THREE-DIMENSIONAL MODELING OF PASSIVE AND ACTIVE MIGRATION OF LIVING CELLS IN A MICROCHANNEL

AN ABSTRACT

SUBMITTED ON THE EIGHTEENTH OF DECEMBER 2013 TO THE DEPARTMENT OF BIOMEDICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE SCHOOL OF SCIENCE AND ENGINEERING OF TULANE UNIVERSITY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

Hongzhi Lan

APPROVED:

Damin B. Khismatullin, Ph.D. Director

Donald P. Gaver, Ph.D.

Walter Lee Murfee, Ph.D.

Ricardo Cortez, Ph.D.
Abstract

The migration of living cells plays an important role in immune response, hemostasis, cancer progression, delivery of nutrients, and microfluidic technologies such as cell separation/enrichment and flow cytometry. Using three-dimensional computational algorithm for multiphase viscoelastic flow and mass transport, this study is focused on the investigation of the effects of cell size, viscoelasticity, cortical tension, fluid inertia and cell-cell interaction on passive migration and deformation of leukocytes, and active deformation of circulating cells during chemotactic migration in a rectangular microchannel. The results of the passive migration modeling show that there is an almost linear increase in the distance between the wall and the lateral equilibrium position of liquid drops or leukocytes with the particle diameter-to-channel height ratio increased from 0.1 to 0.5. Drops with different bulk viscosities can be efficiently separated if their interfacial tension is low or the flow rate is sufficiently high. The microfluidic technology is well suited for the separation of leukocytes with different cytoplasmic viscosities and relaxation times, but it is much less sensitive to cortical tension. When a series of closely spaced cells with same size are considered, they generally undergo damped oscillation in both lateral and translational directions until they reach equilibrium positions where they become evenly distributed in the flow direction (self-assembly phenomenon). For a series of cells with different sizes, bigger cells could collide repeatedly with smaller ones and enter the other side of the channel (above or below the centerline). For a series of cells with different deformability, more deformable cells upon impact with less deformable cells move to an equilibrium position closer to the centerline. The results of our study provide better understanding of cell margination in bloodstream and cell
separation/enrichment in microfluidic devices. The simulation data on active migration of cells show the formation of a finger- or lamellipodium-like projection of the cell membrane towards the chemoattractant source and indicate that lowering the cortical tension facilitates cell protrusion.
THREE-DIMENSIONAL MODELING OF PASSIVE AND ACTIVE MIGRATION
OF LIVING CELLS IN A MICROCHANNEL

A DISSERTATION
SUBMITTED ON THE EIGHTEENTH OF DECEMBER 2013
TO THE DEPARTMENT OF BIOMEDICAL ENGINEERING
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
OF THE SCHOOL OF SCIENCE AND ENGINEERING
OF TULANE UNIVERSITY
FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY

BY

Hongzhi Lan

APPROVED

Damir B. Khismatullin, Ph.D. Director

Donald P. Gaver, Ph.D.

Walter Lee Murfee, Ph.D.

Ricardo Cortez, Ph.D.
©Copyright by Hongzhi Lan, 2013

All Rights Reserved
Acknowledgements

I would like to sincerely thank my advisor, Dr. Damir B. Khismatullin, for his guidance, patience and support throughout the time of my dissertation research. I was introduced into the exciting areas of cellular biomechanics and computational fluid dynamics with his great help. I appreciate Dr. Donald P. Gaver, Dr. Walter Lee Murfee and Dr. Ricardo Cortez for taking time to serve on my committee and providing suggestions and encouragement. I thank Dr. Hideki Fujioka in Center for Computational Science for his advice during my code development. My thanks also go to our collaborators, Dr. Dino Di Carlo (UCLA), Dr. Soojung Claire Hur (Harvard University) and Dr. Wonhee Lee (KAIST), for being willing to provide original experimental data and giving me inspiration on the simulation work.

I thank all the lab members and friends for their help, friendship and joy they brought to me during my graduate studies.

Lastly, I am deeply grateful to my parents and wife for their endless love, encouragement and trust over all these years. They always cared about me and never complained, even when they needed help themselves and even we were separated by thousands of miles for years.
# Table of Contents

Acknowledgements ........................................................................................................................................ ii

Table of Contents ..................................................................................................................................... iii

List of Tables ........................................................................................................................................... v

List of Figures .......................................................................................................................................... vi

Chapter 1 Introduction ..............................................................................................................................1

1.1 Motivation and objective ................................................................................................................1

1.2 Background .....................................................................................................................................7

1.2.1 Migration and deformation of a deformable particle .............................................................7

1.2.2 Hydrodynamic interaction between particles .........................................................................11

1.2.3 Cell mechanics ......................................................................................................................14

1.2.4 Active migration of living cells ...........................................................................................15

Chapter 2 Methods ..................................................................................................................................19

2.1 Cell model ......................................................................................................................................19

2.2 Navier-Stokes equations ..............................................................................................................20

2.3 Volume-of-fluid method .............................................................................................................23

2.4 Active migration ..........................................................................................................................28

2.5 Summary of computational algorithms ....................................................................................31

2.6 Parallel programming .................................................................................................................32

Chapter 3 A numerical study of the lateral migration and deformation of drops and leukocytes in a rectangular microchannel .............................................................................................................35

3.1 Abstract .........................................................................................................................................35

3.2 Simulation framework ..................................................................................................................36

3.3 Results and discussion ................................................................................................................40

3.4 Conclusions ...................................................................................................................................69

Chapter 4 Numerical simulation of deformable cell interaction during migration in a microchannel .....................................................................................................................................................70

4.1 Abstract .........................................................................................................................................70

4.2 Simulation framework ..................................................................................................................71

4.3 Results and discussion ................................................................................................................72

4.4 Conclusions ...................................................................................................................................89

Chapter 5 Active migration of circulating cells in a microchannel explored by three-dimensional numerical simulation .................................................................................................................................91

5.1 Abstract .........................................................................................................................................91
5.2 Simulation framework ........................................................................................................92
5.3 Results and discussion ....................................................................................................94
5.4 Conclusions ....................................................................................................................102
Chapter 6 Summary ..............................................................................................................104
References ............................................................................................................................106
List of Tables

Table 3-1: Grid convergence test for $D/H=0.5$, $\gamma=1$, $Re=10$, $Ca=0.14$..............................40
Table 5-1: Parameters used in the simulation of active migration.................................................93
List of Figures

Fig. 1-1 Lateral equilibrium positions of different types of living cells .........................4
Fig. 1-2 Cellular deformation by hydrodynamic stretching .............................................5
Fig. 1-3 Segre-Silberberg effect: lateral migration of particles in a channel ....................9
Fig. 1-4 Neutrophils with a lamellipodium or finger-like projection during active migration .............................................................................................................16
Fig. 1-5 The treadmilling and dendritic nucleation of actin filaments ..............................17
Fig. 2-1 Variables in a MAC grid cell ...........................................................................23
Fig. 2-2 2-D sketch of reconstructed interface by the Piecewise-Linear Interface Calculation (PLIC) method and calculation of C advection in x-direction ..........24
Fig. 2-3 Comparison of Continuous Surface Force (CSF) method and height function (HF) method ...........................................................................................................27
Fig. 2-4 Performance comparison between MPI code and OpenMP code ...................34
Fig. 3-1 Schematic of the computational domain showing geometrical dimensions and initial and boundary conditions used for passive migration ......................36
Fig. 3-2 Velocity disturbances caused by a Newtonian drop ........................................37
Fig. 3-3 Schematic of lateral migration and deformation of a deformable drop in a microchannel ............................................................................................................38
Fig. 3-4 Comparison of the numerical and analytical shapes of a Newtonian fluid drop moving at the centerline of the channel .................................................................42
Fig. 3-5 Comparison of the numerical and analytical data on the x-component of the Newtonian fluid drop velocity .................................................................................44
Fig. 3-6 Migration of a drop in a rectangular microchannel ........................................47
Fig. 3-7 Lateral equilibrium position, deformation index, and x-component velocity of a Newtonian drop perfused through a microchannel for different drop diameters and viscosities .................................................................50
Fig. 3-8 Lateral equilibrium position, deformation index, and orientation angle of a Newtonian drop perfused through a microchannel for different drop viscosities and capillary numbers .........................................................53
Fig. 3-9 Lateral equilibrium position, deformation index, and orientation angle of a Newtonian drop perfused through microchannel for different flow rates and drop viscosities .................................................................................55
Fig. 3-10 Effect of the Reynolds number on the lateral equilibrium position of Newtonian drops ...........................................................................................................57
Fig. 3-11 Lateral equilibrium position and deformation index of leukocytes perfused through a microchannel for different cell diameters and cytoplasmic viscosities. ........................................................................................61
Fig. 3-12 Lateral equilibrium position and deformation index of leukocytes perfused through a microchannel for different cell diameters and relaxation times ....................................................................................................63
Fig. 3-13 Lateral equilibrium position, deformation index, and orientation angle of leukocytes perfused through a microchannel for different flow rates and cytoplasmic viscosities ........................................................................................................65
Fig. 3-14 Comparison of lateral equilibrium position of cells and Newtonian drops with different sizes between simulation and experimental results ..........68
Fig. 4-1 Schematic of the simulated problem on pairwise interaction of cells ..........71
Fig. 4-2 Lateral equilibrium position of a series of identical and periodically spaced cells ................................................................................................................................................73
Fig. 4-3 Migration of cells during pairwise interaction when located at the bottom half of the microchannel ................................................................................................................................................76
Fig. 4-4 Migration of only two cells during pairwise interaction when located at the bottom half of the microchannel ................................................................................................................................................79
Fig. 4-5 Migration of only two cells during pairwise interaction when located at the bottom half of the microchannel with larger cell diameters ................................................................................................................................................81
Fig. 4-6 Migration of cells during pairwise interaction when located at the top and bottom halves of the microchannel respectively ................................................................................................................................................83
Fig. 4-7 Migration of cells during pairwise interaction when located at the bottom half of the microchannel with a large initial z-distance ................................................................................................................................................85
Fig. 4-8 Migration of cells with different diameters during pairwise interaction when located at the bottom half of the microchannel ................................................................................................................................................87
Fig. 4-9 Migration of cells with different deformability during pairwise interaction when located at the bottom half of the microchannel ................................................................................................................................................88
Fig. 5-1 Sketch of the channel dimensions for active migration ........................................................................................................................................................92
Fig. 5-2 Boundary conditions for polymerization messenger and chemoattractant in the simulation of finger-like projection ........................................................................................................................................................94
Fig. 5-3 3-D and 2-D views of cell finger-like projection ........................................................................................................................................................95
Fig. 5-4 Elongation of the projection with time ........................................................................................................................................................96
Fig. 5-5 Boundary conditions chemoattractant in the simulation of lamellipodium-like extension ........................................................................................................................................................97
Fig. 5-6 3-D and 2-D views of cell lamellipodium-like extension ........................................................................................................................................................98
Fig. 5-7 Concentration of chemoattractant and polymerization messenger at the center point of the front end of the cell ........................................................................................................................................................99
Fig. 5-8 The “vision” of the cell on the chemoattractant near the membrane and the corresponding polymerization messenger ........................................................................................................................................................101
Chapter 1 Introduction

1.1 Motivation and Objective

Blood is the most important bodily fluid in all vertebrates including human beings. It plays a key role in delivery of nutrients to cells in tissues and removal of byproducts of cell metabolism from the tissues, in the initiation of immune response to invading pathogens, and in body thermoregulation. All these functions depend on rheological properties of blood, i.e., its flow characteristics under various conditions (blood vessel diameter, blood pressure, protein and blood cell content). Significant changes in the blood rheological properties result in serious pathophysiological conditions such as aneurysm, sickle cell disease, thrombosis, thromboembolism and atherosclerosis (leading cause of deaths in the United States) and contribute to inflammatory and autoimmune diseases, cancer and diabetes.

From a mechanical point of view, whole blood is a highly complex multiphase fluid consisting of blood plasma (continuous phase) and circulating cells (dispersed phases) [1]. The disperse phases are blood cells such as erythrocytes (red blood cells), thrombocytes (platelets), and leukocytes (white blood cells) and other individual cells under certain conditions (e.g., cancer/tumor cells during hematogenous metastasis [2]). Circulating cells are the major determinant of blood rheology. They take ~45% of the whole blood volume, and they are much more viscous and elastic than blood plasma,
which is a dilute solution of proteins, glucose, salt and other solutes in water and thus can be treated as a Newtonian fluid with viscosity slightly higher (1.2 times) than the water viscosity. Circulating cells are the primary reason why whole blood is a non-Newtonian fluid with yield stress, shear thinning and viscoelastic properties. The rheological properties of whole blood are highly dynamic properties because of different level of deformation of circulating cells in vessels of different diameter and drastic differences in geometric and mechanical properties between circulating cells of different type [3], because of the ability of the circulating cells to adhere to each other and form aggregates directly in blood flow, and because of passive and active migration of these cells in blood flow due to their strong hydrodynamic interactions and adhesion and activation. These features complicate the development of realistic biophysical models of blood flow that can describe the conditions leading to diseases mentioned above and predict possible therapeutic interventions that can overcome these conditions. The focus of my work is on modeling passive and active migration of deformable circulating cells, which are important processes in immune responses, hemostasis, cancer progression, and delivery of oxygen and nutrients [2, 4-7] and play a central role in recent biotechnological applications such as flow cytometry and cell separation/enrichment in microfluidic devices [8-13].

Microfluidics has gained significant advances in various biochemical, clinical and industrial applications because of its advantages such as reduced sample consumption, laminar flow, portability, and parallelization over conventional cell handling processes [14]. One application of this technology is to exploit cell migration for the separation or enrichment of living cells, e.g., 1) the isolation of leukocytes from red blood cells in
whole human blood to reducing sample size and handling [10], 2) the capture of circulating tumor cells for the identification of cancer metastases [11], 3) the detection of bacteria in biological solutions for the investigation of microorganisms living within human bodies [12], and 4) the derivation of a stem cell line from tissue aspirates for regenerative medicine [13].

Many microfluidic methods has been developed for the separation of cells (and pathogenic microorganisms), such as microfabricated fluorescence-activated cell sorter (µFACS), microfluidic magnetic cell separation, transient cell-ligand adhesion, electrophoresis and free flow acoustophoresis, which require the external assistance of fluorescence, electro-magnetic force, acoustic force, or biochemical labeling, or adhesion molecule coating [15]. However, the separation of particles or cells of different size and/or deformability can be achieved by a purely hydrodynamic approach in a microfluidic flow chamber [10, 16-22]. A particle or cell experiences a lateral drift during its perfusion through a flow channel to reach a specific position (lateral equilibrium position) between the walls and centerline of the channel, which depends on the size and deformability of the particle [21, 23] and its interaction with other cells if multiple cells exit in the channel[4]. As different types of cells could have different lateral equilibrium positions, they can be separated with a special design of channel outlet (Fig. 1-1). Since the aforementioned external assistance is not required, the hydrodynamic method can be a simple, low-cost and high-throughput assay for the particle separation problem.
Fig. 1-1 (a) Lateral equilibrium positions of different types of living cells. Malignant breast cancer cells are closer to the centerline than benign ones. (b) Special design of the channel outlet for separation of different types of cells. (Copy from Hur et al. [21])
Another promising application of the microfluidic technology is deformability-based flow cytometry [8, 9]. Cellular deformation has been used as a distinctive biomarker for certain cell types and pathological conditions [24, 25]. This includes sickle cell disease where red blood cells become less deformable and more sticky [26], sepsis where circulating leukocytes are less deformable because of their activation [27], and cancer metastasis, which is associated with a change in the deformability of tumor cells [28]. Living cells with different deformability can be assessed from the statistical analysis of their deformed shape in a microchannel due to hydrodynamic stretching (Fig. 1-2) [29]. Their mechanical properties can further be extracted from these data by using realistic computational models for cellular deformation in a microchannel. This application can avoid the issue that leukocytes and other blood cells can be activated and have different rheology when exposed to large external forces in other approaches[3], including micropipette aspiration [30-32], atomic force microscopy [33, 34], nanoindentation [35], optical tweezers [36, 37], and magnetic twisting cytometry [38, 39].

Fig. 1-2 (a) Hydrodynamic stretching of living cells during perfusion through a cross flow channel. (b) The deformed shape of a cell with the long and short principal axes. (c) Deformation profile of human embryonic stem cells. (Copy from Gossett et al. [29])
Besides passive migration, many living cells including leukocytes can migrate actively during embryogenesis, immune surveillance and wound healing [40]. One typical example is chemotaxis, the directed movement of cells toward the source of chemoattractant [41], which plays a crucial role in both innate and adaptive immune responses of the body, and cancer metastasis [42, 43]. After contact and adhesion to endothelium, circulating leukocytes in the blood are activated and migrate across the endothelium and migrate in the interstitial tissue to pathogens [44]. During metastasis, malignant cancer cells experience the similar behavior when they travel to a distant site of the body through the cardiovascular or lymphatic system [43]. The cell’s active migration is a dynamic process of cytoskeleton remodeling, involving various biochemical reactions. In general, the active migration consists of protrusion of the cell leading edge, adhesion of the cell front to the substrate through integrins, and retraction of the rear of the cell [45]. However, in three-dimensional environments, integrin binding is not necessary for active migration [46, 47]. Therefore, the migration is mainly driven by intracellular forces generated by polymerization and depolymerization of actin filaments (F-actin) [48].

The specific objective of my research is to develop fully three-dimensional parallel algorithms for passive and active migration of living cells and then use these algorithms to numerically simulate passive and active migration of leukocytes and tumor cells in a microchannel with rectangular cross section. The specific aims include:

Specific Aim 1 (SA1): To develop a three-dimensional volume-of-fluid (VOF) based algorithm for viscoelastic multiphase flow with parallel programming using
Message Passing Interface (MPI) to implement high performance computation of the flow and deformation of drops and cells.

Specific Aim 2 (SA2): To simulate passive migration and deformation of leukocytes during perfusion through a rectangular microchannel, and:

(a) To study the effects of leukocyte properties (size, bulk shear viscosity and elasticity, cortical tension) and fluid inertia on leukocyte passive migration and deformation in a rectangular microchannel;

(b) To study the effect of cell-cell interaction on passive migration of multiple leukocytes in a rectangular microchannel;

Specific Aim 3 (SA3): To extend the VOF-based algorithm to model active cell migration by introducing an “intracellular active force” term into the momentum equations;

Specific Aim 4 (SA4): To study active migration of leukocytes and tumor cells driven by a chemoattractant stimulus in a rectangular microchannel.

1.2 Background

1.2.1 Migration and deformation of a deformable particle

During perfusion in a microchannel, a particle moves relative to the surrounding flow in the lateral (transverse) and translational directions. The lateral drift of a deformable particle in shear flow is driven by the lift force in the lateral direction, which changes with the lateral location of the particle. When the particle is near the wall the lift
force is positive due to the lubrication pressure field at the wall side of the particles [49-54] and particle rotation [55, 56], leading to a drift away from the wall. The lift force decreases in magnitude with increasing the distance between the particle and the wall until it becomes zero at the lateral equilibrium position [55]. This position is either the centerline or between the centerline and the wall [50]. The latter could be explained by nonlinearity of the velocity field in confined channels that causes asymmetry in the pressure field, with a higher pressure on the centerline side of the particle than that on the wall side [57]. As a result, particles located near or at the centerline drift to a certain position between the centerline and the wall, where the velocity nonlinearity and lubrication pressure contributions to the lift force compensate each other.

This effect is observed for both rigid and deformable particles: small rigid spherical particles migrate in a tube to the equilibrium position at approximately 0.4 times the tube radius from the wall in low Reynolds number (~10) [58], and move closer to the wall with the spacing as little as 0.1 times the tube radius as Reynolds number increases to 700 [59]. This tubular pinch of particles in Poiseuille flow is called Segre-Silberberg effect (Fig. 1-3a). Similarly, oil drops [21, 23, 60] and living cells [21] move in a rectangular channel to certain mid-way position between the centerline and the wall dependent on size mechanical properties, or Reynolds number (Fig. 1-3b).
Fig. 1-3 (a) Segre-Silberberg effect: randomly distributed particles in a tube move to an equilibrium position with a constant distance from the wall [58]. (b) Similar to Segre-Silberberg effect, particles randomly distributed in a rectangular channel (channel cross section $W \geq 2H$) move to the middle plane in the width direction with a constant distance from the channel wall [21].
The deformation of drops was extensively studied under simple shear flow conditions [61-63]. However, the assumption that the velocity profile is linear is not appropriate when describing flow in a microchannel, especially when the channel height is comparable with the drop size. Several experimental studies were conducted on the deformation of large drops in a tube or a rectangular channel [64, 65]. As discussed by Olbricht [65] and Goldsmith and Mason [66], drops with the undeformed radius between 0.95 to 1.13 of the tube radius assume a bullet-like shape, with increased elongation and larger gaps between the wall and the drop surface at higher capillary numbers (Ca). When reaching a critical value of Ca, drops experience shape instability that eventually leads to drop breakup [67]. Interestingly, less viscous drops show more deformation in confined flows, though the effect of drop viscosity is less pronounced than the effect of the capillary number [68]. Experimental analysis of small drop deformation in Poiseuille flow is scarce. One recent study shows, for example, that drops with the diameter less than half of the channel height deform to an ellipsoid-like shape at high Re, while they experience very little deformation when Re is low [21].

A number of mathematical models were proposed to describe the motion of a Newtonian fluid drop in Poiseuille flow. Some of them are analytical models restricted to the cases of very low Reynolds number, infinitesimally small perturbations from a spherical shape, and the ratio of the drop diameter to the channel height much less than one [69-71]. These models predict that drops migrate to approximately the mid-way between the centerline and the wall at the drop-to-fluid viscosity ratio between 0.5 and 10 and to the centerline for other values of the viscosity ratio. They also show that the axial velocity of drops is lower than the velocity of the undisturbed flow at the location of a
drop centroid. Several numerical models were proposed to simulate drop deformation and migration in a tube or a parallel-plate flow chamber under Stokes flow conditions. These include boundary integral methods in both two and three dimensions [72-75]. They show that if $Re$ is low, drops migrate toward the centerline after initial rapid deformation and during this migration the difference between the drop axial velocity and the undisturbed flow velocity increases with drop size, drop distance from the wall, and viscosity. With higher $Ca$, drops have higher deformation and steady-state velocities. Front tracking algorithms as well as lattice Boltzmann and volume-of-fluid (VOF) methods were exploited to study the drop dynamics in the presence of fluid inertia [76-78]. The predictions of these models are in line with experimental data. For example, the numerical simulation shows that at $Re < 1$, drops with low drop-to-fluid viscosity ratio (~0.1) migrate to the centerline and those with higher viscosity ratio (> 1.0) move to about the mid-way. At higher $Re$, even low-viscosity drops migrate to the mid-way. The numerical models also predict that an increase in $Ca$ leads to larger deformation of drops.

1.2.2 Hydrodynamic interaction between particles

Much research on the migration of circulating cells and deformable particles in flow channels was conducted in the simplest scenario of negligible intercellular or interparticle interactions where the cell-to-cell or particle-to-particle distance was much larger than the size of cells or particles (scenario A). In fact, cell interaction plays an important role when investigating cell migration in blood flow or microchannels when multiple cells appear with close distance. Experimental works have been done on interaction between white and red blood cells, which leads to erythrocyte aggregation and
leukocyte margination due to their different size, shape and deformability [4-7]. Therefore, scenario A does not work well for blood flow because of the large volume fraction of circulating cells and thus a close spacing between cells. For example, the volume fraction of erythrocytes (hematocrit) reaches 45% in humans [79].

In the scenario of dense suspension, called as scenario B, the migration of circulating cells significantly depends on hydrodynamic cell-cell interactions. Hydrodynamic interactions between erythrocytes and leukocytes and between erythrocytes and thrombocytes were studied in a number of experimental works [4-7, 80, 81]. These studies revealed that the geometric (size, shape) and mechanical properties (deformability) of the cells contribute to erythrocyte aggregation and leukocyte and thrombocyte margination [82]. Erythrocyte aggregation near the centerline leads to the formation a thin cell-free layer adjacent to the capillary wall. This layer is responsible for the Fahraeus effect, i.e., a decrease in hematocrit and apparent viscosity of the blood with a decrease in the capillary diameter [83]. Leukocyte margination, i.e., their lateral migration to vessel wall margins, is necessary for leukocytes to adhere to the wall and initiate the immune response [5]. Similarly, the migration of thrombocytes to vessel wall margins is a necessary step in blood clotting [84]. In terms of microfluidic applications for scenario B, it is important to mention biomimetic separation of leukocytes during whole blood perfusion in a microfluidic channel [10]. Lattice Boltzmann and immersed boundary methods have been used to model the flow of a dense suspension of deformable particles or cells [85-87]. The results of these computational studies indicate that highly deformable particles or erythrocytes aggregate during circulation, which pushes out solid
particles or leukocytes from the center, which enables the initial contact between leukocytes and endothelial cells on the vessel wall.

There exists another scenario (scenario \(AB\)) where the interactions between particles or cells may be significant but the average separation distance is larger than in the case of a dense suspension (scenario \(B\)). One example is the “single chain” flow of particles or cells in a small channel where a moving particle/cell interacts with only two neighbors located at some distance from the cell [88]. This self-assembly of circulating particles/cells has been employed in biomedicine, material synthesis, and logical computation [89-91] and shown to be very important for microfluidics-based flow cytometry and cell separation [92, 93]. Recent experimental studies show that circulating particles migrate to a specific lateral position where they are uniformly spaced in the flow direction (like beads on a string) as a result of the balance of attractive and repulsive interactions imposed by nearby particles [88, 94-96]. The analysis of scenario \(AB\) is very important for optimization of cell separation/enrichment microfluidic technologies because of the potential to increase the efficiency of cell separation when the cell concentration or volume fraction is between that of the dense and dilute suspensions. The pairwise interactions of spherical and nonspherical rigid particles in linear or quadratic shear flows have already been investigated [88, 97-99]. It was theoretically established that the flow disturbances induced by a rotating particle could repel the neighboring particle maintaining a finite distance between the particles [88]. The interactions of two deformable particles have not been analyzed in the context of cell migration in confined channels. Previous work was about the interaction of two elastic solid particles close to
the wall in linear shear flow [100] and the effect of the collision of two elastic capsules or drops on their deformation in linear shear flow [101, 102].

1.2.3 Cell mechanics

Living cells have a very complex and dynamic structure. Their mechanical properties strongly depend on the biochemical composition of the cell cytoskeleton and plasma membrane and the presence and properties of cell organelles (e.g., nucleus). Blood cells of different type differ in mechanical properties. For example, mature red blood cells lack nucleus and have a rich cytoskeleton only in the vicinity of the plasma membrane. These cells are highly deformable and have an equilibrium biconcave shape, as compared to leukocytes that have most organelles and three-dimensional cytoskeleton that spans all space from the nucleus to the plasma membrane [103]. Leukocytes are of spherical shape when not exposed to shear flow. In addition to a difference in passive mechanical properties, leukocytes (and circulating tumor cells) can deform actively, i.e., their deformability can change with time as a result of cytoskeleton remodeling induced by outside-in signaling via chemoattractant molecules [104].

In this study, I focused on modeling the dynamics of leukocytes such as neutrophils. More than 50% of all the leukocytes in human blood are neutrophils and most of investigation about mechanical properties of leukocytes were conducted on neutrophils [104]. Neutrophils play a central role in acute inflammation, and could transmigrate during circulation through the endothelium activated by proinflammatory mediators [3]. It is known that neutrophils deform to a teardrop shape during rolling on the endothelium in vivo [105] and behave as a highly viscous liquid drop when aspirated
into a micropipette [106]. Neutrophils are other leukocytes have a ruffled plasma membrane with microvilli, which produce excess surface area and protect the cells against the dilation caused by deformation [107]. It’s also observed that their cortical layer (plasma membrane together with actin cytoskeleton) possesses cortical tension similar to interfacial tension in drops [31, 32]. Based on these observations, these cells were modeled as a viscous drop in the simplest form[108]. A thorough investigation of the cell behavior indicates that the cytoskeleton (a polymer network with a number of cross links) can be modeled as a viscoelastic or non-Newtonian material [109, 110]. The viscoelastic deformation of leukocytes was described by different constitutive equations including Maxwell [111] and Giesekus models [112] as well as a power-law fluid model [109]. Several computational models were developed for elastic capsules and fluid vesicles [54, 113, 114]. In these models, the fluid inside a deformable particle has the same properties as the external fluid, which makes them unsuitable for leukocytes and other deformable particles with bulk properties different from that of the external fluid.

**1.2.4 Active migration of living cells**

During chemotaxis, the chemoattractant receptors on the cell membrane trigger outside-in signaling, which can lead to cytoskeleton remodeling. When chemoattractant binds its receptor, second messengers such as phosphoinositide 3-kinases (PI3-Ks), Rho family GTPases, cyclic AMP and so on are produced [115-117]. PI3-Ks induce the phosphorylation of phosphatidylinositol lipids, such as PtIns(3,4,5)P$_2$ or PIP$_3$. Both the lipids and Rho GTPases activate Arp2/3 complex [118], which is required for actin filament growth [119]. A neutrophil can develop a large polarized shape called
lamellipodium at the front of the cell and a contracted tail known as uropod at the back
[120, 121], or extend a finger-like projection called pseudopod towards the
chemoattractant source [46] (Fig. 1-4).

Fig. 1-4 (a) A neutrophil with a lamellipodium during active migration. (Copy from Eddy et al. [121]) (b) A neutrophil with a finger-like projection. (Copy from Zhelev et al. [46])
Two popular hypotheses were made to explain how F-actin polymerization and depolymerization produces protrusive and contractile forces inside the cell. One of them is treadmilling, in which actin filaments add its globular monomers to the plus (barbed) end and dissociate the monomers from the minus (pointed) end to create the directional movement of actin filaments (Fig. 1-5a) [118, 122]. But the treadmilling velocity is too slow to explain rapid motion of leukocytes [123]. Another hypothesis is dendritic nucleation of actin filaments mediated by Arp2/3 complex protein [119, 124]. Arp2/x complex is activated in leukocytes by chemoattractant receptor signaling. The complex caps the pointed ends and initiates the growth of daughter filaments at the barbed direction of the mother filament with other binding proteins (Fig. 1-5b).

**Fig. 1-5** (a) The treadmilling of actin filament. (Copy from Lowery and Vactor [122]) (b) The dendritic nucleation of actin filaments mediated by Arp2/3 complex protein. (Copy from Machesky et al. [124])
Mathematical models were developed to explain the generation of active force for migration. To describe contractile biological polymer networks, the cell was regarded as a multiphase reactive system (reactive interpenetrating flow formalism), including cytosol (aqueous solvent) and cytoskeleton (network of filaments) [125]. The internal active force was derived from a stress tensor due to the cytoskeleton dynamics, which produces either an interfilament force (networking swelling model) or a network-to-membrane interaction force (polymerization force model) [126]. The network-to-membrane interaction also depends on the polymerization messenger concentration. Other models on active force generation include Brownian ratchet model [127], treadsevering model [128], stochastic branching model [129], molecular motors model [130].

Although cytoskeleton remodeling can drive the cell motion and deformation, integrin binding is still necessary to constrain the cell to migrate on a 2-D surface, such as leukocyte migration on endothelium after firm adhesion. Firm adhesion of a leukocyte is mediated by $\beta_2$ and $\alpha_4$ integrins on its membrane and corresponding ligands such as Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) [131]. The formation and rupture of bonds between integrin (receptor) and ligand of endothelium was described by the spring-peeling kinetic model [132]. When the separation distance between the leukocyte and the endothelium is less than the total length of the unstretched receptor and ligand, receptor-ligands bonds can form and impose a tensile force (bond force) on the leukocyte. Leukocyte adhesion has been studied theoretically as a 3-D rigid particle or numerically as a viscoelastic drop by 2-D or 3-D models [112].
Chapter 2 Methods

2.1 Cell model

The numerical models developed to simulate drop dynamics can be applied to study leukocyte biomechanics [112, 133, 134]. The fluid inside a cell includes two compartments: Newtonian solvent (described by viscosity $\mu_s$) and a polymer matrix. The viscoelasticity of the polymer matrix is captured by the Giesekus model [135]:

$$\lambda\left(\frac{\partial \mathbf{T}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{T} - (\nabla \mathbf{u}) \mathbf{T} - \mathbf{T} (\nabla \mathbf{u})^T\right) + \lambda \kappa \mathbf{T}^2 + \mathbf{T} = \lambda G(0)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T). \quad (2-1)$$

Here $\mathbf{u} = (u, v, w)$ is the velocity vector; $\mathbf{T}$ is the extra stress tensor that represents the polymer contribution to the shear stress field; $G(0)$ is the elastic modulus at $t = 0$; $\lambda$ is the relaxation time, i.e., the ratio of the polymer viscosity $\mu_p$ to $G(0)$. In the current study, the Giesekus nonlinear parameter $\kappa$ was assumed to be zero, so, more specifically the Oldroyd-B model was used. The total viscosity of a deformable particle $\mu_d = \mu_s + \mu_p$.

This model can handle the simulation of shear-induced deformation of high-viscosity drops provided the polymer viscosity of the drop is a dominant contributor to the total viscosity of the drop [112, 136, 137]. This is because the polymer viscosity is included in the Giesekus model, which is solved using an unconditionally stable scheme [112, 138]. The viscoelastic algorithm was previously tested against the experimental
data and small deformation theory for drop breakup [138], and it was also shown to realistically describe leukocyte deformation in parallel-plate and microfluidic flow chambers [112, 136, 137]. Other three-dimensional algorithms for viscoelastic fluid-fluid flow were developed by Aggarwal and Sarkar [139], Pillapakkam and Singh [140], Hooper et al. [141], Yue et al. [142], and Zhou et al. [133]. These algorithms were not applied to the problem of drop migration. Recently, a two-dimensional numerical algorithm for solid particle migration in a viscoelastic fluid has been developed [143]. Viscoelasticity is a property of all living cells and it is crucially important to take it into account in the simulation of leukocyte motion and deformation [3, 106, 144].

The Giesekus model was solved by a semi-implicit scheme in which the advection term and the last term in the left-hand side of the equation were in the implicit part, but the contravariant and nonlinear terms were treated explicitly [112, 138]. By this way, a factorized scheme could be used to solve the equation. For a more accurate solution, the extra stress tensor is solved twice. First, the intermediate \( T^* \) is derived based on previous velocity field \( u^{(n)} \) as Eq. (2-2). Then, \( T^{(n+1)} \) is solved in terms of new velocity field \( u^{(n+1)} \).

\[
(\Delta t + \lambda^{n+1}) \left( 1 + \frac{\Delta t \lambda^{n+1}}{\Delta t + \lambda^{n+1}} u^n \frac{\partial}{\partial x} + \frac{\Delta t \lambda^{n+1}}{\Delta t + \lambda^{n+1}} v^n \frac{\partial}{\partial y} + \frac{\Delta t \lambda^{n+1}}{\Delta t + \lambda^{n+1}} w^n \frac{\partial}{\partial z} \right) T^* = \lambda^{n+1} T^n - \Delta t \lambda^{n+1} \kappa T^{n+2} + \Delta t \lambda^{n+1} ((\nabla u^n)^T T^n + T^n (\nabla u^n)^T) + G(0) \lambda^{n+1} (\nabla u^n + (\nabla u^n)^T)
\]

\(2-2\) Navier-Stokes equations

The velocity field is found from the solution of the Navier-Stokes equations:
\[ \nabla \cdot \mathbf{u} = 0, \quad (2-3) \]

\[ \rho \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = \nabla \cdot \mathbf{T} - \nabla p + \nabla \cdot (\mu_s (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)) + \mathbf{F}. \quad (2-4) \]

\( \rho \) is the mass density, \( \mathbf{F} \) is the body force due to interfacial tension, and \( p \) is pressure. All components of the extra stress tensor are zero in the external fluid, i.e., we model the external fluid as a purely Newtonian fluid with viscosity \( \mu_{\text{ext}} \) replacing \( \mu_s \). Since the cell solvent viscosity-to-external fluid viscosity ratio is \( O(1) \), the numeric solution of the Navier-Stokes equations is stable. The numeric instability appears when the drop solvent viscosity is more than 50 times higher than the external fluid viscosity.

The Navier-Stokes equations were solved by a semi-implicit predictor-corrector-type projection method [138] on a staggered Marker-and-Cell (MAC) grid (Fig. 2-1). In the predictor-corrector approach, the explicit scheme was used for the intermediate velocity \( \mathbf{u}_{\text{expl}}^* \):

\[ \frac{\mathbf{u}_{\text{expl}}^* - \mathbf{u}^*}{\Delta t} = -\mathbf{u}^* \cdot \nabla \mathbf{u}^* + \frac{1}{\rho_{n+1}^*} (\nabla \cdot \mathbf{T}^* + \nabla \cdot (\mu_s^{n+1} (\nabla \mathbf{u}^* + (\nabla \mathbf{u}^*)^T)) + \mathbf{F}^{n+1}). \quad (2-5) \]

\( \rho_{n+1}^* \) and \( \mathbf{F}^{n+1} \) were calculated explicitly based on the new interface. Based on this velocity, the pressure field \( p^* \) was solved:

\[ \nabla \cdot \left( \frac{\nabla p^*}{\rho} \right) = \frac{\nabla \cdot \mathbf{u}_{\text{expl}}^*}{\Delta t}. \quad (2-6) \]
Then, a new intermediate velocity field $u^*$ was recalculated via a semi-implicit scheme using this intermediate pressure field $p^*$ (for instance, $x$-component):

$$
\left(1 - \frac{\Delta t}{\rho^{n+1}_s} \frac{\partial}{\partial x} \left(2\mu^{n+1}_s \frac{\partial}{\partial x}\right) - \frac{\Delta t}{\rho^{n+1}_s} \frac{\partial}{\partial y} \left(\mu^{n+1}_s \frac{\partial}{\partial y}\right) - \frac{\Delta t}{\rho^{n+1}_s} \frac{\partial}{\partial z} \left(\mu^{n+1}_s \frac{\partial}{\partial z}\right) \right) u^* =
$$

$$
u^n - \Delta t (u^n \cdot \nabla) u^n + \frac{\Delta t}{\rho^{n+1}} \left(F_{i}^{n+1} - \frac{\partial p^*}{\partial x}\right) + \frac{\Delta t}{\rho^{n+1}} \left(\frac{\partial T_{11}^*}{\partial x} + \frac{\partial T_{12}^*}{\partial y} + \frac{\partial T_{13}^*}{\partial z}\right) + \frac{\Delta t}{\rho^{n+1}_s} \frac{\partial}{\partial y} \left(\mu^{n+1}_s \frac{\partial v^n}{\partial x}\right) + \frac{\Delta t}{\rho^{n+1}_s} \frac{\partial}{\partial z} \left(\mu^{n+1}_s \frac{\partial w^n}{\partial x}\right) .
$$

(2-7)

The final correction $p_{corr}^*$ to the pressure was found based on the new the intermediate velocity field:

$$
\nabla \cdot \left(\frac{\nabla p_{corr}^*}{\rho^*}\right) = \frac{\nabla \cdot u^*}{\Delta t} .
$$

(2-8)

The resulting velocity field at given time step was determined by adding the gradient of the correction pressure to the intermediate velocity:

$$
\frac{u_{n+1} - u^*}{\Delta t} = -\frac{\nabla p_{corr}^*}{\rho^*} .
$$

(2-9)

The final pressure is:

$$
p^{n+1} = p^* + p_{corr}^* .
$$

(2-10)
2.3 Volume-of-fluid method

In the multiphase algorithm [112, 138], the fluid-fluid interface is tracked by the volume-of-fluid (VOF) method, i.e., by reconstructing the interface between two immiscible liquids from a concentration function that takes the value 1 in the first phase and the value 0 in the second phase:

\[ C(t, x) = \begin{cases} 1, & \text{in the first phase} \\ 0, & \text{in the second phase} \end{cases} \quad (2-11) \]

This function of time and coordinates is advected by the velocity field using a transport equation:

\[ \frac{\partial C}{\partial t} + u \cdot \nabla C = 0. \quad (2-12) \]
Here $\mathbf{x} = (x, y, z)$ is a position vector, and $\nabla C = \left( \frac{\partial C}{\partial x}, \frac{\partial C}{\partial y}, \frac{\partial C}{\partial z} \right)$ is the gradient of $C$. The grid cells which the interface passes through have values between 0 and 1 for the concentration function. The transport equation (2-12) is solved by a Lagrangian method [145, 146]. In this method, the interface is first reconstructed by the Piecewise-Linear Interface Calculation (PLIC) method [146]. In PLIC, the outward normal of the interface is derived by $\nabla C$ for the grid having the interface across it, and the linear interface position is calculated according to the value of $C$ in this grid. Based on the reconstructed interface and the velocity field (for instance, in the $x$-direction), the new value of $C$ in next time step is calculated by (Fig. 2-2):

$$C^{n+1} = vof_1 + vof_2 + vof_3.$$  

(2-13)

Here $vof_1$ and $vof_3$ are the contribution from the left and right neighboring cells. $vof_2$ is the remained fraction from its previous value.

**Fig. 2-2** 2-D sketch of reconstructed interface (dashed lines) by the Piecewise-Linear Interface Calculation (PLIC) method and calculation of $C$ advection in $x$-direction.
One of the advantages of the VOF method is that the whole velocity field of the immiscible fluids is found by solving one set of Giesekus constitutive equations and Navier-Stokes equations with variable parameters (mass density, shear viscosity, etc.) for different fluids. The averaged values of these parameters are used over each grid cell which has multiple phases when the interface passes through it.

Because of the averaging on the cells containing the interface, the boundary conditions at the interface cannot be applied directly and, therefore, the interfacial tension force should be included in the Navier-Stokes equations as a body force that acts on interface. The methods used for calculating the body force include PLIC-based methods [147, 148], parabolic reconstruction of surface tension (PROST) [149], C-based method (only concentration function used without considering explicit interface). PROST is the most accurate method but needs massive calculation, which is time-consuming. The PLIC-based methods can give good approximation in 2-D but become unsuitable or time-consuming to implement in 3-D. The C-based methods including Continuous Surface Force (CSF) method [150] and height function (HF) method [151] are relatively simple and easy to implement in 3D, and they provide good approximation.

In the CSF method, discontinuities in the concentration function at the interface are smoothed artificially by the interpolation function, which decreases monotonically with the distance from the interface [112]. The outward unit normal \( \mathbf{n} = \mathbf{n}(t, \mathbf{x}) = \nabla c / \| \nabla c \| \) and mean curvature \( \tilde{\kappa} = \tilde{\kappa}(t, \mathbf{x}) = -\nabla \cdot \mathbf{n} \) of the interface are calculated from the smoothed concentration function (color function) \( c = c(t, \mathbf{x}) \). The body force is then approximated as
\[ F = \sigma \tilde{\kappa} \| \nabla c \| \hat{n}, \]  

(2-14)

where \( \sigma \) is the surface tension coefficient. The body force acts on the phases within a transition region around the interface.

In the case of the HF method, it is assumed that the absolute value of the \( z \)-component of the interface normal vector is largest in interfacial grid cells \((0 < C < 1)\). Then, the local distribution of a height function \( H \) is calculated as

\[ H_{r,s} = \sum_{t=t_{\text{down}}}^{t_{\text{up}}} C_{i+r,j+s,k+t} \Delta z_{k+t}, \]  

(2-15)

where \( r=-1, 0, 1 \) and \( s=-1, 0, 1 \), \( t_{\text{down}} \) and \( t_{\text{up}} \) are adaptively adjusted from 0 to 3, \( \Delta z \) is the grid height. The curvature of the interface at this grid is determined as

\[ \tilde{\kappa} = \frac{H_{xx} + H_{yy} + H_{xx} H_y^2 + H_{yy} H_x^2 - 2 H_{xy} H_x H_y}{(1 + H_x^2 + H_y^2)^{3/2}}. \]  

(2-16)

These two methods were compared on the curvature calculation and spurious currents induced near the interface. In the comparative analysis, a spherical Newtonian drop with diameter \( D = 10 \mu m \) and viscosity \( \mu_d = \mu_{\text{ext}} = 1 \text{ cP} \) was located at the center of a rectangular computation domain \((20 \mu m, 20 \mu m, 20 \mu m)\). The surface tension coefficient \( \sigma = 1 \text{ mN/m} \). As the mesh becomes finer, the HF method produces very accurate value (error < 1\%) for the curvature, while the CSF gives a small error (~5\%) for the average value but have very large deviation for some grids (Fig. 2-3a). However, the maximum velocity of the spurious currents induced by the CSF is about 1/3 of that by the HF (Fig. 2-3b).
Fig. 2-3 Comparison of the numerical solution produced by the Continuous Surface Force (CSF) and height function (HF) methods. (a) The maximum, minimum and average value of the relative error of curvature calculation with different mesh resolutions for CSF (black error bar) and HF (red error bar). $D$ is the drop diameter, $\Delta = \Delta x = \Delta y = \Delta z$ is the grid size). (b) The maximum value of spurious current velocity in the computation domain. $D/\Delta = 40$. 
Since the VOF method was developed specifically for multiphase fluid flow problems [152, 153], it is more advantageous in modeling drop and leukocyte dynamics than other numerical approaches. The VOF approach makes the inclusion of a new fluid phase straightforward and releases the necessity for the generation of a separate mesh or marker points to track a fluid-fluid interface as in front-tracking methods [154, 155]. There are other VOF-like methods that use other simple functions to track the interface, e.g., phase-field and level set methods [156, 157]. As compared to the classical VOF method, they do not conserve mass [157-159] and this drawback makes these methods inaccurate in modeling cell mechanics or the dynamics of nearly incompressible fluid drops.

### 2.4 Active migration

To simulate active migration of a cell, an additional “intracellular active force” term $F_{\text{act}}$ is introduced into the momentum equation Eq. (3). It is derived according to Dembo’s reactive interpenetrating flow formalism [125] and Herant’s polymerization force model [126]. The cell consists of two compartments: cytosol (solvent) and cytoskeleton (network), and their conservation of mass is written as

$$\frac{\partial}{\partial t} \phi_n(x,t) + \nabla \cdot (\phi_n u_n) = J(\phi_n, \ldots), \quad \frac{\partial}{\partial t} \phi_s(x,t) + \nabla \cdot (\phi_s u_s) = -J(\phi_n, \ldots). \tag{2-18}$$
\( J \) is the rate of specific volume change between two compartments and uses a logistic type of law:

\[
J = \frac{\varphi_n}{\varphi_{n0}} \left[ \frac{\varphi_{n0}(1 + m_p) - \varphi_n}{\tau_n} \right] .
\]  

(2-19)

\( \varphi_{n0} \) is the equilibrium volume fraction of the network, \( \tau_n \) the life time of network, \( m_p \) the dimensionless concentration of polymerization messengers. The convection-diffusion-reaction equation for the messengers is as following:

\[
\frac{dm_p}{dt} + \nabla \cdot (m_p \mathbf{u}) = D_p \nabla^2 m_p - \frac{m_p}{\tau_p}
\]  

(2-20)

with the von Neumann boundary condition

\[
D_p \mathbf{n} \cdot \nabla m_p = \varepsilon_p .
\]  

(2-21)

Here, \( D_p \) is the diffusion coefficient of the messengers, \( \tau_p \) the lifetime of polymerization.

It is assumed that the receptors are uniformly distributed on the membrane and the emissivity of polymerization messenger \( \varepsilon_p \) is a linear increasing function of chemoattractant concentration \( m_c \) on the membrane. The convection-diffusion equation for \( m_c \) is:

\[
\frac{dm_c}{dt} + \nabla \cdot (m_c \mathbf{u}) = D_c \nabla^2 m_c .
\]  

(2-22)

\( D_c \) is the diffusion coefficient of chemoattractant. The active force \( \mathbf{F}_{act} \) is expressed as
\[
\mathbf{F}_{act} = -\nabla \cdot \Psi. \tag{2-23}
\]

\(\Psi\) is the stress tensor due to the network-membrane interaction as below:

\[
\Psi = \psi \hat{n} : \hat{n}, \quad \psi = \psi_0 m_p \varphi_n, \tag{2-24}
\]

where \(\hat{n} : \hat{n}\) is the dyadic product of the unit outward normal vector to the membrane, and \(\psi_0\) is the equilibrium value of the network-membrane interaction stress. This equation connects the intracellular active force to external chemoattractant through the polymerization messengers.

In the VOF method, the boundary conditions for the emissivity of polymerization messengers at the interface cannot be applied directly. To do so, Eq. 2-21 is converted to reaction rate \(\varepsilon_p \| \nabla c \|\) and integrated into Eq. 2-20 as a new boundary condition. Since the cell and the external fluid are two immiscible phase and \(m_c\) and \(m_p\) (or \(\varphi_n\)) only exist outside the cell and inside the cell respectively, Eqs. 2-20 and 2-22 are calculated by two steps. First, the diffusion-reaction part is solved via a semi-implicit scheme. Then, similar to the solution of the transport equation for concentration function \(C\), the transport equations for \(m_c\) and \(m_p\) are numerically solved by the Lagrangian method based on PLIC. For example, the convection of \(m_p\) is calculated by:

\[
m_{p,i+1} = \left( m_{p,j-1} \text{vo}f_1 + m_{p,i} \text{vo}f_2 + m_{p,i+1} \text{vo}f_3 \right) / C_{i}^{n+1}, \tag{2-25}
\]

where \(m_p^*\) is calculated from the diffusion-reaction part according the value at the previous time step.
2.5 Summary of computational algorithms

Here is the summary of the computational algorithms for passive and active migration:

1. Initialization: initial values of concentration function $C$, velocity $u$, pressure $p$, extra stress tensor $T$, chemoattractant concentration $m_c$, polymerization messenger concentration $m_p$, volume fraction of actin filament network $\phi_n$

2. Solution of the diffusion-reaction equations for $m_c$, $m_p$ and $\phi_n$ with the semi-implicit scheme excluding convection terms: $m_c^n, m_p^n, \phi_n^n \rightarrow m_c^*, m_p^*, \phi_n^*$

3. Reconstruction of the interfaces by Piecewise-Linear Interface Calculation (PLIC) and advection of $m_c$, $m_p$, and $\phi_n$ based on the previous velocity field: $m_c^*, m_p^*, \phi_n^* \rightarrow m_c^{n+1}, m_p^{n+1}, \phi_n^{n+1}$

4. Active force calculation based on network volume fraction and polymerization messenger concentration: $\phi_n^{n+1}, m_p^{n+1} \rightarrow F_{act}^{n+1}$

5. Calculation of surface tension force $F_{act}^{n+1}$ by the Continuous Surface Force (CSF) or height function (HF) method

6. Calculation of the intermediate extra stress tensor from the solution of the Giesekus equation with the semi-implicit scheme based on the previous velocity field $u^n : T^n \rightarrow T^*$
7. Calculation of an intermediate velocity by the explicit solver of the pressure gradient-free Navier-Stokes equations: $$\mathbf{u}^n \rightarrow \mathbf{u}_{\text{expl}}^*$$

8. Calculation of pressure through the solution of the Poisson equation: $$\mathbf{u}_{\text{expl}}^* \rightarrow p^*$$

9. Calculation of the intermediate velocity through the solution of the Navier-Stokes equations containing the gradient of $$p^*$$ with the semi-implicit scheme: $$\mathbf{u}^n \rightarrow \mathbf{u}^*$$

10. Determination of the correction to pressure through the solution of Poisson equation: $$\mathbf{u}^* \rightarrow p_{\text{corr}}^*, \ p_{n+1}^* = p^* + p_{\text{corr}}^*$$

11. Correction of $$\mathbf{u}^*$$ by the pressure term: $$p_{\text{corr}}^* : \mathbf{u}^* \rightarrow \mathbf{u}_{n+1}^*$$

12. Calculation of the extra stress tensor from the solution of the Giesekus equation with the semi-implicit scheme based on the new velocity field: $$\mathbf{u}_{n+1}^* : \mathbf{T}^n \rightarrow \mathbf{T}_{n+1}^*$$

13. Calculation of adaptive time step length based on the new velocity field: $$\mathbf{u}_{n+1}^*$$

14. Return to Step 2 until reaching the final time step.

2.6 Parallel programming

The entire code was developed in C language with Message Passing Interface (MPI) implementation for parallel programming. Portable, Extensible Toolkit for Scientific Computation (PETSc) is used to provide parallel data management and numerous methods and options for preconditioners and solvers of linear equations, such as Conjugate Gradient, Generalized Minimal Residual, Geometric Multigrid, Algebraic
Multigrid (through High Performance Preconditioners hypre) and so on. In general, the code has a number of advantages and features:

- Three-dimensional multiphase viscoelastic or Newtonian fluid simulation, including the simulation of multicellular flow in a microchannel;
- Changeable boundary conditions: constant velocity, constant pressure or periodic conditions at boundary;
- High performance parallel computation, which makes the code extendable to large computer clusters;
- Adaptive time step length during simulation, especially for high velocity flow;
- Various channel geometries: rectangular, circular, expansion, cross channels;
- Mass transfer simulation in multiphase fluid;
- Active force generation of a cell.

The previous code was developed in Fortran with Open Multi-Processing (OpenMP). To compare computational performance and scalability, two cases (one with a Newtonian drop, the other with a viscoelastic cell) were run by the old and new code with different number of processors. The old code runs at the Ares cluster of Tulane Center for Computational Science (CCS) for shared-memory computers, and has a limited number of processors available. The new code runs at the Sphynx cluster of CCS for distributed-memory computers with a large number of processors available, and has excellent scalability even up to 128 processors as shown in Fig. 2-4. When using the same number of processors (<8), the old code is faster than the new code because the old code uses factorized scheme to solve linear equations, which takes less time for
calculation. For higher accuracy, the new code uses a non-factorized scheme. Especially for the viscoelastic case, the new code takes much more time than the old code because the extra stress tensor is solved twice in the new code. However, the computational time can be easily decreased when running the new code on a large number of processors.

Fig. 2-4 Performance comparison between old code and new code. The old code runs at the Ares cluster for shared-memory computers, and has a limited number (<8) of processors available. The new code runs at the Sphynx cluster for distributed-memory computers with a large number (up to 128) of processors available. The computational domain: height $H = 70 \, \mu m$, width $W = 70 \, \mu m$, length $L = 140 \, \mu m$. Mesh number is 130 x 66 x 66. Time step length is 0.5 $\mu s$. The number of time step is 100. Velocity at the centerline $U_c = 0.1429 \, m/s$. Boundary condition at the inlet and outlet is constant velocity calculated by analytical solution of fully developed flow. External fluid: $\mu = 1 \, cP, \rho = 10^3 \, kg/m^3$. Newtonian drop: $\mu = 1 \, cP, \sigma = 1 \, mN/m, \rho = 10^3 \, kg/m^3$. Viscoelastic cell: $\mu_s = 1 \, cP, G(0) = 50 \, Pa, \lambda = 0.2 \, s, \sigma = 0.03 \, mN/m, \rho = 10^3 \, kg/m^3$. 
Chapter 3 A numerical study of the lateral migration and deformation of drops and leukocytes in a rectangular microchannel

3.1 Abstract

When deformable particles (e.g., drops or living cells) are perfused through a flow channel, they drift into a specific lateral position that depends on their size and mechanical properties. This characteristic can be used for deformability-based particle sorting. Using a fully three-dimensional algorithm for viscoelastic drop dynamics, I studied numerically the effects of particle size, bulk shear viscosity and elasticity, interfacial (or cortical) tension, and fluid inertia on lateral migration and deformation of small liquid drops and leukocytes (white blood cells) in a rectangular microfluidic flow chamber. The numerical data show that there is an almost linear increase in the distance between the wall and the lateral equilibrium position of liquid drops or leukocytes with the particle diameter-to-channel height ratio increased from $0.1 \leq \frac{D}{H} \leq 0.5$. Excluding the case of drops with high interfacial tension, an increase in bulk viscosity of these particles led to a closer-to-wall equilibrium position. Overall, the results of this work indicate that 1) drops with different bulk viscosities can be separated in a rectangular microchannel if their interfacial tension is low or the flow rate is sufficiently high; and 2) the microfluidic technology is well suited for the separation of leukocytes with different cytoplasmic viscosities and relaxation times, but it is much less sensitive to cortical
tension. 3) This investigation opens up the possibility of using microfluidic systems for deformability-based flow cytometry.

3.2 Simulation framework

Fig. 3-1 Schematic of the computational domain showing geometrical dimensions and initial and boundary conditions used.

In this study, only one cell is simulated without considering cell-cell interaction. In the simulation, an initially spherical drop/cell is initially located in an external fluid at the middle plane ($y = W/2$) of a rectangular microchannel with parallel upper/lower and front/back no-slip plates (Fig. 3-1). The channel geometry is based on a Bioflux microfluidic system (Fluxion Biosciences, San Francisco, CA) installed in our laboratory. The initial velocity field as well as the velocity field at the inlet and outlet were calculated from the series solution for the fully developed flow in a rectangular duct [160]:

$$U_x = \frac{4k^2}{\pi^3} \frac{H^2 \Delta \rho}{\mu_{eq} L} \frac{1}{(2n+1)^3} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)^3} \left[ \frac{\cosh\left(\frac{2n+1}{k} \cdot \pi z_1\right)}{\cosh\left(\frac{2n+1}{2k} \cdot \pi\right)} \right] \cos((2n+1)\pi y_1),$$

(3-1)
Here $z_1 = \frac{z - H/2}{H}$, $y_1 = \frac{y - W/2}{W}$, and $k = \frac{W}{H}$. In all runs, $N = 100$, which is large enough to calculate accurate values. Equations (3-1) and (3-2) also describe the velocity of the undisturbed flow when no drops exist in the channel and are used to calculate the centerline velocity $U_c$.

When a drop is introduced, there is a strong flow disturbance in the velocity field near the drop (Fig. 3-2). We found that this disturbance becomes less than 1% of undisturbed flow velocity at 2.5 drop diameters away from the drop centroid. Therefore, we positioned the drop at least $2.5 D$ from the inlet/outlet to avoid the boundary effect. For smaller drop, we considered a narrower computational domain with the velocity at front/back boundaries calculated by Eq. (3-1).

![Fig. 3-2 Velocity disturbances caused by a Newtonian drop. (a) changes in the $x$-component of the velocity relative to the undisturbed velocity in the $x$-direction, $u_x/U_x - 1$; (b) changes in the $z$-component of the velocity relative to the undisturbed velocity in the $x$-direction, $u_z/U_x$. Drop diameter $D = 14 \text{ \mu m}$, centerline velocity $U_c = 0.13 \text{ m/s}$, external fluid viscosity $\mu_{\text{ext}} = 1 \text{ cP}$, drop viscosity $\mu_d = 10 \text{ cP}$, and interfacial tension $\sigma = 1 \text{ mN/m}$.](image-url)
In this numerical study, we simulated the motion of viscous or viscoelastic drops with different initial vertical displacements (z-coordinate of their centroid) from the bottom wall of the channel. The equilibrium position $z_{eq}$ was determined from the trajectories data as the threshold height at which the lateral migration velocity of all the drops reaches zero. Specifically, the drops with the centroid located below or above $z_{eq}$ migrate upward or downward to the equilibrium position, respectively (Fig. 3-3). Fluid shear stresses induce their elongation with an acute orientation angle $\theta$ between the first (long) principal axis of deformation and the $xy$-plane. We quantified the drop deformation by the Taylor deformation index:

$$D_{xz} = \frac{D_{max} - D_{min}}{D_{max} + D_{min}},$$

where $D_{max}$ and $D_{min}$ are the lengths of the drop in the $xz$-plane along the long and short principal axes of deformation.

**Fig. 3-3** Schematic of lateral migration and deformation of a deformable drop in a microchannel.
The centroid coordinates, velocity vector, deformation index, and orientation angle of the drop at the equilibrium position are functions of the channel height, drop size and rheological properties (viscosity, relaxation time), interfacial tension between the external fluid and the drop, and flow rate and viscosity of the external fluid. For example, the equilibrium position \( z_{eq} \) of a viscoelastic drop can be expressed as

\[
 z_{eq} = f(H, D, \rho_{ext}, \rho_d, \mu_{ext}, \mu_d, \sigma, U_c, \lambda).
\]  

According to dimensional analysis, Eq. (3-4) can be written in the following dimensionless form:

\[
 \frac{2z_{eq}}{H} = \prod \left( \frac{D}{H}, \gamma, Re, Ca, Wi \right).
\]  

In this expression, \( \gamma = \frac{\mu_d}{\mu_{ext}} \) is the drop-to-external fluid viscosity ratio, \( Re = \frac{\rho_{ext} U_c H}{\mu_{ext}} \) is the channel height-based Reynolds number, \( Ca = \frac{\mu_{ext} U_c}{\sigma} \) is the external fluid viscosity-based capillary number, and \( Wi = \frac{2\lambda U_c}{\gamma H} \) is the drop-based Weissenberg number. Here, we assume that the drop density is equal to the external fluid density (a neutrally buoyant case): \( \frac{\rho_d}{\rho_{ext}} = 1 \). The deformation index and the orientation angle of the drop also depend on these five dimensionless parameters: \( D/H, \gamma, Re, Ca, \) and \( Wi \). Since we define the polymer viscosity as a product of the relaxation time and the elastic modulus, the limiting case of \( \lambda = 0 \) s (\( Wi = 0 \)) corresponds to a Newtonian drop with the total viscosity \( \mu_d \) equal to its solvent viscosity \( \mu_s \). In this work, we changed the solvent viscosity only when
we simulated Newtonian drops. For the viscoelastic drop simulation, $\mu_d/\mu_s$ was always one, and thus the code was stable when $Wi$ approached zero.

### 3.3 Results and discussion

**Validation of the numerical method**

For particles with $D/H$ from 0.3 to 0.8, the size of the computational domain was (280 µm, 140 µm, 70 µm), as shown in Fig. 3-1. The grid number was 256×128×64. In the case of smaller particles, $D/H = 0.2$ and 0.1, the computational domain length and width were decreased to (140 µm, 70 µm) and (70 µm, 35 µm), respectively. We kept the same grid size for $D/H = 0.2$, i.e., we considered 128×64×64 grid cells in this case. A finer mesh (128×64×128) was used for $D/H = 0.1$. Table 3-1 shows the grid convergence test for $D/H = 0.5$. Decreasing the number of grid cells from 256×128×64 to 128×64×32 resulted in 0.4% change in the equilibrium position, 4.7% change in the deformation index, 0.7% change in the orientation angle, and 0.1% change in the particle axial velocity. More significant changes were observed for a courser mesh 64×32×16.

Table 3-1: Grid convergence test for $D/H=0.5$, $\gamma=1$, $Re=10$, $Ca=0.14$

<table>
<thead>
<tr>
<th>Grid number</th>
<th>$2z_{eq}/H$</th>
<th>$D_{xz}$</th>
<th>$\theta_{eq}$</th>
<th>$u_{ax}/U_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>256×128×64</td>
<td>0.706</td>
<td>0.061</td>
<td>41.7</td>
<td>0.841</td>
</tr>
<tr>
<td>128×64×32</td>
<td>0.703</td>
<td>0.064</td>
<td>42</td>
<td>0.840</td>
</tr>
<tr>
<td>64×32×16</td>
<td>0.696</td>
<td>0.075</td>
<td>40</td>
<td>0.842</td>
</tr>
</tbody>
</table>
Two benchmark tests of the computational algorithm were conducted. In the first test, we compared the numeral data on the deformation of a Newtonian fluid drop moving at the centerline of the microchannel with the corresponding analytical solution for the problem of drop motion and deformation in an infinitely wide and long channel between two parallel plates [71]. The drop viscosity was the same as the viscosity of the external fluid, i.e., $\mu_d = \mu_{ext} = 1$ cP, and two values of the capillary number were considered: $Ca = 0.28$ and 1.4. With a smaller value of $Ca$, the drop has small deformation and the drop shape calculated by the numerical model (dashed line in Fig. 354) matches well the analytical formula (solid line):

$$r = 1 + Ca \left( \frac{D}{H} \right)^2 \frac{10 + 11 \gamma}{8(1 + \gamma)} f(\varphi),$$

(3-6)

where $r$ is the radial coordinate, $\varphi$ is the angular coordinate measured from the $x$-axis, and $f(\varphi)$ is a shape correction function:

$$f(\varphi) = 0.2 \cos \varphi (\cos^2 \varphi - 4 \sin^2 \varphi),$$

(3-7)

Equations (3-6) and (3-7) describe the shape of the drop in the $xz$-plane (side view) with the center at the origin that moves in an unbounded quadratic flow field at a low Reynolds number. Both the numerical and analytical models predict the drop deformation to a smoothed triangular shape at a larger value of $Ca$ (Fig. 3-4b). However, the analytical shape is characterized by concave regions at the top and bottom of the drop located closer to the drop front. The numerical shape is akin to a bullet with a small concave region on the back, which is completely consistent with the previous boundary integral simulation by Griggs et al. [75] (cf. Fig. 5 in that paper). It should be noted that
Eqs. (3-6) and (3-7) were derived under the assumption of infinitesimally small perturbations from the spherical shape and thus they are invalid when the drop experiences large deformation.

**Fig. 3-4** Comparison of the numerical (dotted) and analytical shapes (solid) of a Newtonian fluid drop moving at the centerline of the channel. (a) $Ca = 0.28$ and (b) $Ca = 1.4$. For both plots, $D/H = 0.5$, $Re = 1$, and $\gamma = 1$. 
In the second test, we compare the numerical results on the drop velocity in the flow direction $u_{dx}$ with the corresponding analytical formula [71]:

$$\frac{u_{dx}}{U_c} = \frac{4z}{H} \left(1 - \frac{z}{H}\right) - \frac{\lambda}{2 + 3\lambda} \left(\frac{D}{H}\right)^2,$$

(3-8)

The dimensionless drop position $2z/H$ in the $z$-direction now varies from 0.3 to 1.0 (centerline). The results of this test are shown in Fig. 3-5 for a small drop ($D/H = 0.2$) and a large drop ($D/H = 0.5$). As seen in this figure, there is an excellent agreement between the numerical simulation and the analytical model when the drop size is small. An increase in the drop size leads to some discrepancy between the numerical and analytical models, especially for drops located closer to the channel walls, but the results agree well with those from Griggs et al. [75] (cf. Fig. 4c in that paper). This could be explained by the fact that the wall effects were not considered when deriving Eq. (3-8). This comparison also indicates that the boundaries in the $y$ direction have a negligibly small effect on the drop dynamics when $W$ is greater than $H$ (in the simulation, $W/H = 2$).
Fig. 3-5 Comparison of the numerical (dashed) and analytical data (solid) on the $x$-component of the Newtonian fluid drop velocity as a function of the $z$-coordinate of the drop centroid. Triangles are the numerical data by Griggs et al. [75]. Two sizes of the drop are considered: $D/H = 0.2$ and $0.5$. The values of other dimensionless parameters are $Re = 10$, $Ca = 0.14$, and $\gamma = 1$. 
Lateral migration of Newtonian fluid drops

The viscosity ratio $\gamma$, capillary number $Ca$, and Reynolds number $Re$ ranged in the Newtonian drop simulation from 1 to 10, 0.1 to 10, and 10 to 100, respectively. These ranges were selected on the basis of previous computational and experimental studies mentioned in the Introduction section, including the data from microfluidic experiments. One application of the results presented in this section could be the use of microfluidic technology for efficient homogenization of milk, the process where large fat globules are separated or break into small globules [161]. The milk viscosity is between 1 and 10 of the water viscosity. Another application is the preparation of kerosene drops with similar size and viscosity for accurate testing combustion systems such as diesel and jet engines [162]. The kerosene viscosity is slightly more than the water viscosity. These results provide the information about the equilibrium position of Newtonian liquid drops at different viscosities and sizes that could be used for the design of microfluidic systems, e.g., for separation of kerosene drops or fat globules in milk.

Figure 3-6 shows typical trajectories as well as lateral (perpendicular to the flow) and translational (along the flow centerline) velocities of a Newtonian liquid drop before it reaches the steady state. The trajectories of the drop migrating upwards from the position close to the wall or downwards from the position close to the centerline asymptotically approach the lateral equilibrium position $z_{eq}$ (Fig. 3-6a). As seen in Fig. 3-6b, the lateral velocity $u_{dz}$ calculated based on the displacement of the drop centroid decreases to zero when the position of the drop approaches the equilibrium position. Another result that follows from Figs. 3-6a and 3-6b is that drops located closer to the
wall migrate faster in the z-direction and therefore need less time and less travelling distance in the x-direction to reach the equilibrium position, as compared to drops closer to the centerline. This phenomenon may be explained by the larger lift force on a drop near the wall [50, 163]. As evident from Fig. 3-6c, the translational velocity $u_{dx}$ is always less than the flow velocity, but the difference between the translational and flow velocities decreases with an increase in the distance of the drop from the wall. For different initial positions of the drop, the dependence of the velocity on the drop centroid coordinates remains the same. Overall, Fig. 3-6 indicates that the length of a microchannel with the traps at the outlet to capture particles of different properties should be determined based on the lateral migration of particles initially located at the centerline. Since the lateral velocity is less than 2% of the centerline velocity $U_c$ (Fig. 3-6b), we estimate that for particle separation applications the length of the microchannel should be at least one order of magnitude more than its height.
Fig. 3-6 (a) A change in the $z$-coordinate of the drop centroid moving in a microchannel at different initial positions. (b) The $z$-component and (c) the $x$-component of the drop velocity as functions of the $z$-coordinate of the drop centroid. For all plots, $D/H = 0.5$, $Re = 10$, $Ca = 0.14$, and $\gamma = 1$. 
Figure 3-7 shows the numerical data on the equilibrium position, deformation, and the translational velocity of liquid drops with different viscosity and size. An increase in the drop diameter $D$ leads to a higher equilibrium position (Fig. 3-7a). Specifically, the equilibrium position increases from 0.44 at the drop diameter-to-channel height ratio $D/H$ of 0.2 to 1.0 (centerline) at $D/H = 0.8$. This effect of the drop size agrees well with the front tracking data by Mortazavi and Tryggvason [164] and the experimental data by Hur et al. [21]. A decrease in the equilibrium position with a decrease in the drop size, coupled with the data in Fig. 3-6, indicates that smaller droplets initially located at the centerline travel much longer distance in the flow direction before settling at their lateral equilibrium position than larger ones. Thus, trapping of the population of smaller drops with specific mechanical properties requires longer microchannels. Shear flow induces deformation of the drop from a sphere to either an ellipsoid-like shape oriented at a specific angle of $35^\circ$-$42^\circ$ to the channel wall if the drop size $D/H \leq 0.5$ or a bullet-like shape (cf. Fig. 3-4b) for drops of size $D/H = 0.8$. The steady state deformation index $D_{xz}$ changes nonlinearly with the drop size, reaching the maximum of 0.06 at $D/H$ between 0.4 and 0.5 (Fig. 3-7b). The translational velocity of very small drops ($D/H \leq 0.2$) at the equilibrium position is almost the same as the x-velocity of the external fluid, but the difference between the velocities increases with an increase in the drop size (Fig. 3-7c). This result is in line with Eq. (3-8). Thus, the flow velocity measured (or estimated from the Poiseuille formula) at the lateral equilibrium position predicts well the steady-state translational velocity of small drops, but not that of large ones.

The drop viscosity has a small effect on drop dynamics provided the capillary number is small (0.14). The equilibrium position slightly increases with an increase in the
drop-to-external fluid viscosity ratio $\gamma$ from 1 to 10 (Fig. 3-7a). This effect could be
explained by the increased lubrication force (and thus the increased hydrodynamic lift) on
high-viscosity drops [164]. It should be noted that the equilibrium position reaches the
peak value at $\gamma = 10$, according to the experimental data for oil drops in water [21]. We,
therefore, do not expect a further increase in the equilibrium position with $\gamma > 10$. Our
numerical data also predict a smaller deformation index at $\gamma = 10$ than at $\gamma = 1$ or 5 (Fig.
3-7b) and a larger deviation of the translational velocity of the drop from the flow
velocity with higher viscosity values (Fig. 3-7c).
Fig. 3-7 (a) Equilibrium position, (b) deformation index, and (c) \(x\)-component velocity of a Newtonian drop perfused through a water-filled microchannel as functions of particle diameter for different values of the drop-to-external fluid viscosity ratio. Here, \(Re = 10\) and \(Ca = 0.14\). The curve fitting is based on the quadratic form.
The interfacial tension can decrease and hence the capillary number $Ca$ can increase in the presence of surfactant molecules. The effects of $Ca$ on the drop lateral migration and deformation were studied in the case of $D/H = 0.2$ (Fig. 3-8). As seen in this figure, an increase in the capillary number dramatically amplifies the effects of drop viscosity on the drop equilibrium position, deformation index, and orientation angle. This could be explained by the fact that the viscous force at higher values of $Ca$ is more dominant in drop dynamics than the interfacial tension force. For low-viscosity ($\gamma = 1$) drops, the equilibrium position increases from 0.44 to 0.72 when $Ca$ increases from 0.14 to 1.4 (Fig. 3-8a). It should be noted that an increase in the capillary number beyond 10 leads to significant elongation and then breakup of the drops (illustrated in Fig. 3-8a in the case of $\gamma = 1$). For higher-viscosity drops, the non-dimensional equilibrium position $2z_{eq}/H$ reaches the maximum of 0.59 at $Ca \approx 5$ (for $\gamma = 5$) and 0.53 at $Ca \approx 0.6$ (for $\gamma = 10$). For the highest value of $Ca$ considered in this study (14), the equilibrium position changes from 0.44 to 0.55 between $\gamma = 5$ and 10. This is a 20% change, while Fig. 3-7a shows only a 0.6% for $Ca = 0.14$ and drops of the same size. Similarly, if $Ca = 14$, the deformation index doubles (from 0.19 to 0.385) with a decrease in the drop-to-external fluid viscosity ratio from 10 to 5 (Fig. 3-8b). However, if it is equal to 0.14, drops of the same size show no change in the deformation index (0.043) between $\gamma = 5$ and 10.

The deformation index increases monotonically with the capillary number for all viscosity values studied. Higher-viscosity drops ($\gamma = 5$ and 10) show a plateau of the deformation index at large values of $Ca$ (Fig. 3-8b). The orientation angle decreases by $30^\circ$ with $Ca$ increased from 0.14 to 14 such that higher-viscosity drops become almost
parallel to the wall at $Ca = 14$ (Fig. 3-8c). For $Ca = 1$, the orientation angle decreases by about 2/3 when the drop viscosity changes from 1 cP to 10 cP (Fig. 3-8c), which is completely consistent with the numerical data by Ghigliotti et al. [165]. The comparison of Figs. 3-8a and 3-8c indicates that a decrease in the orientation angle below 10º will result in the reduction of the lift force on the drop, thereby leading to a lower equilibrium position at higher capillary numbers. The decrease of lift force due to a smaller orientation angle was also discussed for vesicles in both computational and experimental works [54, 166, 167].

There are two possible outcomes of the observed effect of the capillary number. First, it would be much easier to 1) separate drops based on their bulk viscosity and 2) measure their viscosity from the images of their shape in a microchannel if their interfacial tension is low. Second, the separation of high-viscosity drops based on interfacial tension is not feasible using microfluidic flow systems because their equilibrium position changes nonmonotonically with interfacial tension. However, it is possible to classify drops with different values of interfacial tension from the measurements of their deformation index and orientation angle.
Fig. 3-8 (a) Equilibrium position, (b) deformation index, and (c) orientation angle of a Newtonian drop perfused through a water-filled microchannel as functions of the capillary number for different drop-to-external fluid viscosity ratios ($D/H = 0.2, Re = 10$).
In microfluidic applications, different flow rates can be used for separation of liquid drops. Higher flow rates are often preferred for fast and efficient separation. Figure 10 illustrates the effect of the flow rate (or the centerline velocity) on the lateral equilibrium position of Newtonian fluid drops. If the flow rate increases from 0.686 µL/s ($Re = 10$) to 3.43 µL/s ($Re = 50$), and then to 6.86 µL/s ($Re = 100$), the equilibrium position of low viscosity (1-5 cP) drops becomes higher (Fig. 3-9a) and they experience larger deformation (Fig. 3-9b). However, the flow rate effects on the drop position and deformation index are reduced at a further increase of the viscosity to 10 cP (Fig. 3-9a, b). On the contrary, a change in the orientation angle with the flow rate becomes more pronounced at higher viscosities (Fig. 3-9c). When the flow rate is low, the equilibrium position increases with the drop viscosity and the deformation index remains essentially the same (cf. solid line in Fig. 3-9a, b). These are consistent with the data from microfluidic experiments [21]. An increase in the flow rate above the values used in these experiments reverses the trend of the viscosity effect on the equilibrium position and increases the viscosity effect on the deformation index (dotted line in Fig. 3-9a, b). These results indicate that the separation of low viscosity drops from high viscosity ones and the drop viscosity measurement will be more effective at high flow rates.
Fig. 3-9 (a) Equilibrium position, (b) deformation index, and (c) orientation angle of a Newtonian drop perfused through a water-filled microchannel as functions of the drop-to-external fluid viscosity ratio for different flow rates. $D/H = 0.2$. Flow rates were determined by integration of Eq. (3-1) over the channel cross section at a given pressure gradient. Re and Ca were calculated from the centerline velocity value in Eq. (3-1).
Both the Reynolds and capillary numbers change linearly with the flow rate and it is unclear which of them contribute most significantly to the flow rate effects. To answer this question, we have studied lateral migration and deformation of drops when both the flow rate and the interfacial tension coefficient increased simultaneously to keep the capillary number constant. This gives us the effect of the Reynolds number (cf. Fig. 3-10). As seen in this figure, an increase in $Re$ leads to a decrease in the equilibrium position of drops, but this effect becomes significant only if their viscosity is low. We also found that the deformation index (~0.045) was nearly independent on $Re$ for all the values of drop viscosity considered (not shown). Additionally, a small increase in the orientation angle (2–6º) was observed with $Re$ changed from 10 to 100. From comparison of these data with Fig. 3-9 it follows that the Reynolds number is not a factor in the flow rate effects, but rather these effects are resulted from an increase in the capillary number. Two conclusions we can make from this analysis. First, the effects of the flow rate on drop migration and deformation are not the same as the Reynolds number effects because the drop dynamics also depends on the capillary number, which changes with the flow rate. Second, the interfacial tension coefficient is one of the mechanical properties that we are interested to measure, while the flow rate is an input parameter that can be well controlled in experiments. Thus, the numerical or analytical studies based on a change in the Reynolds number only are insufficient in understanding the separation of drops in a microchannel.
Fig. 3-10 The effect of the Reynolds number on the equilibrium position of Newtonian drops ($D/H = 0.2$, $Ca = 0.14$).
Lateral migration of leukocytes

The leukocyte was modeled as a viscoelastic drop with interfacial tension (known as cortical tension) due to the elasticity of the actin cytoskeleton in the cell cortex [3]. Viscoelastic fluid models with cortical tension were widely used for the determination of rheological properties of human neutrophils and other leukocytes from the micropipette aspiration data and for modeling leukocyte biomechanics [111, 112, 136, 168, 169]. The basic values of the model parameters were taken from experimental measurements. Specifically, the viscosity and relaxation time of the leukocyte cytoplasm were respectively 100 P and 0.2 s, in line with step aspiration studies [30]. The cortical tension coefficient is 30 pN/µm which is in the range of values measured by Tsai et al. [109] and other researchers [170, 171]. The basic value of the leukocyte diameter was selected to be 14 µm. This describes well the size of circulating monocytes [172] and the cells from monocytic cell lines such as Mono Mac 6 [173]. The range of values considered was also within the limits of the experimental data. For example, the cytoplasmic viscosity changed in our study from 100 P to 1000 P, while the experimental range is between 65 and 2100 P [3, 30, 31, 106, 109, 111, 174].

The lateral equilibrium position and deformation index of the leukocyte are shown in Fig. 3-11 as a function of the cell diameter-to-channel height ratio $D/H$ for different viscosity values. As in the case of a Newtonian fluid drop, the equilibrium position of the leukocyte increases almost linearly with $D/H$. An increase in the cytoplasmic viscosity $\mu_d$ leads to a shift of the equilibrium position toward the lower wall of the microchannel and a decrease in the deformation index. This corresponds to the
behavior observed for the drop in the high capillary number case (compare Figs. 3-8 and 3-11). If the channel height is 70 µm and the cell diameter is half the height, a 10-fold increase in the cytoplasmic viscosity (from 100 to 1000 P, i.e., when $\gamma = 10^4$ to $10^5$) results in a decrease in the equilibrium position from 32.20 µm to 24.85 µm (7.35 µm difference). Although this difference $\Delta z_{eq}$ becomes smaller with a decrease in the cell diameter, its ratio to the cell diameter ($\Delta z_{eq} / D$) becomes larger. For example, $\Delta z_{eq} / D = 0.21$ and 0.33 for $D = 35$ µm and 14 µm, respectively. This ratio is an important factor in the problem of separation of living cells with different mechanical properties. If we consider $\Delta z_{eq}$ as the separation distance between cells, $\Delta z_{eq} / D$ will determine the strength of their hydrodynamic interaction [175, 176]. The cell-cell separation is more difficult to achieve with more closely spaced cells because of the increased interaction force between cells. Thus, our analysis indicates that the efficiency of a microfluidic flow chamber for the separation of particles with different mechanical properties increases with a decrease in the particle size.

As about the deformation of leukocytes, we did not see any significant changes in the orientation angle (43º) with the cell viscosity or diameter. The cell deformation index decreases with the cell diameter-to-channel height ratio when the cytoplasmic viscosity is at its basic value (solid line in Fig. 3-11b), but no significant effect of the cell size was observed at higher values of the viscosity (dashed and dotted lines). This behavior is different from that of a Newtonian fluid drop. There are two reasons for this difference. First, the equilibrium position of the leukocyte is much closer to the centerline than that of the drop. This is especially true for large cells ($D/H = 0.5$; Fig. 3-11a), which
experience less deformation than small cells located close to the channel wall because of reduced fluid shear stresses. Second, the cortical tension coefficient is very small for leukocytes and the leukocyte viscosity is several orders of magnitude higher than the water viscosity. Thus, the capillary number for leukocytes is much higher than that for drops we considered in the previous section. We therefore hypothesize that the bulk mechanical properties such as viscosity and relaxation time but not the surface mechanical properties such as cortical tension play an important role in the passive migration and deformation of leukocytes. Indeed, our numerical simulation in the case of $\gamma = 10^4$, $D/H = 0.2$, $Re = 10$, and $Wi = 0.082$ shows very small changes in the leukocyte equilibrium position (from 0.62 to 0.65) and deformation index (from 0.04 to 0.07) when the capillary number changes from 0.48 to 47.6. This effect is much less than the effects of the cytoplasmic viscosity and relaxation time (see below). If we consider the leukocyte cytoskeleton as a series of parallel cross-linked springs, the number of which increases with the cell diameter, then the elastic force produced by this network will be higher in a larger cell. Thus, a larger cell will have more resistance to shear-induced deformation if our hypothesis is true.
Fig. 3-11 (a) Equilibrium position and (b) deformation index of leukocytes perfused through a water-filled microchannel as functions of the cell diameter for different values of the cytoplasmic viscosity. Here, $Re = 10$ and $Ca = 4.76$. 
Figure 3-12 illustrates the effect of the cytoplasmic relaxation time \( \lambda \) on the equilibrium position and deformation index of leukocytes. Here, four values of the relaxation time were considered: 0.05 s, 0.1 s, 0.2 s, and 0.5, which correspond to the Weissenberg number \( Wi = 0.020, 0.041, 0.082, \) and 0.204, respectively. An increase in the relaxation time leads to a closer-to-centerline equilibrium position (Fig. 3-12a) and larger deformation (Fig. 3-12b). For the largest value of the relaxation time considered (0.5 s), the cells with size \( D/H \geq 0.4 \) reach the centerline at steady state, with deformation to a bullet-like shape. We should point out that the relaxation time is not an equivalent to the elastic modulus in viscoelastic solid models. We can approximate this modulus as \( \mu_d / \lambda \) at high values of the cytoplasmic viscosity and the relaxation time [177]. From this formula it follows that the cell elasticity in sense of elastic solid deformation and thus the elastic restoring force decreases with an increase in the relaxation time. This explains why leukocytes are more deformed at higher values of the Weissenberg number (Fig. 3-12b).
Fig. 3-12 (a) Equilibrium position and (b) deformation index of leukocytes perfused through a water-filled microchannel as functions of the cell diameter for different values of the relaxation time. Here, $Re = 10$, $Ca = 4.76$, $\gamma = 10^4$. 
Figure 3-13 shows the effect of the flow rate on leukocyte migration and deformation in a microchannel and also illustrates which of the dimensionless groups is dominant in these processes. The lateral equilibrium position increases significantly (from 0.58 to 0.71) with the flow rate changed from 0.686 µL/s \((Re = 10)\) to 6.86 µL/s \((Re = 100)\), with a plateau at higher values of the flow rate (Fig. 3-13a). The deformation index is a linearly increasing function of the flow rate (Fig. 3-13b) and the cells become more oriented in the flow direction at higher flow rates (Fig. 3-13c). Except the orientation angle, the observed dependence of these parameters on the flow rate follows the Weissenberg number effects in Fig. 3-12.

Coupled to the results in Fig. 3-11, these data indicate that the migration of living cells such as leukocytes significantly depends on their bulk viscoelastic properties. Additionally, the equilibrium position of leukocytes is a monotonic function of both the viscosity and relaxation time of the cell cytoplasm (cf. Figs. 3-11a and 3-12a). Thus, it is feasible to use a microfluidic flow system to separate cells with different mechanical properties or to measure these properties including the viscosity and relaxation time from the information about the equilibrium position and deformation index of the cells.
Fig. 3-13 (a) Equilibrium position, (b) deformation index, and (c) orientation angle of leukocytes perfused through a water-filled microchannel for different centerline velocities Other parameters: $D/H = 0.2$. 
To compare with experimental data, simulation was done according to the experimental setup of Hur et al. [21]. The microchannel was rectangular with height $H = 38 \, \mu m$ and width $W = 85 \, \mu m$. The flow is fully established with the centerline velocity $U_c = 0.4 \, m/s$. Figure 3-14 shows both the numerical data and experimental results on the lateral migration of living cells with different viscoelasticity and size (breast cancer cell lines MCF7 and modMCF7 and white blood cells from human blood). MCF7 are benign breast cancer cells, which were found to be less deformable than chemically modified modMCF7 that have an increased metastatic potential [25, 28].

According to the numerical simulation, if the relaxation time is fixed, the cell equilibrium position moves closer to the wall with increasing polymer viscosity (dashed lines in Fig. 3-14). When the polymer viscosity changes from 100 P to 10000 P, the equilibrium position of the cell with $D/H = 0.2$ and 0.5 changes from 0.72 to 0.48 and from 1.0 to 0.67, respectively. As in the case of drops, larger cells move closer to the centerline. These trends are consistent with the experimental data (inverted triangles, solid circles, and solid squares in Fig. 3-14). Less deformable MCF7 (solid circles) have an equilibrium position closer to the wall than modMCF7 (solid squares). From the comparison of the simulation and experiment, the polymer viscosity of the cells studied falls within the range chosen in the simulation (100-10000 P). The cells with polymer viscosity of 10000 P (asterisks) behave similarly to rigid particles (diamonds). On the contrary, the cells with polymer viscosity of 100 P (crosses) have an equilibrium position reaching the centerline at $D/H > 0.4$. From comparison of the numerical and experimental data in Fig. 3-14, it follows that the polymer viscosity of white blood cells and MCF7 cells is slightly higher than 1000 P, while it is between 100 P and 1000 P for modMCF7.
cells. The majority of micropipette aspiration measurements also predict the values close to 1000 P for the cytoplasmic viscosity of white blood cells [106, 109, 174]. It should be noted that the cells exposed to conditions of in vitro experiments can be easily activated and thus become less deformable. Therefore, leukocytes may experience much higher deformation in vivo than in vitro [3].

Interestingly, the cells with the polymer viscosity less than 1000 P have an equilibrium position closer to the centerline than Newtonian liquid drops with much lower viscosity (0.83~9.3 cP). This seemingly conflicting observation can be explained by the dominance of solvent viscosity when cells undergo small deformation during passive migration. This is especially true when the elastic modulus $G(0)$ is sufficiently small. In this situation, cortical tension still contributes to the lateral migration and since it is much less for cells than the interfacial tension for drops, the cells go to a higher equilibrium position than drops with similar internal viscosity.
Fig. 3-14 Comparison of equilibrium position of cells and Newtonian drops with different sizes between our simulation (dashed lines) and the experimental results of Hur et al. [21]. $\sigma = 0.5 \text{ mN/m}$. 

Fig. 3-14 Comparison of equilibrium position of cells and Newtonian drops with different sizes between our simulation (dashed lines) and the experimental results of Hur et al. [21]. $\sigma = 0.5 \text{ mN/m}$. 

- Malignant cancer cells
- Oil droplets 9.3 cP
- Benign cancer cells
- Oil droplets 0.83 cP
- Rigid particles
3.4 Conclusions

The comprehensive computational study has been carried out to investigate lateral migration and deformation of fluid drops and leukocytes in a rectangular microchannel. Our simulation shows that the external force field-free microfluidic technology can be used for separation of drops with different bulk viscosities if their interfacial tension is low or the flow rate is sufficiently high. Living cells with different bulk mechanical properties such as cytoplasmic viscosity and relaxation time can be separated in a microchannel. It is possible to classify cells with different mechanical properties by imaging their deformed shape during perfusion through a microchannel. These open up the possibility of using microfluidics for deformability-based flow cytometry. The microfluidic technology, however, could be much less effective in the separation of high-viscosity drops based on interfacial tension. Additionally, our study shows that if the majority of particles are close to the centerline when entering a microchannel (e.g., when we perfuse blood through a microchannel and need to separate red blood cells from other blood cells), they reach their lateral equilibrium position at larger distances from the inlet than particles initially located close to the wall. Thus, the length of microchannels for deformability-based cell sorting should be determined from the traveling distance of particles migrating from the centerline. Our data also indicate an inverse relationship between the particle size and the traveling distance. This means that the deformability-based separation of smaller particles requires longer microchannels. Sorting of cells with different deformability could be more effective with a low cell size-to-channel height ratio.
Chapter 4 Numerical simulation of deformable cell interaction during migration in a microchannel

4.1 Abstract

When the blood flows in vessels and channels, circulating cells deform and move relatively to the blood flow in the lateral and translational directions. This migratory property plays a key role in immune response, hemostasis, cancer progression, delivery of nutrients, and microfluidic technologies such as cell separation/enrichment and flow cytometry. Using our three-dimensional computational algorithm for multiphase viscoelastic flow, we have investigated the effect of pairwise interaction on the lateral and translational migration of deformable cells in a microchannel. The numerical simulation data show that when two cells with same size and close distance interact, they repel each other until they reach a same lateral equilibrium position where their equilibrium separation distance depends on their location relative to the centerline. When a series of closely spaced cells with same size are considered, they generally undergo damped oscillation in both lateral and translational directions until they reach equilibrium positions where they become evenly distributed in the flow direction (self-assembly phenomenon). For a series of cells with different sizes, bigger cells could collide repeatedly with smaller ones and enter the other side of the channel (above or below the centerline). For a series of cells with different deformability, more deformable cells upon impact with less deformable cells move to an equilibrium position closer to the centerline.
The results of our study offer insight on cell margination in bloodstream and cell separation/enrichment in microfluidic devices.

### 4.2 Simulation framework

In the simulation, two initially spherical cells with diameters $D_1$ and $D_2$ suspended at the middle plane ($y=W/2$) of a rectangular microchannel with height $H = 70 \, \mu m$ and width $W = 140 \, \mu m$ (Fig. 4-1). Initially, the flow is fully established with the centerline velocity $U_c = 0.14 \, m/s$. All the walls of the rectangular duct are considered to be no slip boundaries and periodic boundary conditions are applied at the inlet and outlet. In most cases, we consider cells with same diameter $D_1 = D_2 = D = 14 \, \mu m$, elastic modulus $G(0) = 50 \, Pa$, relaxation time $\lambda = 0.2 \, s$, and cortical tension coefficient $\sigma = 0.03 \, mN/m$. We have also included the data for different size cells ($D_1 = 28 \, \mu m$, $D_2 = 14 \, \mu m$) and for the cells with different deformability ($G_1(0) = 25 \, Pa$, $G_2(0) = 250 \, Pa$).

Fig. 4-1 Schematic of the simulated problem. Two interacting cells in a rectangular microchannel. The cells may have different size and deformability and may have different spacing in the x-direction (translational direction) and/or the z-direction (lateral direction). They are subject to shear flow created by a pressure difference between inlet and outlet. Periodic boundary conditions have been applied at the inlet and outlet. No-slip boundary conditions were applied to the channel walls in the y- and z-directions. Channel height $H$ and width $W$ are 70 $\mu m$ and 140 $\mu m$, respectively. Channel length $L$ is variable.
4.3 Results and discussion

The simplest case in our simulation of cell-cell interaction is one cell in a microchannel with periodic boundaries. Physically, this case represents a long series of the cells equally spaced in the translational direction while having the same lateral position. By changing the channel length in the simulation, the distance between the cells can be changed. As seen in Figure 4-2, the later equilibrium position becomes closer to the channel wall as the cell-cell separation distance shortens, in line with the computational work on Newtonian drops by Mortazavi et al. [164]. When the distance between cell centers (here, it is denoted as the $x$-distance) is below 70 µm, its effect on the lateral equilibrium position remains strong. The slope of the lateral equilibrium position vs. the $x$-distance becomes low when the $x$-distance increases beyond 70 µm, indicating that 70 µm can serve as a threshold cell-to-cell distance for a transition from scenario AB to scenario A provided the cell diameter is between 14 and 21 µm. The difference in the lateral equilibrium position between scenario A [178] and the current simulation of scenario AB at the $x$-distance of 140 µm is only 0.04 µm for $D = 14$ µm (0.2 %) and 0.2 µm for $D = 21$ µm (0.8%). Figure 4-2 also shows a decrease in the slope for smaller cells ($D = 14$ µm) when the $x$-distance decreases below 21 µm. This effect is most likely caused by the wall repulsion that becomes higher as the cells get closer to the wall and the close proximity of the cells.
Fig. 4-2 Lateral equilibrium position of cells as a function of x-distance (separation) for different cell diameters (dashed line: $D = 14 \, \mu m$, solid line: $D = 21 \, \mu m$) for a series of identical and periodically spaced cells. Dash-dot lines show the equilibrium position values for single cell migration without interaction (Scenario A, as mentioned in the Introduction).
Next, two equal diameter \((D = 14 \, \mu\text{m})\) cells are put in one computational domain. The cells had the same initial lateral position \(z_0\) and different translational position \(x_0\). In Figure 4-3a, the channel length was 140 \(\mu\text{m}\), i.e., due to periodic boundary conditions, the average \(x\)-distance was \(140/2 = 70 \, \mu\text{m}\). The initial lateral position for both cells was 21.2 \(\mu\text{m}\), which is expected to be the equilibrium position according to the data in Fig. 4-2. The initial \(x\)-distance between the cells was 42 \(\mu\text{m}\). As shown in Fig. 4-3a, the lateral position of both cells experienced damped oscillation around a specific lateral position during migration in the channel. The oscillations of the two cells were in antiphase (i.e., the phase shift is 180°) and had a period of about 25 ms and the maximum amplitude of about 1.9 \(\mu\text{m}\) (Fig. 4-3a). For another case (the channel length was 84 \(\mu\text{m}\) and the average \(x\)-distance of cells was 42 \(\mu\text{m}\)), the period of the oscillations was 15 ms (Fig. 4-3b) and the maximum amplitude was 2.5 \(\mu\text{m}\). In each case, the distance (separation) between the cells in the \(x\)-direction also oscillated around the average \(x\)-distance with time (Fig. 4-3c). This oscillatory dynamics of cells occurs while the cells are dragged by bulk flow toward the outlet, i.e., two interacting cells behave as two beads attached to a string with a dashpot that regularly contracts and relaxes during its movement in shear flow. The oscillation of the cells in the translational direction was out of phase (at a phase shift of 90°) with the oscillation in the lateral direction, but the periods of these oscillations were same. The maximum amplitude was 47 \(\mu\text{m}\) for the average \(x\)-distance of 70 \(\mu\text{m}\) (solid line in Fig 4-3c) and 34 \(\mu\text{m}\) for 42 \(\mu\text{m}\) (dashed line), which was more significant than the amplitude of the lateral oscillation. After the decay of the oscillations, the cells reached the same lateral equilibrium position and same \(x\)-distance between them, as discussed in Figure 4-2 and agreed with the experimental results of Lee et al. [88] on
particle self-assembly in a microchannel. These oscillations in Fig. 3-3 were caused by the repulsive and attractive interaction with the cells of the neighboring computation domains since the periodic condition was used in the inlet and outlet of the channel.
Fig. 4-3 (a) Time evolution of the lateral positions of identical cells with the average $x$-distance of 70 $\mu$m. The initial position for Cell 1 and Cell 2 is $x_0 = 49$ $\mu$m and $z_0 = 21.2$ $\mu$m and $x_0 = 91$ $\mu$m and $z_0 = 21.2$ $\mu$m, respectively. (b) Time evolution of the lateral positions of cells with the average $x$-distance of 42 $\mu$m. The initial position for Cell 1 and Cell 2 are $x_0 = 31.5$ $\mu$m and $z_0 = 21.2$ $\mu$m and $x_0 = 52.5$ $\mu$m and $z_0 = 21.2$ $\mu$m, respectively. (c) The $x$-distance between the cells as a function of time for cases (a) (solid line) and (b) (dashed line). In both cases, the cell diameter is 14 $\mu$m.
To reduce the effect of periodic boundaries on the interaction of two migrating cells, we increased the channel length to 280 \( \mu \)m. In this simulation, the initial \( x \)-distance between the cells was fixed at 140 \( \mu \)m to ensure that the cell-cell interaction was initially negligible (cf. Fig. 4-2). By changing the initial \( z \)-distance (initial distance between the cell centroids in the lateral direction), we then investigated the migration of both cells in a microchannel. When the initial \( z \)-distance was 7 \( \mu \)m (Fig. 4-4a), the cells first moved slowly towards the lateral equilibrium position as expected according to our previous study on single cell migration [178]. Cell 1 was closer to the centerline and moved faster in the \( x \) direction than Cell 2. This led to a decrease in the \( x \)-distance (Fig. 4-4c) with a concurrent decrease in the \( z \)-distance (Fig. 4-4a). Just before \( t = 10 \) ms, the rate by which the cells approached each other in the lateral direction increased. Then, at \( t = 10 \) to 11 ms, the \( x \)-distance became almost zero and the strong interaction between the cells led to strong cell deformation and to Cell 1 jumping upward and Cell 2 jumping downward (Fig. 4-4a). After \( t = 11 \) ms, Cell 1 passed Cell 2 and the cell-cell interaction weakened. The cells recovered their pre-collision shapes and moved back rapidly to the lateral position slightly higher (in the case of Cell 1) or lower (Cell 2) than they had before \( t = 10 \) ms. For the initial \( z \)-distance of 7 \( \mu \)m, Cell 1 remained to be closer to the centerline and faster in the translational direction than Cell 2, and thus the absolute value of the \( x \)-distance continued to increase with time and the interaction gradually vanished (solid line in Fig. 4-4c). However, when the initial \( z \)-distance became 4 \( \mu \)m, a different migratory behavior was observed. First, these cells never collided. Their \( x \)-distance was no less than 45 \( \mu \)m. When they reach this minimum distance (at \( t = 13 \) ms), they had the same lateral position (Fig. 4-4b). After \( t = 13 \) ms, Cell 1 continued to move downward, while Cell 2 moved
upward (Fig. 4-4b). As a result, Cell 1 went below Cell 2 and Cell 2 started moving faster than Cell 1, as evident by positive and increasing values of the x-distance after $t = 13 \text{ ms}$ (dashed line in Fig. 4-4c). At $t = 40 \text{ ms}$, the x-distance was 163 µm, which was 11.6 times higher than the cell diameter. The cells eventually reached their lateral equilibrium position, which was higher than the initial z-position for Cell 2 and much lower than the initial z-position for Cell 1. This case illustrates that although the cells at the end are far from each other and their position equilibrates, they can be “swapped” during transient interactions occurring before reaching this equilibrium position. The disturbance flow reflected from the nearby channel wall by the lagging cell pushed the leading cell across the streamlines of the external flow towards the centerline direction and caused the “swapping” [88]. In Fig. 4-4, the channel length in the computation domain is large enough and there are no repulsive and attractive interactions with the cells of the neighboring computation domains, so there was no oscillation during migration.
Fig. 4-4. The effect of the initial $z$-distance on the migration of two identical cells. (a) Time evolution of the cell lateral positions when the initial $z$-distance is 7 $\mu$m ($z_0$ for Cell 1 and 2 is equal to 29 $\mu$m and 22 $\mu$m, respectively). (b) Time evolution of the cell lateral positions when the initial $z$-distance is 4 $\mu$m ($z_0$ for Cell 1 and 2 is equal to 26 $\mu$m and 22 $\mu$m, respectively). (c) Time evolution of the $x$-distance between the cells in cases (a) (solid line) and (b) (dashed line). In both cases, the cell diameter is 14 $\mu$m; the channel length, width, and height are 280 $\mu$m, 140 $\mu$m, and 70 $\mu$m; and the initial translational position $x_0$ for Cells 1 and 2 is 70 and 210 $\mu$m, respectively.
Figure 4-5 shows the results of the simulation of two cells with low deformability. This case was selected to test the model against the experimental data on the interaction of two solid particles by Lee et al. [88]. To match the experimental conditions, the flow velocity increased to $U_c = 0.53$ m/s and the ratio of cell diameter to channel height increased to 0.4. The initial $x$-distance of two equal size (here, $D = 10 \, \mu m$) cells was fixed at 100 $\mu m$ to have negligible interaction between cells initially, as in the previous case. When the initial $z$-distance was 6 $\mu m$, Cell 1 moved downward slowly with time and Cell 2 moved upward much faster than Cell 1 because of the wall repulsion (Fig. 4-5a). As in Fig. 4-4b, cell “swapping” was observed. However, the cells quickly reached the same lateral equilibrium position and their final $x$-distance was about 54 $\mu m$, i.e., less than 6 times of the cell diameter (solid line in Fig. 4-5c). With a decrease in the initial $z$-distance to 2 $\mu m$, “swapping” disappeared (Fig. 4-5b). Cell 1 moved initially upward and then slightly downward. Cell 2 also moved upward. Both cells eventually reached the same lateral equilibrium position at $t \approx 3$ ms and the $x$-distance monotonically decreased to about 70 $\mu m$ at this time instant. These simulation results are consistent with the experimental results [88]. The difference in the migratory behavior between Figs. 4-4 and 4-5 is due to a higher cell diameter-to-channel height ratio in the data shown in Fig. 4-5.
Fig. 4-5 The effect of the initial z-distance on the migration of two identical cells with higher cell diameter-to-channel height ratio than that in Fig. 4-4. a) Time evolution of the cell lateral positions when the initial z-distance is 6 \( \mu m \) (\( z_0 \) for Cell 1 and 2 is equal to 12 \( \mu m \) and 6 \( \mu m \), respectively). (b) Time evolution of the cell lateral positions when the initial z-distance is 2 \( \mu m \) (\( z_0 \) for Cell 1 and 2 is equal to 10 \( \mu m \) and 8 \( \mu m \), respectively). (c) Time evolution of the x-distance between the cells in cases (a) (solid line) and (b) (dashed line). In both cases, the cell diameter is 10 \( \mu m \), the channel length, width, and height are 300 \( \mu m \), 90 \( \mu m \), and 25 \( \mu m \); and the initial translational position \( x_0 \) for Cells 1 and 2 is 100 and 200 \( \mu m \), respectively.
Our next simulation deals with two cells initially located in the top and bottom halves of the channel (Fig. 4-6). The channel height was 70 µm. The bottom and top cells were initially at \( z = 24 \) µm and 48 µm, respectively. In the data shown in Figure 4-6a, the channel length was 280 µm and thus the average \( x \)-distance was \( 280/2 = 140 \) µm. The initial \( x \)-distance between the cells was 130 µm. According to this simulation, both cells experience damped oscillation in both lateral and translational directions. During these in-phase (phase shift = 0°) oscillations with the maximum lateral amplitude of about 2 µm, they were slowly drifting to their expected lateral equilibrium positions. These positions were symmetric around the centerline, i.e., if the bottom cells goes to the equilibrium position \( z_{eq} \), the top cell has the equilibrium position \( H-z_{eq} \). As seen in Fig. 4-6c, Cell 1 moved faster in the \( x \)-direction than Cell 2 because it was closer to the centerline than Cell 2. This resulted in a decrease in the \( x \)-distance with time until \( t \approx 20 \) ms, when the damped oscillation in the \( x \)-distance around 42 µm began (solid line in Fig. 4-6c). Since the cells did not move far from each other and the channel was long enough to avoid the influence of cells outside the computational domain (due to channel periodicity), the final \( x \)-distance of 42 µm was much less than the expected average \( x \)-distance of 140 µm. The final \( x \)-distance did not change until the average \( x \)-distance became less than the final \( x \)-distance (Fig. 4-6c). It should be noted that when the average \( x \)-distance was 42 µm and the initial \( x \)-distance was 2 µm, the oscillation amplitude of top and bottom cells (initially located at \( z = 50.2 \) µm and 19.8 µm, respectively) became much higher (Fig. 4-6b) than for cells with larger initial and average \( x \)-distances (Fig. 4-6a). This occurred due to stronger cell-cell interaction at smaller \( x \)-distance.
**Fig. 4-6** Migration of two identical cells located in different halves of the channel. (a) and (b) Time evolution of the cell lateral positions when the average $x$-distance is 140 µm and 42 µm, respectively. In (a), the initial positions for Cells 1 and 2 are $(x_0, z_0) = (80, 24)$ µm and $(210, 48)$ µm. In (b), the initial positions for Cells 1 and 2 are $(x_0, z_0) = (41, 19.8)$ µm and $(43, 50.2)$ µm. (c) Time evolution of the $x$-distance between the the cells in cases (a) (solid line) and (b) (dashed line). In both cases, the cell diameter is 14 µm.
Figure 4-7 shows the simulation data when two cells have very large initial z-distance but are still located in the same half (bottom or top) of the channel. As seen in Fig. 4-7, the upper cells repeatedly collided with the lower one. For comparison, the migration data for a single cell (dotted and dash-dot-dot lines) is also plotted in Fig. 4-7a. Similarly to Fig. 4-4a, during every collision, the cells have opposite spikes in their lateral position. After collision, the cells moved toward the single-cell lateral equilibrium position, but, during this motion, they again collided. These repeated collisions kept them from reaching the single-cell equilibrium position. The spike height for the lower cell (Cell 1) was shorter due to the wall repulsion, i.e., Cell 1 stayed closer to the trajectory of the single cell migration than the upper cell (Cell 2). Eventually, due to longer spikes, Cell 2 moved further and further from the trajectory of the single cell migration. It finally crossed the centerline and entered another half of the channel, where it underwent damped oscillation with larger amplitude than before (Fig. 4-7b), similar to what was shown in Fig. 4-6b.
Fig. 4-7 The migration of a series of identical and periodically spaced cells (two lines of cells with a large initial $z$-distance between them). (a) and (b) show the short- and long-term evolution of the lateral positions of these two lines (denoted as Cell 1 and Cell 2). In (a), dotted and dash-dot-dot lines are the time evolution of the lateral positions according to scenario $A$ (single cell). Here, the cell diameter is 14 $\mu$m; the channel length, width, and height are 84 $\mu$m, 140 $\mu$m, and 70 $\mu$m; $z_0$ for Cells 1 and 2 is 13 $\mu$m and 27 $\mu$m; and $x_0$ for Cells 1 and 2 is 63 and 21 $\mu$m, respectively.
In reality, cells may differ in their size and/or deformability. Figure 4-8 shows the numerical data on the interaction of two different-diameter cells. In this simulation, the length of the computational domain was small enough for periodic boundary conditions (and thus cells outside the domain) to influence the cell migration dynamics. In Figure 4-8a, the average $x$-distance was 42 µm. The initial lateral position of the bigger cell (Cell 2) was higher than that of the smaller one (Cell 1). This situation was considered because bigger cells generally migrate to higher lateral equilibrium positions (i.e., closer to the centerline) than smaller ones [178]. Similar to Fig. 4-3a, both cells experienced damped oscillation in antiphase, and the oscillation amplitude was much larger for the smaller cell than that for the bigger cell. At the end of oscillation, the bigger cell remained at a lateral position closer to the centerline than the smaller cell, i.e., no “swapping” occurred. This result indicates that cells with different sizes can be separated using inertial microfluidics even when the spacing between the cells is small and the cell-cell interaction is significant. Interestingly, as the average $x$-distance decreased to 28 µm (Fig. 4-8b), the bigger cell collided and then passed the smaller cell repeatedly (because of periodic boundaries). This led to the oscillation of the cells and also the entrance of the bigger cell to the top half of the microchannel, while the smaller cell remained in the bottom half (the case similar to what is shown in Fig. 4-7b). Because of this phenomenon, the $z$-distance between the cells increased dramatically: from 6 µm at the average $x$-distance of 42 µm to 28 µm at 28 µm (Fig. 4-8).
Fig. 4-8 Time evolution of the lateral positions of two cells of different size and the average x-distance of 42 µm (a) and 28 µm (b). In (a), the initial translational positions for Cells 1 and 2 are $x_0 = 21$ µm and $63$ µm. In (b) $x_0 = 10.5$ and $38.5$ µm for Cells 1 and 2, respectively. The diameters of Cell 1 and 2 are $28$ µm and $14$ µm, and their initial lateral positions $z_0 = 22$ µm and $28$ µm.
The effect of deformability on pairwise cell interaction and migration in a microchannel is displayed in Fig. 4-9. In this simulation, the average $x$-distance was 70 µm. The initial lateral position of Cell 2 (more deformable than Cell 1) was higher than that of Cell 1. This case was selected because more deformable cells generally have lateral equilibrium positions closer to the centerline [178]. As in Fig. 4-8, Cell 2 collided and passed Cell 1 repeatedly until reaching the equilibrium position closer to the centerline than Cell 1. Cell 2 was unable to enter the top half of the channel, but the lateral distance between the cells increased significantly from the initial value. When compared to the scenario A simulation [178], this result indicates that cell-cell interaction facilitates separation of cells with different deformability.

**Fig. 4-9** Time evolution of the lateral positions of two cells of different deformability and the average $x$-distance of 70 µm. The initial translational positions for Cells 1 and 2 are $x_0 = 35$ and 105 µm. The diameter and relaxation time of both cells is 14 µm and 0.2 s. The elasticity of Cells 1 and 2 is 250 Pa and 25 Pa, and their initial lateral positions $z_0 = 18$ µm and 25 µm.
4.4 Conclusions

In this computational study, we have investigated the effect of pairwise interaction of deformable cells on lateral and translational migration in a microchannel. When two identical cells are located in the bottom or top half of the channel at different lateral positions and not interacting with other cells, they pass each other or “bounce back” in the translational direction, eventually reaching the same lateral equilibrium position and a large separation distance (more than 10 times the cell diameter). The transient interaction between these cells leads to cell “swapping” in the lateral direction provided the lateral distance between the cells is small enough. “Swapping” can deleteriously influence cell separation or enrichment, and our study indicates that there is a threshold value in the lateral distance of cells entering the channel for this phenomenon to occur. When the cells become bigger, or when they are located in different halves of the channel, they keep a close $x$-distance between each other (3 to 7 times the cell diameter).

When considering a series of identical and periodically spaced cells with close $x$-distance, these cells generally undergo damped oscillation in both the lateral and translational directions until they reach equilibrium positions and become evenly distributed in the translational direction (self-assembly phenomenon). For a series of cells with different sizes, bigger cells have the equilibrium position closer to the centerline than smaller ones. When their $x$-distance becomes small enough, bigger cells collide with smaller ones and, as a result, enter the other half of the channel. This indicates that the efficiency of separation/enrichment of cells with different size can be improved when the cells are sufficiently close to each other when entering the channel, i.e., when their migration follows scenario AB. For a series of cells with different deformability, the cell-
cell interaction leads to the migration of more deformable cells to the equilibrium position closer to the centerline, thereby facilitating cell separation. This supports the hypothesis that leukocytes margination occurs because of the interactions of these cells with much more deformable red blood cells in the bloodstream.
Chapter 5 Active migration of circulating cells in a microchannel explored by three-dimensional numerical simulation

5.1 Abstract

Circulating cells such as leukocytes can deform and migrate actively toward the source of chemoattractant. This directed movement of cells, known as chemotaxis, plays a crucial role in the immune response and cancer metastasis. The cell’s active deformation and migration are driven by intracellular forces generated by polymerization/depolymerization of cytoskeletal filaments due to various signaling pathways. To numerically study these processes, we have implemented the Dembo’s reactive interpenetrating flow formalism and Herant’s polymerization force model into the three-dimensional computational algorithm for passive cell deformation. The resulting computational model takes into account passive mechanical properties of the cell, extracellular diffusion of chemoattractant molecules, intracellular release and diffusion of signaling molecules, intracellular active force generation. We have applied this algorithm to study circulating cell chemotaxis in a rectangular microchannel. The simulation shows that the active stress near the membrane in the region facing the chemoattractant source drives the cell to deform and migrate towards the chemoattractant source, with the formation of a finger-like projection or a large lamellipodium, similar to those observed in the experiments of chemoattractant-induced migration of motile cells.
5.2 Simulation framework

In this study the active migration and shape change of a non-adherent cell (e.g., leukocyte or cancer cell) was explored under different conditions in a rectangular microchannel under static flow. An initially spherical cell is initially located in an external fluid with the centroid position \((x_c, y_c, z_c) = (10, 10, 10) \, \mu m\) and diameter \(D = 10 \, \mu m\) (Fig. 5-1). No-slip condition with zero velocity on all the channel wall and inlet/outlet is applied. Boundary values for polymer messenger and chemoattractant is set zero except the outlet where \(m_c\) could be non-zero to simulate chemoattractant source.

Fig. 5-1 (a) 3-D sketch of the channel dimensions for active migration. (b) View at the middle plane \(y = W/2\).
The other parameters used in active migration of a cell are chosen from Herant’s simulation [126] and listed in Table 5-1.

Table 5-1: Parameters used in the simulation of active migration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$</td>
<td>fluid density (for all phases), $10^3$ kg/m$^3$</td>
</tr>
<tr>
<td>$\mu_s$</td>
<td>solvent viscosity, 1 cP</td>
</tr>
<tr>
<td>$G(0)$</td>
<td>Giesekus elasticity of cell, 0.5 Pa</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>relaxation time of cell, 0.2 s</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>cortical tension, $3 \times 10^7$ N/m</td>
</tr>
<tr>
<td>$D_c$</td>
<td>chemoattractant diffusivity, $10^{-9}$ m$^2$/s</td>
</tr>
<tr>
<td>$D_p$</td>
<td>polymerization messenger diffusivity, $10^{-13}$ m$^2$/s</td>
</tr>
<tr>
<td>$t_p$</td>
<td>polymerization messenger decay time, 1 s</td>
</tr>
<tr>
<td>$e_p$</td>
<td>polymerization messenger emissivity rate, $5.0 \times 10^{-6}$ m/s</td>
</tr>
<tr>
<td>$\varphi_{n0}$</td>
<td>network baseline fraction, 0.001</td>
</tr>
<tr>
<td>$t_n$</td>
<td>network decay time, 20 s</td>
</tr>
<tr>
<td>$\Psi_{nM}^{MN}$</td>
<td>Network-network stress constant, $0.5 \times 10^5$ Pa</td>
</tr>
</tbody>
</table>
5.3 Results and discussion

Simulation of a finger-like projection

Here, only a small cap of the membrane facing the chemoattractant source was stimulated with constant polymerization messenger concentration \( m_p = m_{\text{stimulated}} \) and mass transport of chemoattractant was not considered (Fig. 5-2). In this study \( m_{\text{stimulated}} \) is set as 0.08 to get a proper value of active stress, according to Herant’s model and parameters used here (Table 5-1).

**Fig. 5-2** Boundary conditions for polymerization messenger and chemoattractant in the simulation of a finger-like projection. In this study, \( m_{\text{stimulated}} = 0.08 \).
For the case of $m_{stimulated} = 0.08$, $\sigma = 3 \times 10^{-7}$ N/m (Fig. 5-3), the cell rapidly extends a small projection and then keeps its elongation with slower velocity. The cross sectional diameter of the front end of this finger-like projection is constant, nearly equal to the size of the stimulated membrane region before the extension. These results are consistent with the simulation of Herant’s work [126] and the experimental observation [179].

Fig. 5-3 (a-c) 3-D view of cell finger-like projection with time ($t = 25\text{ms}, 250\text{ms}, 475\text{ms}$); (d) the view at the middle plane $y = W/2$ ($t = 475\text{ms}$). $m_{stimulated} = 0.08$, $\sigma = 3 \times 10^{-7}$ N/m.
Several other cases were simulated to investigate the effect of different cortical tension on the cell projection (Fig. 5-4). For $m_{stimulated} = 0.08$, when cortical tension is high ($\sigma = 3 \times 10^{-6}$ N/m), the cell can only produce a very short projection (less than 1 µm in length); while, with reduced cortical tension ($\sigma = 3 \times 10^{-8}$ to $3 \times 10^{-7}$ N/m), the cell can extend a sufficient long projection with almost constant velocity (1 to 2 µm/s) after initial rapid protrusion. Therefore, lowering the cortical tension facilitates the active migration of the cell.

**Fig. 5-4** The elongation of the projection with time for different $m_{stimulated}$ and cortical tension.
Simulation of a lamellipodium-like extension

Here, chemoattractant transport is considered for two more realistic cases. One has a lower constant concentration in the whole outlet for chemoattractant and the other use a spot source with small diameter size but very high concentration value to imitate the chemoattractant releasing from a micropipette in the experiment [179] (Fig. 5-5).

Fig. 5-5 Boundary conditions of chemoattractant on the outlet for two different cases.
For both cases, the simulation shows that after some initial short time, the cell elongates a bit like an ellipsoid and then its front end expands in the directions perpendicular to the chemoattractant gradient, which is similar to a lamellipodium in the view at the middle plane $y = W/2$ (Fig. 5-6)

**Fig. 5-6** (a,b) 3-D view of cell deformation with time ($t = 275\text{ms, 400ms}$); (c) the view at the middle plane $y = W/2$ ($t = 400\text{ms}$). $m_c = 20$ at the whole outlet, $\sigma = 3\times10^{-8}\text{ N/m}$. 
The simulation result does not produce a finger-like projection in this more realistic situation, but presents a lamellipodium-like extension, which was also observed in experiments [120]. To find the reason, further analysis was done on the temporal and spatial change of chemoattractant and polymerization messenger. As Fig 5-7 shows, the relative change and trend of $m_c$ or $m_p$ with time at the center point of the front end of the cell is very similar for the two cases. Initially, the fluid is static in the whole system, and, after very short time (about $t = 15 ms$) of chemoattractant diffusion from the source, the cell starts producing polymerization messengers.

![Graph showing concentration change over time](image)

**Fig. 5-7** The concentration of chemoattractant molecules and polymerization messengers at the center point of the front end of the cell for the two cases with different profiles of chemoattractant concentration on the outlet.
At $t = 15$ms, what does the cell “see”? The cell can only sense the concentration of chemoattractant at the region near the membrane. Hence, the spatial distribution of chemoattractant around the cell membrane was plotted based on the data at the middle plane $y = W/2$ for both cases (Fig. 5-8). The distribution is very similar for these two cases, which indicates that when the distance of the cell front end to the chemoattractant source is 1.5 times cell diameter, the chemoattractant source profile does not influence the distribution of chemoattractant on the cell membrane. At the central part of the front end, the chemoattractant concentration is much higher than that at the other region, and polymerization messengers have similar distribution because their emissivity linearly depends on the chemoattractant concentration. Even though it looks like only small region have very high concentration for $m_c$ and $m_p$, a relatively large area about (from $-30^\circ$ to $30^\circ$ from the central point) still has $m_c$ and $m_p$ above 80% of its maximum value respectively, and the area diameter is equal to the cell radius. Therefore, a large part of the front end can still migrate towards the chemoattractant source. As the cell move closer to the source, more area on the front end has higher $m_c$ and $m_p$, which finally lead to a lamellipodium-like extension.
Fig. 5-8 The “vision” of the cell on the chemoattractant near the membrane and the corresponding polymerization messenger at $t = 15$ ms. 0º is the center point of the front end, -90º and 90º are the center point of the top end and bottom end, respectively, and ±180 º is the center point of the back end.
Based on experimental investigations, during a finger-like projection (pseudopod), chemoattractant receptors on the cell membrane, such as interleukin 8 receptor alpha and beta in neutrophils, are dynamically distributed and enriched near the projection region [180, 181]. More complicatedly, actin dynamics also affect chemoattractant receptor sensitivity [182]. Hence, the assumptions that receptors are distributed uniformly on the membrane and the emissivity of polymerization messenger linearly depends on the chemoattractant concentration near the membrane are not suitable for this situation. A more realistic model needs to consider the effect of chemoattractant on the receptor distribution and the sensitivity change of the receptor during migration.

5.4 Conclusions

The computational model for cell active migration has been developed by introducing the active force term into the established 3-D multiphase viscoelastic fluid algorithm, based on a simplified representation of signaling pathways during polymerization, including the chemoattractant transport, polymerization messenger production, and filament network remodeling.

When only a small region of membrane is stimulated, the model captures a finger-like projection of the cell, as observed in micropipette experiments of chemoattractant-induced leukocyte migration. The cortical tension was shown to be an important factor to cell motility during migration. In a general case of chemotaxis, the simulation shows the cell moves toward the chemoattractant source with a lamellipodium-like projection. This demonstrates the assumption of the linear dependence of polymerization messenger production on the chemoattractant
concentration near the membrane is not suitable to describe the cell biological response resulting in a finger-like projection.
Chapter 6 Summary

In this dissertation study, a high-performance parallel three-dimensional computational algorithm for multiphase viscoelastic fluid dynamics including mass transport has been developed (Chapter 2). Numerical simulations were conducted on: (1) passive lateral migration and deformation of a single drop or leukocyte during perfusion in a rectangular microchannel (Chapter 3), (2) hydrodynamic interactions of passively migrating cells in a rectangular microchannel (Chapter 4) and (3) active migration of cells during chemotaxis (Chapter 5).

The simulation work on passive migration shows drops or living cells with different bulk mechanical properties such as cytoplasmic viscosity and relaxation time can be separated in a microchannel. It is possible to classify cells with different mechanical properties by imaging their deformed shape during perfusion through a microchannel. Cell-cell interaction could facilitate the separation of cells with different sizes or deformability. These studies support the possibility of using microfluidics for deformability-based flow cytometry and can give certain suggestion to optimize microfluidic design for cell separation/enrichment. The exploratory work on the simulation of active migration of a living cell reproduced some experimental observations such as a finger-like and lamellipodium-like projection during chemotaxis.

There are some limitations in this study:
(1) Only a simple rectangular microchannel was considered, while some novel microfluidic devices have started to use special channel geometry to improve effectiveness and efficiency of cell sorting/separation.

(2) Although the viscoelastic drop model can describe the mechanical behavior of leukocytes during passive migration in the microchannel, it may not suitable for very deformable cells like red blood cells, which could easily break up if modeled as a liquid drop.

(3) The simulation of active migration did not consider the effects of cell adhesion, which is indispensable for various living cells to migrate on 2-D surface.

(4) Regular structured mesh was used for numerical simulation, which is difficult to use for geometrically complex computation domains and for tracking the cell membrane.

For future study, the current code, without significant modification, can be used to investigate cell deformation during aspiration into a micropipette, cell migration in the expansion channel, and cell deformation in the cross-flow channel. By including the adhesion model, the code will be able to simulate cell migration on a vessel wall and transmigration across the endothelium.
References:

107


R. Fahraeus, The suspension stability of the blood, 9 (1929), 241-274.


Y.C. Tan and A.P. Lee, Microfluidic separation of satellite droplets as the basis of a monodispersed micron and submicron emulsification system, Lab on a Chip, 5 (2005), 1178-1183.


D.R. Subramaniam, D.J. Gee and M.R. King, Deformable cell-cell and cell-substrate interactions in semi-infinite domain, J. Biomech., 46 (2013), 1067-1074.


D. Needham and R.M. Hochmuth, Rapid flow of passive neutrophils into a 4 microns pipet and measurement of cytoplasmic viscosity, J Biomech Eng, 112 (1990), 269-276.


Biography

I was born in Qingzhen, a small city in the southwest of China. I received my bachelor degree in science in 1999 and master degree in science in 2002 in Mechanical Engineering from Tsinghua University in Beijing, China. During the study in Tsinghua University, I obtained broad background on mathematics, mechanics, electronics, mechanical design, materials processing, computer programming. After two years of work in industry for software development, I decided to continue my academic study and research in US. I was admitted by Tulane University to the PhD program in Mechanical Engineering to study solid mechanics on micro-indentation of alloys and piezoelectric materials. Unfortunately, the program was cut later in the aftermath of Hurricane Katrina. Luckily, after I received a master degree in science in Mechanical Engineering, I had a chance to switch to Biomedical Engineering in 2007 to study biotransport in complex networks of bone cells. My current research is focused on numerical computation of passive and active migration of living cells in a microfluidic channel, using three-dimensional algorithms for multiphase viscoelastic fluid dynamics and mass transport. I would like to carry on my career in the field of biomedical engineering, with strong interest on computational and experimental research on cell migration and deformation related to microfluidic applications.