\text{m}^2$, and (c) $D_e = 15$ $\mu\text{J}/\text{m}^2$, in which the dashed lines with symbols show the centroid trajectories.

The different cell performance induced by the intercellular interaction strength is attributed to the competition between the DEP force and intercellular interaction force acting on the RBCs. Fig. 5.62 plots the y components of intercellular interaction and DEP forces acting on the RBCs with the different intercellular interaction strengths. At $D_e = 1.0$ and $10 \mu\text{J}/\text{m}^2$, the y component of DEP force is positive for the RBC I, and larger than the absolute value of y component of intercellular interaction force, such that the upward resultant force drives the RBC I moving up. However, for the RBC II, the y component of DEP force is negative, but its absolute value is still larger than that of intercellular interaction force, and therefore the downward resultant force drives the RBC II going down. Moreover, for both the RBCs I and II, the intercellular interaction and DEP forces are almost identical at $D_e = 1.0$ and $10 \mu\text{J}/\text{m}^2$ respectively, and therefore they exhibit the similar motion and deformation. At $D_e = 15 \mu\text{J}/\text{m}^2$, the y component of intercellular interaction force is negative for the RBC I before about 160 ms, and its absolute value is smaller than that of the DEP force, and thus the RBC I moves up. From about 160 to 170 ms, its absolute value becomes larger than the y component of DEP force, leading to the RBC I slightly goes down. Subsequently, a repulsion behavior happens between the RBCs I and II at about 180 ms due to the decreasing intercellular distance, resulting in the y component of intercellular interaction force acting on RBC I becomes positive. Hence, the RBC I moves up again after about 180 ms. For the RBC II, the absolute value of the y component of intercellular interaction force is positive and quite larger than that of DEP force after about 160 ms, and therefore the RBC II also moves up although it experiences the p-DEP. In brief, if the intercellular interaction strength is large enough, the expected trend of cell motion may be changed, for example the cell exhibiting the p-DEP moves away from the region of strong electric field. Therefore, the intercellular interaction strength also has a direct effect on the motion and deformation of multiple cells, except the dielectric properties. It should be pointed out in Fig. 5.62 that the y component of intercellular interaction force on the RBC I doesn't match that on the RBC II at $D_e = 15 \mu\text{J}/\text{m}^2$, which results from the numerical error in simulations. When the intercellular interaction strength is significantly strong, for example $D_e = 15 \mu\text{J}/\text{m}^2$ remarkably larger than the physical value of about $1.0 \mu\text{J}/\text{m}^2$, the intercellular interaction force strength is very large acting on the local part of cell membrane, leading to the local distortion of

cell membrane. As a result, the Lagrangian nodes used to track the cell membrane in simulations are disordered, resulting in the inaccurate intercellular force, and even the membrane deformation force. This is a limitation of the present simulation, generated by the disorder of Lagrangian nodes. When the intercellular interaction strength is close to the physical value, such as $D_e = 1.0$ and $10 \mu\text{J}/\text{m}^2$, however, the intercellular interaction force acting on two cells exactly matches each other, as shown in the inset of Fig. 5.62(b).

Fig. 5.62 Variations of the y component of intercellular interaction force acting on (a) the RBC I and (b) RBC II, and the y component of DEP force acting on (c) the RBC I and (d) RBC II under the different intercellular interaction strengths. The inset in (b) shows the low region of the y component of intercellular interaction force acting on the RBCs I and II at $D_e = 10 \mu\text{J}/\text{m}^2$.

Fig. 5.63 plots the extensions and orientations of the two RBCs with the different intercellular interaction strengths in the nonuniform electric field. The RBC I has the similar extension and orientation at the different intercellular strengths, meaning that the

RBC I undergoes the similar stretching and rotation, which is also exhibited on the RBC II. However, the variations of extension and orientation of RBC I are larger than those of RBC II, indicating that the deformation and rotation of RBC I are also larger, as shown in Fig. 5.61

Fig. 5.63 Variations of the extensions of (a) the RBC I and (b) RBC II, and the orientations of (c) the RBC I and (d) RBC II with the different intercellular interaction strengths in the nonuniform electric field.

5.4 Motion and deformation of cells in the hydrodynamic-electric field

5.4.1 Introduction

To our knowledge, the majority of previous studies on cells in the hydrodynamic-electric coupled field have focused on the electrical field-flow fractionation (ElFFF) [170], in which the DEP FFF is of particular interest. However, most of them didn't concern the deformation behavior of cells in that the cells are usually assumed as rigid

particles. Therefore, the present study numerically designs first a microfluidic-microelectric hybrid chip, and then manipulates a single deformable RBC or two deformable RBCs using this hybrid chip, for investigating the behavior of deformable cells in the hydrodynamic-electric coupled field. As the most complete case, the present study includes the shear force of hydrodynamic flow, membrane mechanical force, DEP force, and intercellular interaction force for two RBCs. Fig. 5.64 illustrated the computational domains of a single RBC or two RBCs in a parabolic hydrodynamic and nonuniform AC electric coupled field. The RBC shape and parabolic velocity profile are given by Eqs. (5.1) and (5.3), respectively.

Fig. 5.64 Computational domains of (a) a single RBC and (b) two RBCs in a parabolic hydrodynamic and nonuniform electric coupled field.

5.4.2 A single cell in a hydrodynamic-electric field

In this subsection, a microfluidic-microelectric hybrid chip is numerically designed as a separator by choosing the suitable electrode width, flow velocity and electrode potential. After that, the separating ability of hybrid chip is broadly evaluated by analyzing the effects of initial shape and position of a single RBC. Finally, a single RBC with different permittivities and conductivities is numerically manipulated by the hybrid chip, and the motion and deformation of RBC are further discussed. The mesh size in the computational domain and particle number on the cell membrane are taken as

1000×200 and 360, respectively. Tab. 5.5 lists typical values of several physical quantities for numerical simulations.

Tab. 5.5 Typical values of several physical quantities in numerical simulations of a single RBC in the parabolic hydrodynamic and nonuniform electric coupled field.

Definition	Symbol	Value
Computational domain length	L	100 μm
Computational domain width	W	20 μm
Maximum flow velocity	$u_{\max}$	0.5 mm/s
Fluid density	ρ_m	$1.0 \times 10^3 \text{ kg/m}^3$
RBC density	ρ_c	$1.125 \times 10^3 \text{ kg/m}^3$
Fluid viscosity	μ_m	$1.0 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC viscosity	μ_c	$1.3 \times 10^{-3} \text{ pa} \cdot \text{s}$
RBC radius	R	3.91 μm [225]
Membrane shear modulus	E_s	$6 \times 10^{-3} \text{ dyn/cm}$ [198]
Membrane bending stiffness	E_B	$1.8 \times 10^{-12} \text{ dyn} \cdot \text{cm}$ [198]
Electrode width	d_1	40 μm
Electrode gap	d_2	10 μm
Fluid permittivity	ξ_m	$80 \xi_0$
Fluid conductivity	σ_m	1.0 mS/m
RBC interior permittivity	ξ_{in}	$57 \xi_0$ [106]
RBC interior conductivity	σ_{in}	0.52 S/m [106]
RBC membrane capacitance	C_{mem}	11.8 mF/m ² [106]
RBC membrane conductance	G_{mem}	271 S/m ² [106]
Electrode potential	V_0	1.5 V
Electric frequency	f	10^9 Hz

5.4.2.1 Validation

The validation of a single cell in the hydrodynamic-electric coupled field is performed by comparing with the experimental results published previously by Huang et al. [175],

where the levitation height of a typical HL-60 cell suspending in a medium was measured as a function of the electrode potential. Huang et al. [175] considered an array of electrodes with equal width d_1 and gap d_2 of 20 μm , and assumed the HL-60 cell as a rigid spherical particle because the channel width of 200 μm was significantly larger than the cell radius of 6.3 μm . The permittivity and conductivity of cell interior used in their work [175] are $\xi_{in} = 75\xi_0$ and $\sigma_{in} = 0.75 \text{ S/m}$, and the capacitance and conductance of the membrane are $C_{mem} = 16.3 \text{ mF/m}^2$ and $G_{mem} = 220 \text{ S/m}^2$. Moreover, the permittivity and conductivity of the suspending medium are $\xi_m = 80\xi_0$ and $\sigma_m = 0.056 \text{ S/m}$, respectively.

In the present validation, the same parameters as those of Huang et al. [175] are used, but no assumption of rigid particle is made for the cell. Fig. 5.65 shows the comparisons among the present numerical predictions, the experimental results and theoretical fitting of Huang et al. [175], in which the levitation height of HL-60 cell exhibits a monotonically increasing relationship to the electric potential. A reasonable agreement is observed among them with the error of about 8%, although the cell is not assumed rigid. Since the chip width is significantly larger than the cell radius, the cell deformation is quite small, and leads to the slight the effect on the levitation height.

Fig. 5.65 Comparisons of the levitation height of HL-60 cell at the electric frequency of 17.8 kHz between the present numerical predictions and the experimental and theoretical results of Huang et al [175].

5.4.2.2 Design of a microfluidic-microelectric hybrid chip

5.4.2.2.1 Effect of electrode width

Fig. 5.66 illustrates the deformed shape of a single RBC located at $x = 92.2 \mu\text{m}$ in the microfluidic-microelectric hybrid chip with different electrode widths d_1 , 10, 25 and 40 μm . At the initial state, the RBC is placed on the centerline of hybrid chip. With increasing the electrode width, the RBC is levitated higher and higher due to the increasing DEP force. However, the RBC under the three different electrode widths is not completely levitated above the centerline of hybrid chip. For convenience, here we define the outlet above the centerline of hybrid chip as the outlet 1, and the outlet below the centerline as the outlet 2. As such, the RBC under the three different electrode widths is not completely transported to the outlet 1. In order to successfully separate cells, therefore, the electrode width $d_1 = 40 \mu\text{m}$, generating the highest levitation height in the three cases, is used in the following simulations.

Fig. 5.66 Deformed shape of a single RBC at $x = 92.2 \mu\text{m}$ in the hybrid chip with different electrode widths.

Fig. 5.67 plots the variations of y value of RBC centroid, and the RBC extension for quantitative analysis of the height and deformation of RBC in the chip with the different electrode widths. With increasing the electrode width, the y value of RBC centroid increases obviously, meaning that the RBC is levitated higher and higher. The RBC extension decreases more rapidly before about 80 ms with increasing electrode width, and then increases more rapidly. As a result, the RBC deformation becomes larger and larger with increasing the electrode width. At the beginning, the lower and

upper parts of RBC are subjected to the similar shear force of fluid flow, and thus the RBC shrinks and approaches towards a circle. With increasing time however, the RBC is levitated gradually, and the shear force acting on the lower and upper parts of RBC are quite different, such that the RBC is stretched and becomes less compact. Therefore, the extension of RBC at the three different electrode widths decreases first and then increases.

Fig. 5.67 Variations of (a) the height and (b) extension of the single RBC in the hybrid chip with the different electrode widths.

5.4.2.2.2 Effect of flow velocity

Fig. 5.68 illustrates the deformed shape of a single RBC at $x = 92.2 \mu\text{m}$ in the hybrid chip subject to different flow velocities u_{max} , 0.5, 1.0 and 1.5 mm/s. The ascending height of RBC increases as the flow velocity decreases, but the RBC in all the three velocities is still not completely transported to the outlet 1. Therefore, the flow velocity $u_{\text{max}} = 0.5$ mm/s is used in the following simulations, because it can provide the highest ascending height.

Fig. 5.69 plots the variations of y value of RBC centroid, and the RBC extension under the different flow velocities. With decreasing the flow velocity, the RBC height increases in that the DEP force has enough time to levitate the RBC. However, the time that the RBC arrives at the outlet increases, which directly affects the separating efficiency of hybrid chip. Moreover, the RBC extension at $u_{\text{max}} = 0.5$ mm/s is larger than that at $u_{\text{max}} = 1.0$ and 1.5 mm/s, meaning that the RBC at $u_{\text{max}} = 0.5$ mm/s is less

stretched along the x direction. Therefore, the RBC deformation becomes smaller and smaller with decreasing the flow velocity due to the small shear force.

Fig. 5.68 Deformed shape of a single RBC at $x = 92.2 \mu\text{m}$ in the hybrid chip subject to different flow velocities.

Fig. 5.69 Variations of (a) the height and (b) extension of the single RBC in the hybrid chip subject to the different flow velocities.

5.4.2.2.3 Effect of electrode potential

Fig. 5.70 demonstrates the deformed shape of a single RBC at $x = 92.2 \mu\text{m}$ in the chip subject to different electrode potential V_0 , 0.5 1.0 and 1.5 V. With increasing the electrode potential, the RBC ascends more quickly. At $V_0 = 1.5 \text{ V}$, the RBC is completely levitated up to $10 \mu\text{m}$, i.e., the RBC moves out through the outlet 1. Therefore, the electrode potential $V_0 = 1.5 \text{ V}$ is selected to separate cells in the following simulations.

Fig. 5.70 Deformed shape of a single RBC at $x = 92.2 \mu\text{m}$ in the hybrid chip subject to different electrode potentials.

Fig. 5.71 plots the variations of y value of RBC centroid, and the RBC extension subject to the different electrode potentials. With increasing the electrode potential, the DEP force acting on the RBC also increases, and thus the RBC is levitated higher and higher. The RBC extension at $V_0 = 1.5 \text{ V}$ is smallest before about 70 ms among the three electrode potentials, meaning that the RBC at $V_0 = 1.5 \text{ V}$ shrinks largest along the y direction. Subsequently, the RBC is levitated above the centerline of hybrid chip due to the large DEP force, and starts to be stretched along the x direction. As a result, its extension increases. However, the RBC at $V_0 = 0.5 \text{ V}$ is slightly levitated due to the small DEP force, such that it continues to shrink after 70 ms. Therefore, its extension exhibits a monotonic decreasing trend.

Fig. 5.71 Variations of (a) the height and (b) extension of the single RBC in the hybrid chip subject to the different electrode potentials.

5.4.2.2.4 Remarks

According to the above analyses, the RBC is levitated higher and higher with increasing the electrode width, decreasing the flow velocity, or increasing the electrode potential. However, it is not profitable to increase the electrode width and potential unboundedly, or decrease the flow velocity without limit.

On one hand, the remarkable increase of potential and electrode width results in the large deformation of cell, which may lead to the cell damage. Especially for the electrode potential, the DEP force changes significantly with increasing it, and therefore induces the large deformation. Moreover, there is a necessary condition for the electrode width $0 < d_1 < L/2$ due to the electrode layout, where L is the length of computational domain.

On the other hand, the remarkable decrease of flow velocity results in the low separating efficiency of hybrid chip, which not only influences the separating time but also the separating ability. If the flow velocity is 0.01 mm/s and the chip length is 100 μm , the separating time is 10 s at least. Furthermore, since the velocity profile is nearly uniform when the flow velocity is small, the time that the cell arrives at the outlet is almost same, and thus reducing the separating ability.

Therefore, in the present work the microfluidic-microelectric hybrid chip is designed as, the electrode width 40 μm , the flow velocity 0.5 mm/s, and the electrode potential 1.5 V in order to manipulate a single cell successfully and efficiently. For the multiple cells, the electrode potential increases to 2.0 V for overcoming the intercellular aggregation.

5.4.2.3 *Evaluation of separating ability of the hybrid chip*

5.4.2.3.1 Effect of cell shape

Fig. 5.72 illustrates the motion and deformation of a single RBC with different initial shapes, namely the biconcave, elliptical, and circular shapes. With increasing time, the RBC with the each initial shape is transported gradually towards the outlet 1, and finally separated completely from the outlet 1. It is therefore concluded that the cell shape has almost no effect on the separating ability of hybrid chip.

Fig. 5.72 Motion and deformation of a single RBC with different initial shapes, (a) the biconcave, (b) elliptical, and (c) circular shapes in the hybrid chip, where the snapshots are taken from 0 to 180 ms with the time interval of 20 ms, and the dash line represents the centerline of hybrid chip.

Fig. 5.73 Variations of (a) the extension and (b) orientation of the single RBC with the different initial shapes in the hybrid chip.

Fig. 5.73 shows the variations of the extension and orientation of the single RBC with the different initial shapes in the hybrid chip. The RBC with the biconcave and elliptical shapes exhibits the similar change trends of the extension and orientation, decreasing first and then increasing. This indicates that the RBC with the biconcave and elliptical shapes shrinks first along the y direction, and is then stretched along the x direction. The RBC tilts clockwise first due to the n-DEP, and then tilts anticlockwise due to the shear force of fluid flow. However, the RBC with the circular shape has a

monotonously increasing trend of the extension, meaning that the circular RBC is stretched along the x direction all the time. Its orientation decreases first to $-\pi/2$, and then jump abruptly to $\pi/2$ at about 50 ms. At first, the circular RBC rotates clockwise due to the n-DEP until its long axis changes from the y axis to x axis at about 50 ms. At the moment, the circular RBC is completely levitated above the centerline of hybrid chip, and will continue rotating clockwise due to the shear force of fluid flow. Based on the definition of orientation, the RBC is then defined as tilting anticlockwise at that moment. As a result, its orientation jumps abruptly from $-\pi/2$ to $\pi/2$, and then decreases gradually as the RBC continuously rotates, as shown in Fig. 5.72.

5.4.2.3.2 Effect of cell position

Fig. 5.74 shows the motion and deformation of a single RBC, whose centroid is initially located at different positions, $(5\ \mu\text{m}, 15\ \mu\text{m})$, $(5\ \mu\text{m}, 10\ \mu\text{m})$ and $(5\ \mu\text{m}, 5\ \mu\text{m})$ in the hybrid chip. There are two distinct phenomena exhibited therein. One is that the RBC arrives at the outlet at the different time. The RBC initially located at $(5\ \mu\text{m}, 15\ \mu\text{m})$ arrives at the outlet most slowly at about 260 ms, while the RBC initially at $(5\ \mu\text{m}, 5\ \mu\text{m})$ and $(5\ \mu\text{m}, 10\ \mu\text{m})$ arrives at about 90 ms. This is because the flow velocity is smaller near the upper and lower boundaries. The other phenomenon is that the RBC initially at $(5\ \mu\text{m}, 5\ \mu\text{m})$ has a very small cusp left behind the centerline of hybrid chip, which is attributed to the shear force of fluid flow [228]. Except the small cusp, the RBC at the different initial positions is completely transported towards the outlet 1. Therefore, the initial position of RBC has a minor effect on the separating ability of the microfluidic-microelectric hybrid chip.

Fig. 5.75 plots the variations of the extension and orientation of the single RBC initially located at the different positions in the hybrid chip. It is found from Fig. 5.74 that the RBC initially at $(5\ \mu\text{m}, 5\ \mu\text{m})$ and $(5\ \mu\text{m}, 15\ \mu\text{m})$ is stretched first and then shrinks along the long axis, such that its extension increases first and then decreases. However, the RBC initially at $(5\ \mu\text{m}, 10\ \mu\text{m})$ shrinks first and then is stretched, and thus the extension decreases first and then increases. The RBCs initially at $(5\ \mu\text{m}, 15\ \mu\text{m})$ and $(5\ \mu\text{m}, 10\ \mu\text{m})$ tilts anticlockwise from the y axis, whereas the RBC initially at $(5\ \mu\text{m}, 5\ \mu\text{m})$ tilts clockwise. Consequently, the orientation is larger than zero for the RBC at the first two positions, but less than zero for the RBC at the last position.

Fig. 5.74 Motion and deformation of a single RBC initially located at different positions, (a) (5 μm , 15 μm), (b) (5 μm , 10 μm) and (c) (5 μm , 5 μm) in the hybrid chip, where the snapshots are taken from 0 ms with the time interval of 20 ms, and the dash line represents the centerline of the hybrid chip.

Fig. 5.75 Variations of (a) the extension and (b) orientation of the single RBC initially located at the different positions in the hybrid chip.

5.4.2.4 Manipulation of a single cell

5.4.2.4.1 Effect of cell permittivity

Fig. 5.76 shows the motion and deformation of a single RBC with different interior permittivities ξ_{in} , $57 \xi_0$, $80 \xi_0$ and $90 \xi_0$ at the high frequency of $f = 10^9$ Hz in the hybrid chip. The corresponding RBC permittivities ξ_c are calculated by the single shell

model in Eq. (2.9) as $56.4 \xi_0$, $78.8 \xi_0$ and $88.5 \xi_0$, respectively. When the RBC permittivity is less than the fluid permittivity set as $80 \xi_0$ here, the RBC ascends gradually away from the electrodes and then moves out from the outlet 1, i.e., it experiences the n-DEP. On the contrary, the RBC moves towards the electrode array and moves out through the outlet 2, when its permittivity is larger, meaning that the p-DEP occurs. However, the RBC moves almost along the centerline of the chip, when its permittivity is close to the fluid permittivity. Fig. 5.77 illustrates the distribution of DEP force strength acting on the single RBC with the different interior permittivities. It is found that the DEP force strength is directed inward in the n-DEP, but outward in the p-DEP. Since the lower part of RBC is closer to the electrode than the upper part of RBC, the DEP force strength is larger at the lower part of RBC. As a result, the RBC is subjected to an upward and downward DEP force in the n-DEP and p-DEP, and moves up and down, respectively. When the RBC permittivity is very close to the fluid permittivity, the DEP force strength is almost equal to zero, such that the RBC moves and deforms subject to the fluid flow only.

Fig. 5.76 Motion and deformation of a single RBC with different interior permittivities, (a) $\xi_{in} = 57\xi_0$, (b) $\xi_{in} = 80\xi_0$, and (c) $\xi_{in} = 90\xi_0$ at the high frequency $f = 10^9$ Hz in the hybrid chip, where the snapshots in each case are taken from 0 to 180 ms at the time interval of 20 ms.

In Fig. 5.76(a) and (c), the RBC exhibiting either the n-DEP or p-DEP tends to deform into a slipper shape gradually, which is attributed to the combined effects of the

nonuniform electric field and parabolic fluid flow. The nonuniform electric field makes the RBC deviate from the centerline of parabolic fluid flow due to the DEP force. In the parabolic fluid flow, the RBC tails closer to the chip wall are subjected to the larger shear force of fluid flow. However, the leading parts of RBCs near the chip centerline undergo the smaller shear force. As a result, the RBCs exhibit the slipper shape, which is the characteristic shape in the asymmetric motion of RBC in the parabolic fluid flow. The RBC with the permittivity close to the fluid permittivity deforms into a parachute shape, as shown in Fig. 5.76(b). This is mainly attributed to the parabolic fluid flow because there is almost no DEP force, such that the RBC experiences the axisymmetric motion.

Fig. 5.77 Distribution of the DEP force strength acting on the single RBC with the different interior permittivities at $t = 180$ ms, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.78 Variations of (a) the extension and (b) orientation of the single RBC with the different interior permittivities in the hybrid chip.

Fig. 5.78 plots the variations of the extension and orientation of the single RBC with the different interior permittivities in the hybrid chip. The extension of RBC with the interior permittivities $80 \xi_0$ and $90 \xi_0$ decreases with time, indicating the RBC is stretched along the x direction, and becomes rounder and rounder. However, the RBC with the interior permittivity $57 \xi_0$ is stretched first and then shrinks, and thus its extension decreases first and then increases. The RBC tilts anticlockwise in the n-DEP, but clockwise in the p-DEP due to the shear force of flow, which is exactly opposite to the RBC in the nonuniform electric field. Therefore, the RBC orientation is positive in the n-DEP, but negative in the p-DEP.

5.4.2.4.2 Effect of cell conductivity

Fig. 5.79 shows the motion and deformation of a single RBC with different interior conductivities σ_{in} , 1.0, 10 and 100 mS/m at the low frequency of $f = 10^3$ Hz in the hybrid chip, which corresponds to the RBC conductivities are 0.52, 0.96, 1.05 mS/m obtained by the single shell model in Eq. (2.9). Here the fluid conductivity is 1.0 mS/m. When the RBC conductivity is smaller than the fluid conductivity, the RBC exhibits a n-DEP, and moves up to the outlet 1. On the contrary, the RBC experiences a p-DEP and goes down to the outlet 2, when the RBC conductivity is larger. When the RBC conductivity is quite close to the fluid conductivity, the RBC moves almost along the centerline of hybrid chip. In addition, the RBC with the lower and higher conductivities, compared with the fluid conductivity, deforms into a slipper shape, while the RBC with the similar conductivity deforms into a parachute shape. Fig. 5.80 shows the distribution of the DEP force strength, where it is directed inward in the n-DEP, but outward in the p-DEP. When the RBC conductivity is quite close to the fluid conductivity, it is almost equal to zero so that the RBC exhibits the symmetric motion. Moreover, the DEP force strength in the n-DEP is larger than that in the p-DEP, because the RBC conductivity in the p-DEP is slight larger than the fluid conductivity. Therefore, the behavior of motion and deformation of RBC with different conductivities at the low frequency is quite similar that of RBC with the different permittivities at the high frequency. In other words, the RBC can be separated according to either the permittivity at the high frequency or the conductivity at the low frequency.

Fig. 5.79 Motion and deformation of a single RBC with different interior conductivities, (a) $\sigma_{in} = 1.0$ mS/m, (b) $\sigma_{in} = 10$ mS/m and (c) $\sigma_{in} = 100$ mS/m at the low frequency of $f = 10^3$ Hz in the hybrid chip, where the snapshots in each case are taken from 0 ms with the time interval of 20 ms.

Fig. 5.80 Distribution of the DEP force strength acting on the single RBC with the different interior conductivities at $t = 180$ ms, where the arrow indicates the direction and magnitude of force strength.

Fig. 5.81 plots the variations of extension and orientation of the single RBC with the different interior conductivities in the hybrid chip. It is observed that the n-DEP is accompanied by the anticlockwise direction (positive orientation), but the p-DEP by the clockwise direction (negative orientation). Moreover, the weaker the DEP force strength is, the closer the RBC moves along the centerline of the chip. As a result, the RBC shape becomes round, and the extension thus becomes small.

Fig. 5.81 Variations of (a) the extension and (b) orientation of the single RBC with the different interior conductivities in the hybrid chip.

Tab. 5.6 Typical values of additional physical quantities in numerical simulations of two RBCs in the parabolic hydrodynamic and nonuniform electric coupled field.

Definition	Symbol	Value
Intercellular interaction strength	D_e	$1.0 \mu\text{J}$ [28]
Scaling factor	β	$3.84 \mu\text{m}^{-1}$ [28]
Zero force distance	r_0	$0.49 \mu\text{m}$ [28]
Fluid permittivity	ξ_m	$80 \xi_0$
Fluid conductivity	σ_m	0.5 mS/m
Electrode potential	V_0	2.0 V

5.4.3 Two cells in a hydrodynamic-electric field

In this subsection, two groups of deformable RBCs are separated by the microfluidic-microelectric hybrid chip according to the different conductivities at the low frequency, and the different permittivities at the high frequency, respectively. The first group of cells has the smaller permittivity but larger conductivity, compared with the corresponding permittivity and conductivity of fluid. On the contrary, the second group of cells has the larger permittivity but smaller conductivity. The mesh size and particle number are taken as 1000×200 and 360 for discretizing the computational domain and RBC membrane, respectively. Tab. 5.6 lists typical values of several physical quantities for numerical simulations, and the others can be found in Tab. 5.5. It should be noted

that the electrode potential is taken as 2.0 V to overcome the intercellular aggregation, instead of 1.5 V in the subsection 5.4.2.

5.4.3.1 Validation

The experiment of Huang et al.[175] is still used to validate the case of two cells in the hydrodynamic-electric coupled field, where the levitation height of a single HL-60 cell suspending in a medium was measured as a function of the electrode potential.

Fig. 5.82 (a) Comparison between the present numerical simulations and the experimental observations of Huang et al. [175], where a good agreement with the error about 3% is found when the intercellular interaction is neglected. (b) Distribution of the intercellular interaction force strength, where the arrow indicates to its direction and magnitude.

In the present validation, we numerically simulate the motion of two HL-60 cells (one is upper and the other is lower) suspending in the medium, and then compare the levitation height of the lower HL-60 cell with the experiment of Huang et al. [175]. Except that the same parameters as those of Huang et al.[175] are used, the intercellular interaction is considered therein. The comparison between the present numerical predictions and experimental results [175] is shown in Fig. 5.82(a), in which the monotonically increasing trend is exhibited in the levitation height of HL-60 cell with respect to the electrode potential. However, the predicted levitation height is

overestimated with the relative error about 10%, compared with the experimental values, which is mainly attributed to the intercellular attraction. In the presence of the intercellular interaction, the lower cell is subjected to an upward attractive force from the upper cell, as illustrated in Fig. 5.82(b). This attractive force causes the increase of levitation height of lower cell. If the intercellular aggregation is neglected, the levitation height of lower HL-60 cell is in a good agreement with the experimental results [175], as plotted in Fig. 5.82(a), where the relative error reduces to 3% only.

5.4.3.2 Separation of two group cells with different permittivities

Cells can be separated according to their different permittivities at the high frequency, as shown in Fig. 5.83. The first group of cells (solid line) with the lower permittivity than the fluid permittivity moves up, while the second group of cells (dotted line) with the higher permittivity goes down, wherever the cells are located in the microchannel. When a cell is polarized lower than the suspending fluid, a n-DEP phenomenon occurs. The DEP force strength acting on the cell is directed inward, and it is slightly larger at the lower part of cell, as illustrated in Fig. 5.84(a). As a result, the DEP force acting on the cell is directed upward, i.e., its y component of DEP force is positive, as plotted in Fig. 5.85(a). This upward DEP force drives the cell away from the electrodes. That is why the first group of cells in Fig. 5.83 moves away from the electrodes. On the contrary, the DEP force strength is directed outward if the cell is polarized higher than the suspending fluid, but it is still larger at the lower part of cell, as illustrated in Fig. 5.84(a). Hence, the DEP force is directed downward, i.e., its y component is negative, as plotted in Fig. 5.85(a). Subject to the downward DEP force, the cell is thus gradually attracted to the electrodes, i.e., the cell exhibits the p-DEP, as shown by the second group of cells in Fig. 5.83. Therefore, the necessary condition for the successful separation of deformable cells is the existence of two opposing DEP forces. In addition, the RBCs tend to deform into slipper shapes gradually, whatever they move down or up, as shown in Fig. 5.83. This deformation behavior is mainly attributed to the combined effects of the nonuniform electric field and parabolic fluid flow, similar to the case of single RBC.

The distribution of membrane mechanical force strength acting on the deformable cells is illustrated in Fig. 5.84(b), where it is larger at the RBC tails due to the larger deformation. Fig. 5.84(c) shows the distribution of intercellular interaction force

strength, which is significantly weak and acts on the opposing parts of membrane only. An attractive trend is observed between the deformable cells, as also demonstrated by the variation of intercellular interaction force in Fig. 5.85(b). At about 12.5 and 162.5 ms, the RBCs are closest to each other, and thus the intercellular interaction force also reaches the maximum. As the external force, the DEP force is significantly larger than the intercellular interaction force, as plotted in Fig. 5.85, and therefore the DEP force mainly determines whether the cells move upward or downward. Besides, since the DEP force strength acts on the RBC membranes relative uniformly, and the intercellular interaction force strength is significantly weak, the membrane mechanical force strength mainly determines the cell deformation.

Fig. 5.83 Separation of the first (solid line) and second (dotted line) groups of deformable cells with different interior permittivities at the high frequency $f = 10^9$ Hz, (a) $\xi_{in}^{c1} = 50\xi_0$ and $\xi_{in}^{c2} = 107\xi_0$, (b) $\xi_{in}^{c1} = 77\xi_0$ and $\xi_{in}^{c2} = 83\xi_0$, (c) $\xi_{in}^{c1} = 60\xi_0$ and $\xi_{in}^{c2} = 90\xi_0$, (d) $\xi_{in}^{c1} = 70\xi_0$ and $\xi_{in}^{c2} = 90\xi_0$, in which the permittivities are smaller than the fluid permittivity of $\xi_m = 80\xi_0$ for the first group of cells but larger for the second group of cells. The snapshots of cell shapes in each subfigure are taken from 0 to 200 ms with the time interval of 40 ms.

Fig. 5.84 Distributions of (a) the DEP force strength, (b) membrane mechanical force strength, and (c) intercellular interaction force strength on the two deformable cells with the interior permittivities of $\xi_{in}^{c1} = 50\xi_0$ and $\xi_{in}^{c2} = 107\xi_0$ at $t = 120$ ms in Fig. 5.83(a).

Fig. 5.85 Variations of (a) the DEP force and (b) intercellular interaction force acting on the two deformable cells with the interior permittivities of $\xi_{in}^{c1} = 50\xi_0$ and $\xi_{in}^{c2} = 107\xi_0$ in Fig. 5.83(a), where the dash line is a dividing line between the positive and negative values.

5.4.3.3 Separation of two group cells with different conductivities

Apart from the cell permittivities, the cell conductivity is also used as a critical control factor in the cell separation at the low frequency, as shown in Fig. 5.86. Unlike the behavior at the high frequency, the first group of RBCs (solid line) with the higher conductivity than the fluid conductivity goes down, while the second group (dotted line) with the lower conductivity moves up. This motion behavior is also independent of the initial location of deformable RBCs in the hybrid chip. The first group of RBCs at the low frequency experiences the p-DEP instead of the n-DEP, because they are conducted

better than the suspending fluid. Oppositely, the second group of RBCs at the low frequency exhibits the n-DEP instead of the p-DEP. The first group of RBCs is subjected to the outward DEP force strength, but the second group to the inward DEP force strength, as illustrated in Fig. 5.87(a). Therefore, the motion trends of deformable RBCs are totally reversed at the low and high frequencies. However, the deformation behavior that the cells change into the slipper shapes is quite similar at the low and high frequencies due to the similar membrane mechanical force strength, as plotted in Fig. 5.87(b). An attractive trend between two RBCs is still observed, as demonstrated by the intercellular interaction force strength in Fig. 5.87(c).

Fig. 5.86 Separation of the first (solid line) and second (dotted line) groups of deformable cells with different interior conductivities at the low frequency $f = 10^3$ Hz, (a) $\sigma_{in}^{c1} = 0.95$ mS/m and $\sigma_{in}^{c2} = 0.49$ mS/m, (b) $\sigma_{in}^{c1} = 1.6$ mS/m and $\sigma_{in}^{c2} = 0.4$ mS/m, (c) $\sigma_{in}^{c1} = 1.2$ mS/m and $\sigma_{in}^{c2} = 0.4$ mS/m, and (d) $\sigma_{in}^{c1} = 1.1$ mS/m and $\sigma_{in}^{c2} = 0.45$ mS/m, where the conductivities are larger than the fluid conductivity of $\sigma_{ex} = 0.5$ mS/m for the first group of cells but smaller for the second group of cells. The snapshots of cell shapes are taken from 0 to 200 ms with the time interval of 40 ms.

Fig. 5.88 plots the variations of DEP force and intercellular interaction force acting on the two RBCs in the Fig. 5.86(b). The DEP force acting on the first group of

RBCs is negative, but positive on the second group of RBCs. As a result, the DEP force attracts the first group of RBCs to the electrodes, but repels the second group of RBCs away from the electrodes. The intercellular interaction force acting on the first group of RBCs is negative before about 130 ms, but positive on the second group of RBCs. Subsequently, since the two groups of RBCs change their locations, the intercellular interaction force also changes its direction. Therefore, the two groups of RBCs attract each other during the whole separating process.

Fig. 5.87 Distributions of (a) the DEP force strength, (b) membrane mechanical force strength, and (c) intercellular interaction force strength on the two deformable cells with the interior conductivities of $\sigma_{in}^{c1} = 0.95$ mS/m and $\sigma_{in}^{c2} = 0.49$ mS/m at $t = 120$ ms in Fig. 5.86(b).

Fig. 5.88 Variations of (a) the DEP force and (b) intercellular interaction force acting on the two deformable cells with the interior conductivities of $\sigma_{in}^{c1} = 0.95$ mS/m and $\sigma_{in}^{c2} = 0.49$ mS/m in Fig. 5.86(b), where the dash line is plotted to divide positive and negative values.

5.4.3.4 *Remarks*

Deformable cells are separated successfully according to either their different permittivities at the high frequency, or their different conductivities at the low frequency. Whichever the high frequency or low frequency is applied, the necessary condition is the existence of two opposing DEP forces for successfully separating the two groups of deformable cells using the microfluidic-microelectric hybrid chip. In other words, if one group of cells experiences the p-DEP or n-DEP, the other group of cells is required to exhibit the opposite n-DEP or p-DEP. Therefore, the selection of suspending fluid becomes of critical importance, making the different groups of cells exhibit different DEP phenomena. Here is a guideline on how to select the suspending fluid. Firstly, if there is a large difference among the cell conductivities, the low frequency is selected to separate the cells. Otherwise, the high frequency is preferred because the hybrid chip is more sensitive to the variation of cell interior permittivity. Secondly, if the low frequency is employed for the separation of two groups of deformable cells, the conductivity of suspending fluid selected should be intermediate between the conductivities of two group cells. If the high frequency is employed however, the permittivity of suspending fluid selected should be intermediate between the permittivities of two group cells.

5.5 **Summary**

The present chapter has investigated the motion and deformation of a single cell or two cells in the hydrodynamic, electric, and hydrodynamic-electric fields, respectively. The differences among the three kinds of fields are listed in Tab. 5.7.

In the hydrodynamic field, the motion and deformation of a single RBC or two RBCs in a parabolic capillary flow are simulated, and several parameters are investigated in detail, including the RBC shape, radius, position, deformability, density, viscosity, intercellular interaction strength, and the flow velocity. The simulation results reproduce well the axisymmetric and asymmetric motion of the RBC in the capillary. In the axisymmetric motion, the RBC exhibits the characteristic parachute shape due to the symmetric shear force acting on the lower and upper parts of RBC, respectively. In the asymmetric motion however, the RBC assumes the distinct slipper shape because of the asymmetric shear force acting on the lower and upper parts of RBC. It is also found that

the RBC density plays a significant role in the RBC deformation, and even a slight variation of cell density will result in the different cell deformation. For example, if the cell density is relatively small, the cell is almost split like a gas bubble. On the contrary, the cell is hard to deform like a rigid body if its density is relatively large. Therefore, the two-fluid model is more accurate than the pure-fluid model to characterize the cell performance in a capillary flow. Besides, it is also showed that the cell deformation and aggregation can interact slightly with each other by the intercellular distance. If the cell deformation is large, the intercellular distance is relatively small so that the cell aggregation is obvious. In turn, if the cell aggregation is obvious, the intercellular distance is small so that the cell deformation is large. However, since the intercellular interaction strength is small in practice, the coupled effect of cell deformation and aggregation is not obvious.

In the electric field, the motion and deformation of a single RBC or two RBCs are simulated and investigated under the condition that the electric field is nonuniform and alternating. Due to the cell polarization, the dielectrophoresis (DEP) force is generated acting on the RBC membrane, depending on the various parameters, including the RBC size, electric potential, electric frequency, RBC permittivity and RBC conductivity. At the low frequency, the RBC exhibits the negative DEP (n-DEP) if it is less conductive than the suspending fluid, where the RBC is repelled away from the region with strong electric field strength, and the lower part of RBC deforms narrower and narrower. On the contrary, the RBC experiences the positive DEP (p-DEP) if it is more conductive than the suspending fluid, in which the RBC is attracted towards that region, and the lower part of RBC deforms wider and wider. At the high frequency, the RBC also experiences the n-DEP if it is less polarized than the suspending fluid, whereas the RBC exhibits the p-DEP if it is more polarized. Furthermore, if the intercellular interaction strength is strong enough, the expected trend of RBC motion may be changed. For example, the RBC exhibiting the p-DEP moves away from the region of strong electric field strength.

In the hydrodynamic-electric coupled field, a microfluidic-microelectric hybrid chip is numerically designed as a separator by choosing the suitable electrode width, flow velocity and electrode potential. After that, the separating ability of hybrid chip is evaluated by analyzing the effects of cell shape and position. Finally, a single RBC or two RBCs are manipulated by the designed hybrid chip, respectively. The working

principle of the hybrid chip is that the nonuniform AC electric field makes the RBC deviate first from the centerline of parabolic fluid flow, and then the parabolic fluid flow makes the RBC transported with the different velocity. The simulation results show that deformable cells can be separated successfully either at low frequency according to their different conductivities, or at the high frequency according to their different permittivities. However, whichever the high frequency or low frequency is applied, a necessary condition is required that both the n-DEP and p-DEP phenomena occur in the separation process. Since the n-DEP cell moves up while the p-DEP cell goes down, the different groups of deformable cells are separated successfully.

Tab. 5.7 Differences among the hydrodynamic, electric and hydrodynamic-electric fields, where the tick (✓) and cross (✗) show the presence and absence in simulations, respectively.

Field kind	Hydrodynamic field		Electric field		Hydrodynamic-electric field	
	One cell	Two cells	One cell	Two cells	One cell	Two cells
Shear force	✓	✓	✗	✗	✓	✓
Membrane mechanical force	✓	✓	✓	✓	✓	✓
Dielectrophoresis force	✗	✗	✓	✓	✓	✓
Intercellular interaction force	✗	✓	✗	✓	✗	✓

Chapter 6

Conclusions and Recommendations

In this chapter, the main conclusions are drawn first from the present research work, and several recommendations are then suggested for the future research work.

6.1 Conclusions from the present work

The present research work concentrates on the numerical investigation of the motion and deformation of a single cell or two cells in the microscale hydrodynamic, electric, and hydrodynamic-electric coupled fields, respectively. The two-fluid model is presented to describe the flow characteristics of cells and suspending fluid, considering the interactions between the cell and fluid field, between the cell and electric field, and between the two cells. Subsequently, the two novel numerical methods are proposed to efficiently solve the two-fluid model, including the modified particle binary level set (MPBLS) method for tracking the cell membrane, and the modified semi-implicit method for pressure linked equations-revised (modified SIMPLER). Finally, taking the red blood cells (RBCs) as cell samples, their motion and deformation are simulated and analyzed in detail from different perspectives in order to enhance the understanding of cell performance in the hydrodynamic, electric, and hydrodynamic-electric coupled fields.

The main conclusions drawn from the present work are highlighted in the numerical methodology including the two novel numerical techniques, and the simulation results exploring several important multiphysics phenomena of cells in

hydrodynamic and electric fields. They are summarized in the following two subsections, respectively.

6.1.1 Numerical methodology

One of the major achievements made is the development of the two novel numerical methods, namely the MPBLS method and the modified SIMPLER algorithm.

- In the present work, the MPBLS method is developed to track the cell membrane for efficiently saving the computational cost by considering a set of Lagrangian particles that are already scattered on the cell membrane to calculate the interfacial forces. There are two main differences between the present MPBLS method and previous particle level set (PLS) approach. One is the level set function, and the other is the particle pattern. The MPBLS method uses the binary level set function only to distinguish different materials, whereas the PLS approach usually employs the signed distance function to distinguish different materials and to calculate the normal direction and curvature. Compared with the signed distance function, the binary level set function is easy for numerical implementation, although it is a discontinuous function requiring high-order numerical schemes to be solved. In terms of particle pattern, the particles in the MPBLS method are scattered only on the interface for the multi-purposes, including the interface correction, calculation of the normal direction and curvature, and calculation of the interfacial forces. In the PLS approach however, the particles are scattered on the both sides of interface to correct the interface only. As a result, the particle number in the MPBLS method is significantly smaller than that in the PLS approach. Taking the Zalesak's disk in rotating flow and the square plate in single vortex flow for example, it is shown that the MPBLS method is more accurate and less time-consuming than the PLS approach. It is more suitable to multiphase and interfacial flow applications with Lagrangian particles. For 3D problems however, the present MPBLS method becomes tedious unlike the PLS approach, since the particles in 3D domain difficultly correct the surface-like interface and determine its normal direction.
- In the present work, the modified SIMPLER algorithm is developed to solve the incompressible Navier-Stokes equations with the very low Reynolds number. Compared with the previous SIMPLER algorithm, the present modified

SIMPLER algorithm includes two distinct techniques. One is the introduction of an improved velocity correction, which can speed up the convergence of momentum equations. The other technique is the integration of a fine adjustment, which can make the continuity equation satisfied with higher accuracy. By taking the Poiseuille flow with different Reynolds number for example, it is demonstrated that the modified SIMPLER algorithm is more accurate and convergent than the SIMPLER algorithm. It is thus more applicable to solve the incompressible Navier-Stokes equations with low Reynolds number.

Apart from the above achievement, the effort is also devoted to improve the characterization of interactions between the cell and fluid field, between the cell and electric field, and between the two cells, respectively.

- The shell model is introduced to characterize the interaction between the cell and fluid field, i.e., to formulate the membrane mechanical force strength. The cell membrane is treated as an incompressible and elastic shell with a uniform thickness, such that both the stretching and bending deformation is taken into account in the shell model.
- The MST approach is employed to characterize the interaction between the cell and electric field, i.e., to formulate the dielectrophoresis (DEP) force strength. The biological cell is modeled as a homogeneous interior enclosed by an insulated membrane. Since the cell deformation is not predictable in advance, the cell shape is arbitrary during the deformation process. Therefore, the MST approach is a better choice for determining the DEP force strength acting on the cell with an arbitrary shape.
- The Morse potential is adopted to characterize the interaction between the two cells, i.e., to formulate the intercellular interaction force strength. The intercellular interaction force is characterized by the Morse potential as a strong repulsive force at near distance, but a weak attractive force at far distance. The Morse potential is used just for simplicity, and does not imply any intrinsic mechanism of the intercellular interaction.

6.1.2 Simulation results

The other major achievement made is the exploration of several important multiphysics phenomena of cells, namely the motion and deformation of a single cell or two cells in the hydrodynamic, electric, and hydrodynamic-electric fields, respectively.

- In the hydrodynamic field, the motion and deformation of a single RBC or two RBCs in a parabolic capillary flow are simulated, and several parameters are investigated in detail, such as the RBC position, density, interaction strength, and so on. The simulation results reproduce the axisymmetric and asymmetric motion of the RBC in the capillary, in which the characteristic parachute and slipper shapes are exhibited, respectively. It is also observed that the RBC density plays a significant role in the RBC deformation, and even a slight variation of cell density will result in the different cell deformation. Therefore, the two-fluid model is more accurate than the pure-fluid model to characterize the RBC performance in a capillary flow. Besides, it is also demonstrated that the cell deformation and aggregation can interact with each other due to the different intercellular distance. In general, the cell deformation increases with increasing the cell aggregation, and vice versa.
- In the electric field, the motion and deformation of a single RBC or two RBCs are simulated and investigated under the condition that the electric field is nonuniform and alternating. This study provides an understanding of DEP mechanism from the standview of DEP force strength acting on a deformable object. The simulation results demonstrate that the RBC performance in the nonuniform electric field depends on the various parameters, including the RBC size, electric potential, electric frequency, RBC permittivity and RBC conductivity. At the low frequency, the RBC exhibits the negative DEP (n-DEP) if it is less conductive than the suspending fluid, where the DEP force strength is directed inward and larger at the lower part of RBC. Therefore, the RBC is repelled away from the region with strong electric field strength, and its lower part deforms narrower and narrower. On the contrary, the RBC experiences the positive DEP (p-DEP) if it is more conductive than the suspending fluid, in which the DEP force strength is directed outward and still larger at the lower part of RBC. Consequently, the RBC is attracted towards the region with strong electric field strength, and its lower part

deforms wider and wider. At the high frequency, the RBC experiences the n-DEP if it is less polarized than the suspending fluid, whereas the RBC exhibits the p-DEP if it is more polarized. Furthermore, if the intercellular interaction strength is strong enough, the expected trend of RBC motion may be changed. For example, the RBC exhibiting the p-DEP can move away from the region of strong electric field strength.

- In the hydrodynamic-electric coupled field, a microfluidic-microelectric hybrid chip is numerically designed, and used to manipulate a single deformable RBC or two deformable RBCs, respectively. The working principle of hybrid chip is that the nonuniform AC electric field makes the RBC deviate first from the centerline of parabolic fluid flow, and then the parabolic fluid flow makes the RBC transported with the different velocities. The simulation results show that deformable RBCs can be separated successfully either at low frequency according to their different conductivities, or at the high frequency according to their different permittivities. However, whichever the high frequency or low frequency is imposed, a necessary condition required for the successful separation is that both the n-DEP and p-DEP phenomena occur. In general, since different groups of cells have different dielectric properties, the necessary condition is easily achieved as long as a suitable suspending fluid is selected. If the high frequency is employed, the permittivity of suspending fluid selected should be intermediate between the permittivities of different group cells. If the low frequency is employed however, the conductivity of suspending fluid selected should be intermediate between the conductivities of different group cells.

6.2 Recommendations for the future work

The present research work has provided a better understanding of the performance of a single cell or two cells in the hydrodynamic, electric, and hydrodynamic-electric coupled field, respectively. Several future research directions are recommended to gain a more comprehensive insight, listed as follows.

- It would be interesting to extend the modified particle binary level set (MPBLS) method to 3D problems. In the present work, although the proposed MPBLS method is more accurate and less time-consuming than the previous particle level

set (PLS) approach, it is used for 2D problems only. It is believed that the MPBLS method in 3D problems is still more accurate and less time-consuming, because only a set of Lagrangian particles is scattered on the interface, instead of two sets of particles like in the PLS approach. However, it may be tedious for the MPBLS method to solve 3D problems, because the Lagrangian particles in 3D domain cannot easily correct the surface-like interface and determine its normal direction.

- In the hydrodynamic field, the present work has simulated the performance of deformable cells in a capillary with the rigid walls. However, the capillary wall is deformable in practice, such that the capillary also has deformation when the cells pass through it. Therefore, it is recommended to investigate the performance of cells in the capillary with the deformable walls, especially for the cells with size comparable with capillary diameter, which is involved in moving boundary problems.
- In the electric field, the present work has simulated the motion and deformation of neutral cells in a nonuniform AC electric field, relating to the dielectrophoresis (DEP) only. Therefore, it would be interesting to investigate the motion and deformation of charged cells in a nonuniform AC electric field, involving both the DEP and electrophoresis (EP).
- In the hydrodynamic-electric coupled field, the present work has numerically designed a microfluidic-microelectric hybrid chip for the cell separation, and a single RBC or two RBCs have been manipulated according to the different permittivities and conductivities. Therefore, it is definitely worth conducting experiments under the guide of the present numerical simulations, and in turn to validate the present numerical results by the experimental observations.

Appendix A

Cubic Smoothing Spline Method

A spline function is a piecewise polynomial constructed from conjoint segments subject to the boundary conditions at the ends and continuous constraints at the joints. It is widely used in various applications requiring the data interpolation and curve smoothing. In this appendix, the basic idea and numerical implementation of the cubic smoothing spline method with a periodic boundary condition are introduced in detail.

Assume we have $N+1$ sets of data (x_i, y_i) ($i=0,1,\dots,N$) with the periodic condition $y_0 = y_N$, and the each set satisfies the function

$$y_i = f(x_i) + e_i \quad (\text{A.1})$$

where e_i is a sequence of independently distributed random variables with the zero mean and standard deviation ζ_i . The purpose of cubic smoothing spline technique is to represent $f(x)$ by constructing a cubic smoothing spline function $S(x)$, which makes the $N+1$ sets of data concentrated in the vicinity of spline to the utmost extent. The detailed procedure is listed as follows.

Step 1: It should be described how rough the data is. One roughness measure function is given by evaluating the curve curvature [229],

$$p(S) = \int_{x_0}^{x_N} (S''(x))^2 dx \quad (\text{A.2})$$

The higher the value of $p(S)$ is, the wigglier the data will be. An objective functional is introduced by adding a smoothing parameter λ and considering the residual sum of squares, expressed as [230]

$$L(S) = \lambda \sum_{i=0}^{N-1} \left(\frac{y_i - S(x_i)}{\zeta_i} \right)^2 + (1 - \lambda) \int_{x_0}^{x_N} (S''(x))^2 dx \quad (\text{A.3})$$

where the parameter λ reflects the relative importance between the residual sum of squares and variability of function $S(x)$. As such, the purpose of cubic smoothing spline technique becomes to find a cubic smoothing spline function $S(x)$, which can minimize the value of $L(S)$. At $\lambda = 0$, the smoothing spline function $S(x)$ becomes a straight line fitting to the data. At $\lambda = 1$, it becomes an interpolating spline exactly passing through the data points, because only the closeness between the spline and data is concerned.

Step 2: The boundary conditions and continuous constraints should be provided for simplifying Eq. (A.3). The cubic spline function on the i -th segment is given by

$$S_i(x) = a_i(x - x_i)^3 + b_i(x - x_i)^2 + c_i(x - x_i) + d_i \quad x \in [x_i, x_{i+1}] \quad (\text{A.4})$$

where the subscript i changes from 0 to $N - 1$. The periodic boundary conditions at the ends are expressed as

$$\begin{aligned} S_0(x_0) &= S_{N-1}(x_N) \\ S'_0(x_0) &= S'_{N-1}(x_N) \\ S''_0(x_0) &= S''_{N-1}(x_N) \end{aligned} \quad (\text{A.5})$$

For convenience of derivation, the spline function $S(x)$ is extended to another two virtual segments with considering the periodic boundary conditions

$$\begin{aligned} S_{-1}(x_0) &= S_{N-1}(x_N) & S_N(x_N) &= S_0(x_0) \\ S'_{-1}(x_0) &= S'_{N-1}(x_N) & S'_N(x_N) &= S'_0(x_0) \\ S''_{-1}(x_0) &= S''_{N-1}(x_N) & S''_N(x_N) &= S''_0(x_0) \end{aligned} \quad (\text{A.6})$$

and thus we obtain

$$\begin{aligned} b_N &= b_0 & b_{-1} &= b_{N-1} \\ d_N &= d_0 & d_{-1} &= d_{N-1} \end{aligned} \quad (\text{A.7})$$

The continuous constraints at the joints are given by

$$\begin{aligned} S_i(x_{i+1}) &= S_{i+1}(x_{i+1}) & i &= 0, 1, \dots, N-2 \\ S'_i(x_{i+1}) &= S'_{i+1}(x_{i+1}) & i &= 0, 2, \dots, N-2 \\ S''_i(x_{i+1}) &= S''_{i+1}(x_{i+1}) & i &= 0, 1, \dots, N-2 \end{aligned} \quad (\text{A.8})$$

Combining Eqs. (A.4), (A.6) and (A.8), we have

$$\begin{aligned} h^3 a_i + h^2 b_i + h c_i + d_i &= d_{i+1} & i &= 0, 1, \dots, N-1 \\ 3h^2 a_{i-1} + 2h b_{i-1} + c_{i-1} &= c_i & i &= 0, 1, \dots, N-1 \\ 6h a_i + 2b_i &= 2b_{i+1} & i &= 0, 1, \dots, N-1 \end{aligned} \quad (\text{A.9})$$

in which $h = x_{i+1} - x_i$ is the constant step size of independent variable. Solving Eq. (A.9) yields

$$\begin{aligned} c_i &= \frac{d_{i+1} - d_i}{h} - \frac{h}{3}(b_{i+1} + 2b_i) & i &= 0, 1, \dots, N-1 \\ \frac{h^2}{3}(b_{i-1} + 4b_i + b_{i+1}) &= d_{i-1} - 2d_i + d_{i+1} & i &= 0, 1, \dots, N-1 \\ a_i &= \frac{b_{i+1} - b_i}{3h} & i &= 0, 1, \dots, N-1 \end{aligned} \quad (\text{A.10})$$

Considering Eq. (A.7), the second formula in Eq. (A.10) is expressed as the matrix form,

$$\mathbf{A}\mathbf{b} = \mathbf{B}\mathbf{d} \quad (\text{A.11})$$

where

$$\mathbf{A} = \frac{h^2}{3} \begin{pmatrix} 4 & 1 & & & 1 \\ 1 & 4 & 1 & & \\ & \ddots & \ddots & \ddots & \\ & & 1 & 4 & 1 \\ 1 & & & 1 & 4 \end{pmatrix}_{N \times N} \quad \mathbf{b} = \begin{pmatrix} b_0 \\ b_1 \\ \vdots \\ b_{N-2} \\ b_{N-1} \end{pmatrix} \quad (\text{A.12})$$

and

$$B = \begin{pmatrix} -2 & 1 & & & 1 \\ 1 & -2 & 1 & & \\ & \ddots & \ddots & \ddots & \\ & & 1 & -2 & 1 \\ 1 & & & 1 & -2 \end{pmatrix}_{N \times N} \quad \mathbf{d} = \begin{pmatrix} d_0 \\ d_1 \\ \vdots \\ d_{N-2} \\ d_{N-1} \end{pmatrix} \quad (\text{A.13})$$

Note that the matrix B is singular, but the matrix A is nonsingular. Consequently, the vector $\mathbf{b}$ is expressed as

$$\mathbf{b} = A^{-1}B\mathbf{d} \quad (\text{A.14})$$

Step 3: The objective functional $L(S)$ should be expressed in terms of the coefficient vectors. The term $S_i''(x)$ is a linear function on the interval $[x_i, x_{i+1}]$, changing from $2b_i$ to $2b_{i+1}$, given by

$$S_i''(x) = 2 \left[b_i + \frac{b_{i+1} - b_i}{h} (x - x_i) \right] \quad (\text{A.15})$$

and thus we have

$$\int_{x_0}^{x_N} (S''(x))^2 dx = \frac{4h}{3} \sum_{i=0}^{N-1} (b_i^2 + b_i b_{i+1} + b_{i+1}^2) = \frac{2h}{3} \mathbf{b}^T A \mathbf{b} \quad (\text{A.16})$$

Moreover, considering

$$S_i(x_i) = d_i \quad (\text{A.17})$$

yields

$$\sum_{i=0}^{N-1} \left(\frac{y_i - S(x_i)}{\zeta_i} \right)^2 = (\mathbf{y} - \mathbf{d})^T W (\mathbf{y} - \mathbf{d}) \quad (\text{A.18})$$

in which

$$W = \begin{pmatrix} 1/\zeta_0 & & & & \\ & 1/\zeta_1 & & & \\ & & \ddots & & \\ & & & 1/\zeta_{N-2} & \\ & & & & 1/\zeta_{N-1} \end{pmatrix} \quad \mathbf{y} = \begin{pmatrix} y_0 \\ y_1 \\ \vdots \\ y_{N-2} \\ y_{N-1} \end{pmatrix} \quad (\text{A.19})$$

The matrix W is often called the weight matrix. For the i -th set of data, the larger the standard deviation is, the less significant the effect on the final results will be, and therefore the cubic spline function $S(x)$ becomes smoother. Combining Eqs. (A.14), (A.16) and (A.18), Eq. (A.3) is rewritten as

$$L(\mathbf{d}) = \lambda (\mathbf{y} - \mathbf{d})^T W (\mathbf{y} - \mathbf{d}) + (1 - \lambda) \frac{2h}{3} \mathbf{d}^T B^T A^{-1} B \mathbf{d} \quad (\text{A.20})$$

Step 4: Differentiating $L(\mathbf{d})$ with respect to $\mathbf{d}$, and setting to zero to find the value of $\mathbf{d}$ minimizing the function $L(\mathbf{d})$, we have

$$-2\lambda W (\mathbf{y} - \mathbf{d}) + (1 - \lambda) \frac{4h}{3} B^T A^{-1} B \mathbf{d} = 0 \quad (\text{A.21})$$

Using Eq. (A.14), Eq. (A.21) is simplified as

$$(1 - \lambda) \frac{2h}{3} B^T \mathbf{b} = \lambda W (\mathbf{y} - \mathbf{d}) \quad (\text{A.22})$$

Eq. (A.22) is further simplified using Eq. (A.14) again, and expressed as a compact form for notational convenience

$$C\mathbf{b} = \mathbf{g} \quad (\text{A.23})$$

where

$$C = \left(\lambda A + (1 - \lambda) \frac{2h}{3} B W^{-1} B^T \right) \quad (\text{A.24})$$

$$\mathbf{g} = \lambda B \mathbf{y}$$

Step 5: The successive over relaxation (SOR) method is employed to numerically solve the linear algebraic system in Eq. (A.23). Since the matrix C is symmetric and diagonally dominant, the iterative scheme is given by,

$$b_i^{(k+1)} = (1 - \chi) b_i^{(k)} + \frac{\chi}{c_{ii}} \left(g_i - \sum_{j=0}^{i-1} c_{ij} b_j^{(k+1)} - \sum_{j=i+1}^{N-1} c_{ij} b_j^{(k)} \right) \quad i = 0, 1, \dots, N-1 \quad (\text{A.25})$$

in which χ is the relaxation factor. Once the value $\mathbf{b}$ is obtained, the value of smoothing spline function $S(x)$ at each joint can be calculated by Eq. (A.22)

$$\mathbf{d} = \mathbf{y} - \frac{1-\lambda}{\lambda} \frac{2h}{3} \mathbf{W}^{-1} \mathbf{B}^T \mathbf{b} \quad (\text{A.26})$$

Note that the value $\mathbf{d}$ is not calculated by Eq. (A.14) due to the singularity of matrix $\mathbf{B}$.

For example, Fig. A.1 shows the smoothed results using the cubic smoothing spline method with the different values of smoothing parameter λ . The spline is a straight line at $\lambda = 0.0$, while the interpolating spline appears at $\lambda = 1.0$, exactly passing through every point. At $\lambda = 0.2$, a smoothing spline is fitted to the data points, where a good smoothing effect is found.

Fig. A.1 Cubic smoothing spline of the 21 sets of data centralizing the function $y = \sin(x)$.

Appendix B

Measures of Deformable Shape

In this appendix, four basic measures are introduced to describe the rheological characteristics of a deformable object based on its moments, including the area, centroid, extension and orientation.

The moments of deformable object are defined as follows.

- One zero-order moment:

$$m_{00} = \iint_D dx dy \quad (\text{B.1})$$

- Two first-order moments:

$$m_{10} = \iint_D x dx dy, \quad m_{01} = \iint_D y dx dy \quad (\text{B.2})$$

- Three second-order moments:

$$m_{20} = \iint_D x^2 dx dy, \quad m_{11} = \iint_D xy dx dy, \quad m_{02} = \iint_D y^2 dx dy \quad (\text{B.3})$$

Here D denotes the domain of deformable object. It is obvious that the area A of deformable object is directly calculated by the zero-order moment

$$A = m_{00} \quad (\text{B.4})$$

and the centroid (x_c, y_c) is expressed as

$$x_c = m_{10}/m_{00}, \quad y_c = m_{01}/m_{00} \quad (\text{B.5})$$

However, the definitions of extension and orientation are quite complex [227]. The second-order central moments are defined as

$$\begin{aligned} \mu_{20} &= m_{20} - m_{10}^2/m_{00} \\ \mu_{11} &= m_{11} - m_{10}m_{01}/m_{00} \\ \mu_{02} &= m_{02} - m_{01}^2/m_{00} \end{aligned} \quad (\text{B.6})$$

and normalized as

$$\eta_{20} = \mu_{20}/m_{00}^2, \quad \eta_{11} = \mu_{11}/m_{00}^2, \quad \eta_{02} = \mu_{02}/m_{00}^2 \quad (\text{B.7})$$

The two rotational invariants are thus defined as

$$\begin{aligned} \alpha_1 &= 2\pi(\varsigma_1 + \sqrt{\varsigma_2}) \\ \alpha_2 &= 2\pi(\varsigma_1 - \sqrt{\varsigma_2}) \end{aligned} \quad (\text{B.8})$$

where

$$\begin{aligned} \varsigma_1 &= \eta_{20} + \eta_{02} \\ \varsigma_2 &= (\eta_{20} - \eta_{02})^2 + 4\eta_{11}^2 \end{aligned} \quad (\text{B.9})$$

Therefore, the extension EX and orientation OR are defined by the above two invariants,

$$\begin{aligned} EX &= \log_2(\alpha_1) \\ \tan(2OR) &= \frac{2\eta_{11}}{\eta_{20} - \eta_{02}} \end{aligned} \quad (\text{B.10})$$

The extension describes how much the deformable object differs from a circle. It takes zero value if the deformable object is circular, and increases without limit as the shape becomes less compact. The orientation is the angle of the long axis of deformable object measured positive anticlockwise from the y axis in the range $-\pi/2$ to $\pi/2$, as shown in Fig. B.1.

Fig. B.1 Definition of the orientation, (a) the positive orientation when the deformable object tilts anticlockwise, and (b) the negative orientation when the deformable object tilts clockwise.

Now we turn our attention to how to numerically calculate the four measures by the binary level set function, which is used in the modified particle binary level set (MPBLS) method. The zero-order moment in Eq. (B.1) is discretized as

$$m_{00} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} A_{i,j} \quad (\text{B.11})$$

where I_x and J_y are the mesh size on the x and y directions, and $A_{i,j}$ is the area occupied by the deformable object in the mesh (i, j) , given by

$$A_{i,j} = \begin{cases} \Delta x \Delta y & \phi_{i,j} \leq -0.25 \\ (0.5 - 2\phi_{i,j}) \Delta x \Delta y & -0.25 < \phi_{i,j} < 0.25 \\ 0 & \phi_{i,j} \geq 0.25 \end{cases} \quad (\text{B.12})$$

in which Δx and Δy are the spatial steps on the x and y directions. According to this formula, the area occupied by the deformable object is approximated as the mesh area for $\phi_{i,j} \leq -0.25$, whereas it is assumed to be zero for $\phi_{i,j} \geq 0.25$, as illustrated in Fig. B.2(a) and (b). For $\phi_{i,j} = 0.0$, the occupied area is approximated as a half mesh, as shown in Fig. B.2(c). A linear relationship is assumed between the occupied area and the binary level set function in the range -0.25 to 0.25 , as plotted in Fig. B.3.

Fig. B.2 Illustration of the area occupied by the deformable object in the mesh (i, j) .

The first-order moments are discretized as

$$m_{10} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} i \Delta x A_{i,j}, \quad m_{01} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} j \Delta y A_{i,j} \quad (\text{B.13})$$

and the second-order moments are

$$m_{20} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} (i \Delta x)^2 A_{i,j}, \quad m_{11} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} ij \Delta x \Delta y A_{i,j}, \quad m_{02} = \sum_{i=1}^{I_x} \sum_{j=1}^{J_y} (j \Delta y)^2 A_{i,j} \quad (\text{B.14})$$

Fig. B.3 Illustration of the relationship between the area occupied by the deformable object and the binary level set function in the mesh (i, j) .

Taking a circle with the radius of 0.5 as an example, the zero-, first- and second-order moments are calculated using the above numerical technique, and the numerical error is evaluated, as listed in Tab. B.1. It is found that each of the numerical moments is quite close to the corresponding exact value, and the largest error is about 3% for the second order moment.

Tab. B.1 Comparison between the exact and numerical results of the zero-, first- and second-order moments for a circular shape.

Moments	m_{00}	m_{10}	m_{01}	m_{20}	m_{11}	m_{02}
Numerical	0.7861	1.025	0.5227	1.387	0.6818	0.3969
Exact	0.7854	1.004	0.5022	1.333	0.6422	0.3702
Error	0.0007	0.021	0.0205	0.054	0.0396	0.0267

Appendix C

Weighted Essentially Non-Oscillatory Scheme

In this appendix, the weighted essentially non-oscillatory (WENO) scheme is introduced to interpolate the binary level set value at the half mesh with high resolution based on its values at the grid point.

Take the interpolation of $\phi_{i+1/2,j}^+$ using the fifth-order WENO scheme for example. Assume that there is a stencil with five grid points, as illustrated in Fig. C.1. By constructing polynomials on the stencil, the value $\phi_{i+1/2,j}^+$ at the half mesh is easily interpolated. There are three widely-used approaches to construct the polynomials. The first approach is to directly construct a fourth-order polynomial using the five grid points on the stencil. However, this approach can't keep the high accuracy when the binary level set function is discontinuous on the stencil. The second approach is to construct a polynomial on a smooth substencil, which is adaptively selected by the Newton divided difference of each substencil. This is the basic idea of the essentially non-oscillatory (ENO) scheme. Since the discontinuous substencil is not used in the interpolation, the corresponding Newton divided difference is thrown away although it is calculated to select the smooth substencil, resulting in enormous waste of computational resources. In view of this disadvantage, the third approach is to construct the polynomials on all the substencils, and then distribute the different weights to the each polynomial according to the smoothness level of substencil. This is the main idea of the WENO scheme, which not only makes full use of the substencils but also improves the computational accuracy.

Suppose that the stencil with the five grid points is divided into three substencils with the three grid points as

$$S_{i-1} = \{i-2, i-1, i\}, \quad S_i = \{i-1, i, i+1\}, \quad S_{i+1} = \{i, i+1, i+2\} \quad (\text{C.1})$$

The procedure of how to interpolate $\phi_{i+1/2,j}^+$ is presented as follows.

Step 1: Construct three second-order interpolation polynomials L_1 , L_2 and L_3 on the substencils S_{i-1} , S_i and S_{i+1} respectively, as shown in Fig. C.1. The polynomial values at the half mesh are thus calculated as

$$\begin{aligned} \phi_{(i+1/2,j),S_{i-1}}^+ &= \frac{1}{3}\phi_{i-2,j}^+ - \frac{7}{6}\phi_{i-1,j}^+ + \frac{11}{6}\phi_{i,j}^+ \\ \phi_{(i+1/2,j),S_i}^+ &= -\frac{1}{6}\phi_{i-1,j}^+ + \frac{5}{6}\phi_{i,j}^+ + \frac{1}{3}\phi_{i+1,j}^+ \\ \phi_{(i+1/2,j),S_{i+1}}^+ &= \frac{1}{3}\phi_{i,j}^+ + \frac{5}{6}\phi_{i+1,j}^+ - \frac{1}{6}\phi_{i+2,j}^+ \end{aligned} \quad (\text{C.2})$$

Step 2: Decide the weights according to the smoothness level of substencil. The widely-used weights are expressed as [212]

$$w_r = \frac{\alpha_r}{\alpha_{i-1} + \alpha_i + \alpha_{i+1}} \quad r = i-1, i, i+1 \quad (\text{C.3})$$

with

$$\alpha_r = \frac{d_r}{(e + \beta_r)^2} \quad (\text{C.4})$$

Here e is the very small error to avoid the denominator to become zero, and d_r is a constant defined as a set of weights of smooth substencils. For the three substencils, d_r is given by [212]

$$d_{i-1} = \frac{1}{10}, d_i = \frac{3}{5}, d_{i+1} = \frac{6}{10} \quad (\text{C.5})$$

In addition, β_r is often called as the smoothness factor, given by [212]

$$\begin{aligned}
 \beta_{i-1} &= \frac{13}{12}(\phi_{i-2,j}^+ - 2\phi_{i-1,j}^+ + \phi_{i,j}^+)^2 + \frac{1}{4}(\phi_{i-2,j}^+ - 4\phi_{i-1,j}^+ + 3\phi_{i,j}^+)^2 \\
 \beta_i &= \frac{13}{12}(\phi_{i-1,j}^+ - 2\phi_{i,j}^+ + \phi_{i+1,j}^+)^2 + \frac{1}{4}(\phi_{i-1,j}^+ - \phi_{i+1,j}^+)^2 \\
 \beta_{i+1} &= \frac{13}{12}(\phi_{i,j}^+ - 2\phi_{i+1,j}^+ + \phi_{i+2,j}^+)^2 + \frac{1}{4}(3\phi_{i,j}^+ - 4\phi_{i+1,j}^+ + \phi_{i+2,j}^+)^2
 \end{aligned} \tag{C.6}$$

It is found that β_r approaches to zero if ϕ^+ is continuous enough on the stencil S_r , and thus the weight w_r is very large. Therefore, the smoother a sub-stencil is, the larger the weight will be, or vice versa.

Step 3: Combine the three interpolated values with the corresponding weights.

$$\phi_{i+1/2,j}^+ = \sum_{r=i-1}^{i+1} w_r \phi_{(i+1/2,j),S_r}^+ \tag{C.7}$$

Similarly, the value of $\phi_{i+1/2,j}^-$ can be interpolated on the substencils

$$S_{i+2} = \{i+3, i+2, i+1\}, \quad S_{i+1} = \{i+2, i+1, i\}, \quad S_i = \{i+1, i, i-1\} \tag{C.8}$$

and the value of $\phi_{i,j+1/2}^+$ in the y direction is also obtained.

Fig. C.1 Illustration of the three interpolation polynomials on the substencils S_{i-1} , S_i , and S_{i+1} in the fifth-order WENO scheme.

For example, consider a linear convection equation

$$\frac{\partial u}{\partial t} + \frac{\partial u}{\partial x} = 0 \tag{C.9}$$

with the initial condition

$$u|_{t=0} = \begin{cases} 0.5 & 0 < x \leq 0.5 \\ -0.5 & 0.5 < x \leq 1 \end{cases} \quad (\text{C.10})$$

where u is the velocity, x and t are the spatial and temporal variables. Its exact solution is easily obtained by the method of characteristics, and given by

$$u(x,t) = \begin{cases} 0.5 & 0 < x-t \leq 0.5 \\ -0.5 & 0.5 < x-t \leq 1 \end{cases} \quad (\text{C.11})$$

Its numerical solution is calculated by the fifth-order WENO scheme for the discretization of the convection term and the third-order TVD Runge-Kutta method for the time discretization. Fig. C.2 shows the comparison between the numerical and exact solutions at $t=0.2$, and a good agreement is exhibited. In the numerical solution, a slight dissipation is observed, but there is no dispersion in the vicinity of discontinuity.

Fig. C.2 Comparison between the numerical solution calculated by the WENO scheme and exact solution obtained by the method of characteristics at $t=0.2$ for a linear convection equation.

Appendix D

Effective Velocity of Lagrangian Particles

This appendix concentrates on the calculation of the effective velocity of Lagrangian particles using the area weighted average, which is the main idea of the particle in cell (PIC) method [205].

Fig. D.1 Four cases for the calculation of intersecting areas between the constructed domain and neighboring meshes.

Assume that the location of a Lagrangian particle is denoted as (x^p, y^p) , and the spatial steps are Δx and Δy in the x and y directions. Firstly, construct a domain with the center of (x^p, y^p) , and the length and width of Δx and Δy . Secondly, calculate the intersecting areas between the constructed domain and neighboring meshes. Thirdly, calculate the effective velocity by the area weighted average. There are four cases for the calculation of intersecting areas according to the location of Lagrangian particle, described as follows.

Case 1: $i\Delta x \leq x^p < (i+1/2)\Delta x$ and $j\Delta y \leq y^p < (j+1/2)\Delta y$

The Lagrangian particle is located in the bottom left part of mesh (i, j) , as shown in Fig. D.1(a). The area of each intersecting part is given by

$$\begin{aligned} S_1 &= ((i+1/2)\Delta x - x^p)((j+1/2)\Delta y - y^p) \\ S_2 &= (x^p - (i-1/2)\Delta x)((j+1/2)\Delta y - y^p) \\ S_3 &= ((i+1/2)\Delta x - x^p)(y^p - (j-1/2)\Delta y) \\ S_4 &= (x^p - (i-1/2)\Delta x)(y^p - (j-1/2)\Delta y) \end{aligned} \quad (D.1)$$

Defining

$$S = S_1 + S_2 + S_3 + S_4 = \Delta x \Delta y \quad (D.2)$$

the effective velocity is thus calculated by the area weighted average,

$$\begin{aligned} u_p &= \frac{S_1}{2S}(u_{i-1/2,j-1} + u_{i-1/2,j}) + \frac{S_2}{2S}(u_{i+1/2,j-1} + u_{i+1/2,j}) \\ &\quad + \frac{S_3}{2S}(u_{i-1/2,j} + u_{i-1/2,j+1}) + \frac{S_4}{2S}(u_{i+1/2,j} + u_{i+1/2,j+1}) \\ v_p &= \frac{S_1}{2S}(v_{i-1,j-1/2} + v_{i,j-1/2}) + \frac{S_2}{2S}(v_{i,j-1/2} + v_{i+1,j-1/2}) \\ &\quad + \frac{S_3}{2S}(v_{i-1,j+1/2} + v_{i,j+1/2}) + \frac{S_4}{2S}(v_{i,j+1/2} + v_{i+1,j+1/2}) \end{aligned} \quad (D.3)$$

Case 2: $(i+1/2)\Delta x \leq x^p < (i+1)\Delta x$ and $j\Delta y \leq y^p < (j+1/2)\Delta y$

The Lagrangian particle is located in the bottom right part of mesh (i, j) , as illustrated in Fig. D.1(b). The area of each intersecting part is given by

$$\begin{aligned}
 S_1 &= \left((i+3/2)\Delta x - x^p \right) \left((j+1/2)\Delta y - y^p \right) \\
 S_2 &= \left(x^p - (i+1/2)\Delta x \right) \left((j+1/2)\Delta y - y^p \right) \\
 S_3 &= \left((i+3/2)\Delta x - x^p \right) \left(y^p - (j-1/2)\Delta y \right) \\
 S_4 &= \left(x^p - (i+1/2)\Delta x \right) \left(y^p - (j-1/2)\Delta y \right)
 \end{aligned} \tag{D.4}$$

The effective velocity is expressed by

$$\begin{aligned}
 u_p &= \frac{S_1}{2S} (u_{i+1/2,j-1} + u_{i+1/2,j}) + \frac{S_2}{2S} (u_{i+3/2,j-1} + u_{i+3/2,j}) \\
 &\quad + \frac{S_3}{2S} (u_{i+1/2,j} + u_{i+1/2,j+1}) + \frac{S_4}{2S} (u_{i+3/2,j} + u_{i+3/2,j+1}) \\
 v_p &= \frac{S_1}{2S} (v_{i,j-1/2} + v_{i+1,j-1/2}) + \frac{S_2}{2S} (v_{i+1,j-1/2} + v_{i+2,j-1/2}) \\
 &\quad + \frac{S_3}{2S} (v_{i,j+1/2} + v_{i+1,j+1/2}) + \frac{S_4}{2S} (v_{i+1,j+1/2} + v_{i+2,j+1/2})
 \end{aligned} \tag{D.5}$$

Case 3: $i\Delta x \leq x^p < (i+1/2)\Delta x$ and $(j+1/2)\Delta y \leq y^p < (j+1)\Delta y$

The Lagrangian particle is located in the top left part of mesh (i, j) , as shown in Fig. D.1(c). The area of each intersecting part is given by

$$\begin{aligned}
 S_1 &= \left((i+1/2)\Delta x - x^p \right) \left((j+3/2)\Delta y - y^p \right) \\
 S_2 &= \left(x^p - (i-1/2)\Delta x \right) \left((j+3/2)\Delta y - y^p \right) \\
 S_3 &= \left((i+1/2)\Delta x - x^p \right) \left(y^p - (j+1/2)\Delta y \right) \\
 S_4 &= \left(x^p - (i-1/2)\Delta x \right) \left(y^p - (j+1/2)\Delta y \right)
 \end{aligned} \tag{D.6}$$

The effective velocity is expressed by

$$\begin{aligned}
 u_p &= \frac{S_1}{2S} (u_{i-1/2,j} + u_{i-1/2,j+1}) + \frac{S_2}{2S} (u_{i+1/2,j} + u_{i+1/2,j+1}) \\
 &\quad + \frac{S_3}{2S} (u_{i-1/2,j+1} + u_{i-1/2,j+2}) + \frac{S_4}{2S} (u_{i+1/2,j+1} + u_{i+1/2,j+2}) \\
 v_p &= \frac{S_1}{2S} (v_{i-1,j+1/2} + v_{i,j+1/2}) + \frac{S_2}{2S} (v_{i,j+1/2} + v_{i+1,j+1/2}) \\
 &\quad + \frac{S_3}{2S} (v_{i-1,j+3/2} + v_{i,j+3/2}) + \frac{S_4}{2S} (v_{i,j+3/2} + v_{i+1,j+3/2})
 \end{aligned} \tag{D.7}$$

Case 4: $(i+1/2)\Delta x \leq x^p < (i+1)\Delta x$ and $(j+1/2)\Delta y \leq y^p < (j+1)\Delta y$

The Lagrangian particle is located in the top right part of mesh (i, j) , as illustrated in Fig. D.1(d). The area of each intersecting part is given by

$$\begin{aligned}
 S_1 &= \left((i + 3/2) \Delta x - x^p \right) \left((j + 3/2) \Delta y - y^p \right) \\
 S_2 &= \left(x^p - (i + 1/2) \Delta x \right) \left((j + 3/2) \Delta y - y^p \right) \\
 S_3 &= \left((i + 3/2) \Delta x - x^p \right) \left(y^p - (j + 1/2) \Delta y \right) \\
 S_4 &= \left(x^p - (i + 1/2) \Delta x \right) \left(y^p - (j + 1/2) \Delta y \right)
 \end{aligned} \tag{D.8}$$

The effective velocity is given by

$$\begin{aligned}
 u_p &= \frac{S_1}{2S} (u_{i+1/2, j} + u_{i+1/2, j+1}) + \frac{S_2}{2S} (u_{i+3/2, j} + u_{i+3/2, j+1}) \\
 &\quad + \frac{S_3}{2S} (u_{i+1/2, j+1} + u_{i+1/2, j+2}) + \frac{S_4}{2S} (u_{i+3/2, j+1} + u_{i+3/2, j+2}) \\
 v_p &= \frac{S_1}{2S} (v_{i, j+1/2} + v_{i+1, j+1/2}) + \frac{S_2}{2S} (v_{i+1, j+1/2} + v_{i+2, j+1/2}) \\
 &\quad + \frac{S_3}{2S} (v_{i, j+3/2} + v_{i+1, j+3/2}) + \frac{S_4}{2S} (v_{i+1, j+3/2} + v_{i+2, j+3/2})
 \end{aligned} \tag{D.9}$$

To sum up, a general expression is provided for the effective velocity of Lagrangian particle,

$$\begin{aligned}
 u_p &= \frac{S_1}{2S} (u_{m-1/2, n-1} + u_{m-1/2, n}) + \frac{S_2}{2S} (u_{m+1/2, n-1} + u_{m+1/2, n}) \\
 &\quad + \frac{S_3}{2S} \left(u_{m-\frac{1}{2}, n} + u_{m-\frac{1}{2}, n+1} \right) + \frac{S_4}{2S} (u_{m+1/2, n} + u_{m+1/2, n+1}) \\
 v_p &= \frac{S_1}{2S} (v_{m-1, n-1/2} + v_{m, n-1/2}) + \frac{S_2}{2S} (v_{m, n-1/2} + v_{m+1, n-1/2}) \\
 &\quad + \frac{S_3}{2S} (v_{m-1, n+1/2} + v_{m, n+1/2}) + \frac{S_4}{2S} (v_{m, n+1/2} + v_{m+1, n+1/2})
 \end{aligned} \tag{D.10}$$

where the area of each intersecting part is given by

$$\begin{aligned}
 S_1 &= \left((m + 1/2) \Delta x - x^p \right) \left((n + 1/2) \Delta y - y^p \right) \\
 S_2 &= \left(x^p - (m - 1/2) \Delta x \right) \left((n + 1/2) \Delta y - y^p \right) \\
 S_3 &= \left((m + 1/2) \Delta x - x^p \right) \left(y^p - (n - 1/2) \Delta y \right) \\
 S_4 &= \left(x^p - (m - 1/2) \Delta x \right) \left(y^p - (n - 1/2) \Delta y \right)
 \end{aligned} \tag{D.11}$$

and the subscripts m and n are defined as

$$\begin{aligned} m &= \begin{cases} i & \text{for } i\Delta x \leq x^p < (i+1/2)\Delta x \\ i+1 & \text{for } (i+1/2)\Delta x \leq x^p < (i+1)\Delta x \end{cases} \\ n &= \begin{cases} j & \text{for } j\Delta y \leq y^p < (j+1/2)\Delta y \\ j+1 & \text{for } (j+1/2)\Delta y \leq y^p < (j+1)\Delta y \end{cases} \end{aligned} \tag{D.12}$$

Appendix E

Simple Preprocessing Software

This appendix introduces the development of simple preprocessing software. In the present work, many parameters are included, and have different values in the different simulation cases. It is quite troublesome to change the parameter values in the main program developed by the C++ programming language when simulating different cases, often resulting in wrong inputs. For convenience therefore, simple preprocessing software is developed using the C++ programming language for setting the parameter values in numerical simulations. After the parameter values are set via the preprocessing software, the main program can read them as the input data, and then start running to obtain the simulation results. Fig. E.1 shows the user interface of the preprocessing software, including a cover and five subinterfaces. The preprocessing software can handle four simulation cases, namely cells in the “Front Tracking”, “Hydrodynamic Field”, “Electric Field”, and “Hydrodynamic-Electric Field”. In the “Front Tracking” and “Hydrodynamic Field” cases, the parameters of cell and hydrodynamic field are required to be set. In the “Electric Field” and “Hydrodynamic-Electric Field” cases, the parameters of electric field are also required, except those of cell and hydrodynamic field. Besides, the cell properties include the parameters of cell interior and membrane in the present preprocessing software, because the cell is modeled as a homogeneous interior enclosed by an elastic and insulated membrane with a finite thickness in the present work. All the values listed in the following subinterfaces are default. If a special value is required for a parameter, we can input it in the corresponding textbox.

(a) Software cover for the operating instructions. The left five buttons are used to perform the corresponding operations. When a button is pressed, a corresponding subinterface appears, as shown in the following subfigures. The right textbox is used to show the instructions on how to use the present preprocessing software. Moreover, the differences among the four simulation cases are briefly described here.

(b) Software subinterface for selecting the simulation cases and setting the several computational parameters. The "Back" button at the bottom right corner is used to go back the software cover for look through the operating instructions.

(c) Software subinterface for setting the several parameters of hydrodynamic field, including the flow and dielectric properties of suspending fluid.

(d) Software subinterface for setting the several parameters of electric field, which is available only when the simulation cases are selected as the “Electric Field” and “Hydrodynamic-Electric Field”. There is a necessary condition for the “Electrode Width” $0 < d_1 < L/2$ due to the electrode layout, where L is the length of computational domain.

(e) Software subinterface for setting the cell parameters, where a single cell or two cells should be selected first. The cell dielectric properties include the permittivity and conductivity of cell interior, and the capacitance and conductance of cell membrane.

(f) Software subinterface for a default case, namely the front tracking of a single cell in a steady parabolic flow with the maximum velocity of 1.0 mm/s.

Fig. E.1 User interface of the simple preprocessing software for setting the parameter values used in simulations, including a cover and five subinterfaces.

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Publications Arising from the Thesis

Journal Papers:

1. **Ting Ye**, Hua Li and K. Y. Lam, *Modeling and simulation of microfluid effects on deformation behavior of a red blood cell in a capillary*, Microvasc. Res. 80 (2010), 453-463.
2. **Ting Ye**, Hua Li and K. Y. Lam, *Numerical design of microfluidic-microelectric hybrid chip for the separation of biological cells*, Langmuir 27 (2011), 3188-3197.
3. Hua Li, **Ting Ye** and K. Y. Lam, *Numerical modeling of motion trajectory and deformation behavior of a cell in a nonuniform electric field*, Biomicrofluidics 5 (2011), 021101.
4. **Ting Ye**, Hua Li and K. Y. Lam, *Motion, deformation and aggregation of two cells in a microchannel by dielectrophoresis*, Electrophoresis 32 (2011), 3147-3156.
5. Hua Li, **Ting Ye** and K. Y. Lam, *Qualitative and quantitative analysis of dynamic deformation of a cell in nonuniform alternating electric field*, J. Appl. Phys. 110 (2011), 104701.
6. Hua Li, **Ting Ye** and K. Y. Lam, *A novel interface tracking method based on Lagrangian particles for deformation analysis of a red blood cell in capillary*, Int. J. Numer. Meth. Fluids, 69 (2012), 1031-1044.

7. **Ting Ye**, Hua Li and K. Y. Lam, *Numerical modeling for the separation of multiple deformable cells using dielectrophoresis*, Phys. Fluids (submitted).
8. Hua Li, **Ting Ye** and K. Y. Lam, *Computational analysis of dynamic interaction of two red blood cells in a capillary*, Cell Biochem. Biophys. (submitted).

Conference Papers:

1. **Ting Ye** and Hua Li, *Axisymmetric and asymmetric behaviors of a red blood cell in capillaries*, Proceedings of the International Conference on Biomedical Electronics and Devices (2011), 97-102, Rome, Italy.
2. **Ting Ye** and Hua Li, *Modeling of cell motion in micro-scale hydrodynamic-electrical field*, Adv. Mat. Res. 74 (2009), 139-142, International Conference on Materials for Advanced Technologies (2009), Singapore.