Design and fabrication of a microfluidic chip for living cell transportation and switching

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DESIGN AND FABRICATION OF A MICROFLUIDIC CHIP FOR LIVING CELL TRANSPORTATION AND SWITCHING

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School of Electrical & Electronic Engineering

A Thesis submitted to Nanyang Technological University
in fulfillment of the requirement for the degree of
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2006

“A Lab-on-a-chip is a microfluidic device fabricated using photolithographic processes developed in the microelectronics industry to create circuits of tiny chambers and channels in a polymer, silica, or glass chip. It directs the flow of liquid chemical reagents just as semiconductors direct the flow of electrons. Reagents can be diluted, mixed, reacted or separated— all on a single chip. It will be a promising analytical tool for analyzing cells, DNA and protein complexes in the biology laboratory of the future.”

-- Andreas Manz

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other university or institution.

Sun Yi

Date

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ABSTRACT

A conventional fluorescent activated cell sorter (FACS) is a machine that can efficiently separate cells in a suspension based on size and color of their fluorescence. Notwithstanding of its high efficiency in sorting cells, the sorter is costly, mechanically complex and requires trained personnel for operation and maintenance. In this study, a highly integrated microfluidic cell sorting device is developed using micromachining technology. The device contains two main parts, an optical system for cell detection and a microfluidic system for cell manipulation. The microfluidic system also includes two units, a focusing structure and a switching structure. The task of sorting cells in this microchip is accomplished by electrokinetic flow. The focusing structure enables a single cell suspension along the center line of a micro-channel through a detection region. The micro-optical system excites and detects the fluorescent labeled cells. The sorting process is realized at the switching structure by adjusting the direction of the electroosmotic flow through switching the voltages at output reservoirs. In this project, the physics of microfluidics has been carefully investigated to accomplish the task of designing this device. Straight microchannels are simulated, fabricated and examined to test the transport capability of Electroosmotic Flow (EOF). Simulation and experiments for focusing and switching structures are also carried out to optimize the design of the cell sorter. The integrated device provides an inexpensive, robust and effective way to perform cell sorting.

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LIST OF ABBREVIATIONS

E	Sorting efficiency
P	Sorting purity
R	Sorting recovery
T	Sorting throughput
Y	Sorting yield
λ	Debye length
ζ	Zeta potential
ρ_e	Charge density
e	Electron charge
z	Valence
k_B	Boltzmann constant
n_o	Ion density
ψ	Electroosmotic potential
ω	Debye–Hückel parameter
Φ	Electric potential
σ	Electric conductivity
μ	Fluid viscosity
E_x	Applied electric field
D_h	Hydraulic diameter
CAD	Computer aided design
CV	Coefficient of variance

DAQ	Data acquisition
DEP	Dielectrophoresis
DI	Deionized
EDL	Electric double layer
EOF	Electroosmotic flow
FACS	Fluorescent activated cell sorter
GFP	Green fluorescent protein
IPA	Isopropyl alcohol
MEMS	Micro-electro-mechanical systems
PDMS	Polydimethylsiloxane
PIV	Particle image velometry
RIE	Reactive ion etching
SEM	Scanning electron micrograph
VLS	Very large scale

CHAPTER 1 INTRODUCTION

The objective of this chapter is to give an introduction of the background of the project. First, development of flow cytometer systems from conventional fluorescent activated cell sorters to miniaturized cell sorters is explored. Then, literature review for various components of a miniaturized cell sorting chip that includes cell transportation, focusing, detection and separation are presented. Finally, research motivation, research objective and structure of the thesis are presented.

1.1 Literature Survey

1.1.1 Fluorescent activated cell sorting (FACS) system

A fluorescent activated cell sorting (FACS) system is a crucial instrument for measurement of the fluorescence and light scattering of individual biological cells and various types of microscopic particles and separating them based on the size and color of their fluorescence. This technique is widely used in a broad range of biomedical and industrial applications such as clinical hematology diagnosis [1-4], bacteria analysis [5], gene diagnosis [6], transfusion medicine [7] and many others [8-11]. The conventional FACS system works as follows. A cell suspension containing cells labeled with a fluorescent dye is directed into a thin stream so that all the cells pass in a single file. The dye is coupled to a monoclonal antibody and binds to those cells coated with the antigen for which the antibody is specific. This stream emerges from a nozzle vibrating at some thousands cycles per second which breaks the stream into discrete droplets. Some of these may contain a cell. A laser beam is directed at the stream just before it breaks up into droplets. As each labeled cell passes through the beam, its resulting fluorescence is detected by a photodetector. If the signals from the two detectors meet either of the criteria set for fluorescence and size, an electrical charge (+ or -) is given to the stream. The droplets retain this charge as they pass between a pair of charged metal plates. Positively-charged drops are attracted to the negatively-charged plate and vice versa. Uncharged droplets (those that contain no cell or a cell that fails to meet the desired

criteria) pass straight into a third container and are discarded later. The apparatus can sort up to 5,000 cells per second [12].

Since its invention, flow cytometry has become a technology that is needed for analyzing and sorting cells just as gel electrophoresis is required to characterize, purify or quantify DNA in every biological laboratory. However, although the conventional FACS provides impressively efficient sorting, it is costly (US\$250,000), mechanically complex and requires trained personnel for operation and maintenance [13]. Additionally it also has the drawbacks of low sensitivity for bacterial cells and DNA, cross contamination between runs and is mechanically inflexible to work with different cell types and sizes. Moreover, the conventional FACS combines the technology of ink-jet printing and electrostatic deflection to achieve droplet generation and high sorting rates. However, this mechanism is delicate and many failures of the instrument can result from problems in the flow chamber. For example, clogging of the orifice and particle absorption and contamination in the tubing can cause turbulent flow in the jet stream, inducing variation in illumination and detection. Sample carryover can occur during consecutive runs when remnants of previous samples flush back into the new sample stream. Sterilizing the system between runs is also time-consuming resulting in machine downtime. Furthermore, cells passing through the orifice may perturb droplet formation as larger cells can change the droplet size, nonspherical cells tend to align with the long axis parallel to the flow axis, and deformable cells may elongate in the direction of the flow. Such perturbations in droplet formation may introduce variation in the time from the analysis to the actual sorting event. Finally, a number of technical problems make it

difficult to generate identically charged droplets which in turn increase the deflection error.

The cost and complexity observed in the existing technology has greatly limited the usage of cell sorting in biological research. Thus, there is an urgent need to call for inexpensive devices that can rapidly sort live cells, particles and single molecules. Microfabrication of flow cytometers using MEMS techniques could be a promising approach to reduce the size and cost of this instrument. With the rapid development of microfabrication and integration techniques, miniaturized cell sorting devices have emerged. From the beginning of the 1990s, a number of micromachining methods [14-16] have been sought to replace the conventional flow chambers in FACS with microfluidic devices that can potentially allow more sensitive optical detection, easier mechanical setup, and innovative sorting schemes. Several miniaturized cell sorting devices have been reported using a microfabrication process in metal [17, 18], silicon [19-21], glass [22, 23] and plastic materials [24-27]. Focusing of a sample stream and even counting and sorting of cells or microparticles have been demonstrated in literature. In the following sections, the literature reviews of cell transportation, focusing, detection and sorting are presented.

1.1.2 Microfluids transportation

One of the most critical elements of any microfluidic system is its fluidic transport system. Such fluid drivers must have the ability to generate flow rates of

hundreds of microliters per minute for high-volume-throughput applications such as flow cytometry [28]. Numerous different fluid driving methods for microfluidics have been reported. Electrokinetics [13, 29], pressure [30], piezoelectric actuation [31], electrostatic actuation [32], centrifugal force [33], electrohydrodynamic (EHD) [34] and many other pumping methods, or various combinations have their individual merits and drawbacks. However, the two most commonly used fluid driving methods are electrokinetic force and hydraulic pressure. Pressure driven flow devices which exploit pressure gradient have been reported [35, 36], but external pumps are used to deliver the focusing and sample flows. Unfortunately, there has been little success in the fabrication of on-chip mechanical pumps for the delivery of fluids. Accordingly, electrokinetic phenomena arising from electric potential gradient, i.e., electroosmotic and electrophoretic transport [37], have a tendency to dominate since they are more readily incorporated into microfabricated devices.

1.1.3 Cell focusing

In flow cytometry, cell focusing is necessary in order to achieve sensitive fluorescence measurements. By incorporating transversal spatial confinement of the sample, the randomly distributed cells are focused to the center line of the channel. As the positions of cells are known, more sensitive measurements could be made. In the conventional FACS, the particles or cells to be analyzed pass an incident laser beam one at a time using hydrodynamic focusing in which confinement is achieved by pressure-driven flow. In microfluidic structures employed in flow cytometry, the

hydrodynamically focused fluid streams are generated by syringe pumps. These devices have been used for the analysis of fluorescently labeled leukocytes [36]. In addition to these hydrodynamic schemes, cells and particles can also be focused on a chip using electric field, which is known as electrokinetic focusing. Electrokinetic transport has been employed for the manipulation of cells on a microchip and to focus ions [29] and fluids into a narrow stream [38].

1.1.4 Cell detection

Cell detection in microfluidic devices is a major challenge owing to the combination of low analyte concentrations and small detector volumes. Although several detection methods, such as mass spectroscopy [39], impedance-based Coulter [40] and electrochemical detection [41] have been explored, fluorescence detection remains as the most popular method thus far. This is presumably because its inherent high sensitivity is a good match for the small detection volumes. Most analyte species do not fluoresce intrinsically and therefore have to be chemically derivatized. The success of fluorescence detection is also due to the facts that the biochemically relevant species of high interest can be derivatized readily with a fluorescent tag. Fu *et al.* [13] has demonstrated laser induced fluorescence (LIF) detection of bacterial cells in the development of the microfabricated FACS device. However, in this and most other systems delicate optical components including focused laser beams, various optical detecting/filtering devices and complicated control circuits make the system relatively expensive. Alternatively, a pair of buried optical waveguides integrated into the device to carry out on-line fluorescent cell

detection was reported [42]. The detection light is propagated through the waveguide structure by a process of total internal reflection. This detection arrangement permits the counting of cells and particles without the need for an expensive microscope, or the delicate optical alignment. A further simplification of fluorescence detection can be obtained, at reduced sensitivity, if a laser diode (LD) and a silicon photodiode are used instead of laser source and photomultiplier tube.

1.1.5 Cell switching

In addition to cell detection and counting, the microfabricated FACS is also capable of cell sorting. A few examples of cell sorting on microfabricated devices have emerged where electroosmotic [13, 43], hydrodynamic [44-47], and dielectrophoretic (DEP) [48-53] forces have been exploited.

Electroosmotic switching is the most popular method due to its easy implementation and fast response. Fu *et al.* [13] has constructed a microfabricated FACS device and demonstrated its effectiveness in sorting microbeads and bacterial cells using electrokinetic flow. The standard “forward” sorting algorithm consists of running the cells from the input channel to the waste channel until a cell’s fluorescence is above a preset threshold, at which point the voltages are temporarily changed to divert the cell to the collection channel. The switching angle of the microchip was designed to be 180°. The sorting time of the system is 50 msec per switch and the throughput of the device is about 20 cells per second.

Dittrich *et al.* [43] demonstrated an integrated micro total analysis system based on a microfluidic on-chip device for reaction, high-sensitivity detection and sorting of fluorescent cells and particles. The sorting process is realized by a perpendicular deflection stream that can be switched electrokinetically. To direct the particle stream into one desired output reservoir, the deflection stream in the perpendicular channel has to be activated. The flow in this narrow deflection channel, likewise with equalized pressures at both ends in the resting state, is controlled electrokinetically. By applying a voltage, a weak electrokinetic flow towards the cathode is induced, pushing the particles only a few micrometers away from the center of the channel, which further enhances their probability of entering into the right channel. This deflection flow can be switched in both directions. For particle sorting, the electroosmotic flow is activated discontinuously, that is, whenever the transit of a cell through the detection volume is indicated by the fluorescence recording unit. For the switching structure, the switching angle was 150° . A throughput of 30 cells per second was reported.

The throughputs of 20 or 30 cells/s achieved by the two groups are considerably slower than the conventional FACS machines. This may be due to the large switching angles. the throughput may be augmented by improving the structure design, such as reducing the switching angle.

Hydrodynamic switching is an alternative way for microfluidic cell sorting. Fu *et al.* [44, 47] has presented a cell sorter. The device incorporated a three-valve peristaltic pump (with dampers to minimize fluid pulsations) and switching valves. An upper

control layer that contains a series of channels for introducing pressurized nitrogen and vacuum are used to actuate the valves and pump. Both cell sorting and cell trapping are demonstrated with this device. Complex channel structures and peripheral equipments are the main limitations in hydrodynamic sorting.

Furthermore, single cells and particles have been fixated and manipulated by DEP. In this method, a predetermined set of properties determines the fate of the cells upon detection instead of their inherent properties. A linear traveling wave dielectrophoretic microchip with an array of integrated electrodes is presented by Cui *et al.* [51]. The electrodes were energized with sequentially phase-shifted AC voltages to produce the traveling waves. The device is demonstrated by separating latex beads and rabbit heart cells. Details of the fabrication and design of the device are presented in an earlier paper [26]. In the approach used by Becker *et al.* [52] single cells can be manipulated in high conductivity buffers through the dielectric properties of the cells. Wang *et al.* [53] presents a similar device with an array of integrated electrodes for dielectrophoretic field-flow-fractionation separation of cells. Although DEP can provide fast separation, the delicate and tedious fabrication process along with the complicated electronic and buffer requirements in DEP have inhibited the silicon microfluidic technologies from gaining popularity among biochemists and biologists.

1.2 Research Motivations

This research project focuses on design and fabrication of a microfluidic chip for cell sorting applications. In literature based on the survey, several groups have demonstrated microfabricated flow cytometry devices for cell sorting or for cell analysis, and however, several problems such as low level integration and lack of proper design guidelines are still observable in the existing μ FACS devices.

The most important advantage of the biochip is the possibility of creating complete analytical microsystems by integrating functional modules onto a single chip. However, there have been scant attempts to integrate fabricated microchannels with other peripheral compounds. For most of the reported devices, pressure is used to induce controllable movement of the fluid in a microchannel. Pumps and valves are necessary to regulate the flow properties. Difficulties arise usually in an attempt to incorporate a mechanical pump or valve into the chip as the scale of the microchannels associated with microfluidic chips is too small. In addition, the various interfaces of microfluidic channels, tubing systems, and valves are difficult to handle. In this project, we aim to develop a self-contained and fully integrated microfluidic chip for cell sorting applications.

Although many have presented working microfabricated flow cytometry devices for cell sorting purpose, most of the works were only presented as a “proof of concept” and most often, geometry and dimensions of structures were given without explanation.

No specific rules have so far appeared in the literature to guide the design of the microfluidic structures. It is time consuming to obtain an optimized structure through trial-and-error. In this project, the physics of microfluidic is extensively studied. The impact of geometries on the performance of cell sorting devices is investigated and key parameters which play important roles in obtaining the optimized structure are identified.

1.3 Research Objective

The objective of this project is to develop a microfluidic chip, where microfluidic manipulation is conducted for living cell transportation, focusing and switching using electroosmotic force. The microfluidic structure is designed and simulated, and the focusing and switching effect of the microchannel structures are optimized. This microfluidic cell sorting device may confer several advantages over the previously reported cell sorting devices:

- (i) The electroosmotic manipulation on-chip eliminates the need for external syringe pump, hence, it is suitable for miniaturization as it is relatively easy to generate and structure an electrical field in a microscale.
- (ii) Since the response of electroosmotic flow to the direction of the applied field is instantaneous, electrical manipulation provides much shorter switching times and thus, yields much higher sorting speeds.

- (iii) Due to the easy fabrication process and inexpensive materials used in soft lithography, this elastomeric microfabricated cell sorter is affordable to every research laboratory and can be disposed just as a gel in gel-electrophoresis, which eliminates any cross-contamination from previous runs. The planar configuration of the microfabricated cell sorter and on-chip detection system also allow for more sensitive optical detection and easier mechanical setup.

1.4 Structure of the Thesis

The thesis consists of five chapters.

Chapter 1 gives a brief introduction of the background of this project. Literature review of conventional FACS system and microfabricated cell sorters are presented. Research motivation and research objectives are also highlighted in this chapter.

Chapter 2 provides an overview of a cell sorting system. Concepts and specifications of the microfluidic cell sorter system are presented. Theories and design of the microfluidic system are discussed in details. Microfabrication procedures for the microfluidic cell sorter are also explored. Experiments are carried out using fabricated microchannels to test the transportation capability of electroosmotic flow (EOF).

Chapter 3 discusses the focusing structure design. The principles of electrokinetic

focusing and an equivalent circuit model are illustrated. Simulation of different configurations is carried out to achieve optimum microchannel structure. The effect of two important parameters, applied voltage ratio and channel width ratio, are intensively studied. The simulated results are experimentally verified.

Chapter 4 explores the switching structure design. Both the electrokinetic switching effect and an equivalent circuit are depicted. Y-junction switching structures are designed and simulated. The effects of switching angles on switching accuracy and switching speed are studied. Based on simulation results, several switching structures are fabricated and tested. Experimental results are discussed.

Chapter 5 draws conclusion for the previous chapters and highlights the important results achieved. Recommendations are given at the end of the thesis.

CHAPTER 2 DESIGN AND FABRICATION OF THE MICROFLUIDIC CHIP

The objective of this chapter is to present the architecture of the microfluidic cell sorting chip. Firstly, concepts and specifications of the cell sorting chip are presented. Then, theories and design of two major parts of the chip, microfluidic system and optical system are explained in details. Finally, microfabrication processes of the microfluidic chip are illustrated and experimental results of straight microchannels are discussed.

2.1 The Microfluidic Cell Sorting Chip and the Specifications

Although the conventional FACS machines have demonstrated impressive performance in detecting and sorting cells, however, they have problems of high cost, low flexibility to work with different cell sizes and complex operation and maintenance procedures. Therefore, in this project, we have developed a novel microfluidic cell sorting chip. Microfluidic cell sorter provides an inexpensive, robust and flexible way to sort and manipulate single cells. Fig. 2.1 shows the schematic of the microfluidic cell sorting chip. The chip consists of two parts, a microfluidic system for cell manipulation and an optical system for cell detection. The microfluidic system includes two other units, a focusing structure and a switching structure.

Fig. 2.1 Schematic of the cell sorting chip.

The working principle of the cell sorting chip is as follows. Samples containing different types of blood cells are prepared off chip where targeted cells are labeled with fluorescent marker. The mixture of labeled and unlabeled cells is injected into the input reservoir by micropipette. Cells are transported with electroosmotic flow (EOF), which is

Chapter 2 Design and fabrication of the microfluidic chip

controlled by platinum electrodes at the input and output reservoirs. The chip comprises a branched channel system for focusing of the sample solution. Cells are focused into a narrow stream using neighboring sheath fluids such that the cell or particle samples can pass through the detection area one at a time, which guarantees a complete particle counting and analysis. The sheath flow fluids are pumped by electric field to flow at the required velocity so that the width of the sample flow can be well-controlled. The pre-focused sample moves down to the detection region where fluorescence is excited by the focused laser beam. A 488-nm laser with a spot size of hundred micrometers was used as the light source to excite the fluorescence-labeled cells. The laser light is guided to the microchip using an optical fiber. Two microlens arrays are designed to improve the quality of the beam. One of them is placed ahead of the microchannel to focus laser light to 6-10 μm , which is around the size of the cell and another set is to collimate the emission of fluorescence. The fluorescent emission from the analyzed sample is collected by another optical fiber and detected by the photodetector. The detector is a highly sensitive spectrofluorometer with a built-in 515 nm band pass filter. The detection window has an area of 10 $\mu\text{m} \times 10 \mu\text{m}$ and the detection limit of the spectrofluorometer is 10^{-8} M. Cells are identified by their distinct fluorescent responses. In the subsequent switching region, the cells can be sorted into two different output channels at the second intersection. An electroosmotic flow switch is exploited to direct the bio-particles into the appropriate outlet channels. Sample solution is defaulted to flow into a waste reservoir. When the fluorescence intensity of a cell exceeds a preset threshold, active sorting occurs, which is realized by adjusting the direction of the electroosmotic flow through electrically switching the voltages at the output reservoirs. Since the

electroosmotic flow responds to the direction of the applied field almost instantaneously, electrical manipulation shortens the switching time significantly.

The performance of a cell sorter is usually assessed based on its throughput, accuracy and recovery [54]. The sorting throughput refers to the sample volume processed within a certain period. The sorting accuracy is defined as the percentage of targeted cells collected from the total number of collected cells. The sorting recovery refers to the success rate of selecting the targeted cells as a percentage of the total number of sortings attempted. In this project, a microfluidic cell sorting system for the application of blood cell counting and sorting is developed. In clinical diagnosis, 5 ml blood samples are taken from patient and only 2-3 μl are used in each test and it is recommended to process the sample within half an hour. This means that the flow rate of the sample is between 0.07 and 0.1 $\mu\text{l}/\text{min}$. To achieve the requirement, the cell sorting chip is designed to have a throughput of 0.1 $\mu\text{l}/\text{min}$ and the sorting accuracy and sorting recovery should be no less than 95%.

2.2 Design and Simulation of Microfluidic System

As mentioned in Chapter 1, the most popular methods for fluid transport are pressure- driven and electroosmotically-driven [30]. In the pressure-driven microfluidic systems, the frictional forces of the mobile phase interact at the wall of the microchannel resulting in radical velocity gradients throughout the microchannel. As a result, the fluid velocity is the greatest in the middle of the microchannel and a substantial pressure drop

along the microchannel. On the other hand, in the electroosmotically-driven systems, the EOF is generated uniformly along the length of the microchannel. In this project, we adopted EOF as the mechanism to transport sample solution through the channel systems for the following reasons:

- 1) Separations appear to be more efficient using electroosmosis than pressure-driven flows, due to lower dispersion by the flow profile.
- 2) Electroosmotic pumping is a convenient tool for tuning liquid flow into individual channels, by varying the applied voltages at the end of each channel.
- 3) Since the response of electroosmotic flow to the direction of the applied field is instantaneous, this electrical manipulation of fluids and cells provides much shorter switching times and thus, yields much higher sorting speeds.
- 4) The electroosmotic manipulation on-chip eliminates the need for an external syringe pump.

Therefore, EOF is more suitable than external pressure injection for miniaturization as it is relatively easy to generate and structure an electrical field in a microscale. Theoretical details of EOF are discussed in the following section.

2.2.1 Electroosmotic flow

EOF is the motion of bulk liquid caused by the application of an electric field to a channel with a charged wall [55, 56]. Formation of an electric double layer (EDL) plays a

key role in all electrokinetic transport processes as shown in Fig. 2.2 [57]. ψ is the electroosmotic potential, ψ_o is the surface potential, ζ is the zeta potential, and y is the distance measured from the wall. For example, when silica is in contact with an aqueous solution, its surface hydrolyzes to form silanol surface groups [58]. These groups may be positively charged as SiOH_2^+ , neutral as SiOH , or negatively charged as SiO^- , depending on the pH value of the electrolyte solution. If the channel surface is negatively charged, the positive ions are attracted towards the surface, and the negative ions are repelled from the surface, keeping the bulk of the electrically neutral liquid further away from the wall. The distance from the wall, where the electroosmotic potential energy is equal to the thermal energy is known as the Debye length (λ_D). Ion redistribution within the Debye length results in a net electric charge, which is characterized by the electroosmotic potential. This region is also known as the EDL, which consists of two distinct zones: the Stern-layer and the diffuse layer. The Stern-layer is formed because of the inherent strong attraction of ions onto the oppositely charged wall surface. The ions are immobilized near the charged surfaces and the electroosmotic potential drops rapidly across the Stern-layer to a value at the edge of the Stern-layer, known as the zeta potential (ζ). The zeta potential, along with the buffer ion concentration, characterizes the electroosmotic transport.

Fig. 2.2 Schematic of the structure of the electric double layer.

The electroosmotic potential distribution in the diffuse layer is governed by the Poisson–Boltzmann equation, which is expressed as

$$\nabla^2 \psi = -\frac{\rho_e}{\varepsilon} \quad (2.1)$$

where ψ is the electro-osmotic potential, ρ_e is the charge density, and ε is the electric permittivity. For a symmetric electrolyte under equilibrium distribution, the charge density is given by the Boltzmann distribution, which is written as

$$\rho_e = -2n_o e z \sinh\left(\frac{e z \psi}{k_B T}\right) \quad (2.2)$$

where e is the electron charge, z is the valence, k_B is the Boltzmann constant, n_o is the ion density of the bulk solution, and T is the temperature. The electroosmotic flow is generated by the interaction of the external electric field with the net charge distribution in the EDL. For the case of a negatively charged surface, for example, the EDL becomes positively charged. Upon the application of an external electric field E , the ions in the EDL become mobilized, resulting in motion towards the cathode. Bulk flow motion is determined from the incompressible Navier–Stokes equation, which can be expressed as

$$\rho_f \left(\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla) \vec{v} \right) = -\nabla P + \mu \nabla^2 \vec{v} + \rho_e \vec{E} \quad (2.3)$$

where ρ_f is fluid density, μ is the absolute viscosity, P is the pressure, and $\vec{v}$ is a divergence free velocity field $\nabla \cdot \vec{v} = 0$. The term $\rho_e \vec{E}$ shows the electrokinetic body forces. The above equation is subject to no-slip boundary conditions on all solid surfaces. The externally applied electric field is given by

$$\vec{E} = -\nabla \phi \quad (2.4)$$

where ϕ is the electric potential. The externally applied electric field is subject to insulating boundary conditions on dielectric surfaces, i.e. $\partial\phi/\partial n = 0$. To derive all the equations above, we assume Newtonian fluid with viscosity and permittivity independent of the local or overall electric field. For the case when the EDL thickness is significantly smaller than the channel dimensions, a plug-like velocity is observed in the bulk of the channel, which is also known as the Helmholtz–Smoluchowski electroosmotic velocity, given by

$$v_{eof} = \frac{-\zeta\epsilon E_x}{\mu} \quad (2.5)$$

where E_x is the applied electric field in the stream-wise direction, and μ is absolute fluid viscosity.

In the ideal case there are no cross-stream velocity gradients in the bulk fluid region because no applied forces exist outside of the diffuse layer. In practice, however, several factors can induce velocity gradients in the bulk liquid including: i) a narrowing or partial blockage anywhere in the capillary; ii) the presence of charged particles in the fluid; iii) capillary forces resulting from different curvatures of the free surfaces in the reservoirs; iv) contact angle hysteresis of a bubble entrapped in the channel; v) a non-uniform wall surface charge distribution; and vi) slight height differences between the free surfaces in the reservoirs due to evaporation or buffer depletion [59].

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Fig. 2.3 The effects of EOF and pressure forces on the velocity profile.

One particular fluid flow problem is the coexistence of unwanted pressure-driven flow with EOF as shown in Fig. 2.3. Effects arising from hydrostatic pressure-induced flow, caused by unequal levels in solution reservoirs, can be a source of problems for liquid handling and separation [60]. Differences in liquid level heights between reservoirs will cause siphoning from reservoirs with higher liquid levels. The siphoning effect is illustrated as follows. Initially, we start to apply electric field until the liquid level in two reservoirs are balanced, so the only fluid transport inside the microchannel is EOF from inlet to outlet. As time goes on, fluid flows from the inlet to the outlet, the liquid level in the inlet reservoir drops while in the outlet reservoir rises, inducing an appreciable height difference. This height difference in turn introduces a pressure driven flow which perturbs the EOF. The linear flow rate due to height differences in a microchannel, v_p , is given as

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$$v_p = \frac{\Delta h \rho g D_h^2}{2 f R_e \mu L} \quad (2.6)$$

where Δh is the difference in liquid levels, ρ is the density of the solution, g is the acceleration due to gravity, $f R_e$ is the fanning friction factor multiplied by the Reynold's number and depends on channel geometry, μ is the solution viscosity, L is the channel length and D_h is the hydraulic diameter of the channel which is given by

$$D_h = \frac{4 \times [\text{Cross Sectional Channel Area}]}{\text{Cross Sectional Channel Perimeter}} \quad (2.7)$$

Wherever electroosmosis and pressure-driven counter flow co-exist in microchannels, the average flow rate v_{ave} consists of the sum of the electrokinetic linear velocity v_{eof} , and the average pressure-induced linear velocity v_p , that can be expressed as,

$$v_{ave} = \frac{-\zeta \epsilon E_x}{\mu} - \frac{\Delta h \rho g D_h^2}{2 f R_e \eta L} \quad (2.8)$$

Eq. (2.8) predicts that the plug profile of EOF and parabolic profile of Poiseuille flow are independent of one another and can be combined to give rise to bi-directional flow.

When the average velocity equals to zero, which means the magnitude of EOF is identical as the pressure-driven flow, back flow will start. The height difference at this time is called threshold height difference and can be calculated as:

$$\Delta h = -\frac{2 f R_e L \zeta \epsilon E_x}{\rho g D_h^2} \quad (2.9)$$

The transport capability of an electroosmotically-driven microchannel is given by:

$$Q = \pi D^2 \Delta h / 8 \quad (2.10)$$

where Q is threshold transported volume, D is the diameter of the reservoir and Δh is the threshold height difference between the two reservoirs.

2.2.2 Microfluidic design and simulation of straight channels

The presence of parabolic, pressure-driven flow detracts from the even radial profile of axial velocity generated by EOF. Given a more complex network of connected channels and much smaller reservoir dimensions, chip-based separations become more complicated compared to capillary electrophoresis, and they are also more susceptible to the effects of evaporation, buffer depletion, and Laplace pressures arising from surface tension. In order to optimize the chips to prevent these effects from occurring, it is important to understand the fluid transport mechanisms when electrokinetic flow is combined with pressure-driven flow. The back flow rate is related to hydraulic diameter of the channel. Therefore, to study the siphoning effect, we design and simulate the straight microchannels with different hydraulic diameters. For convenience, channels widths are fixed at 100 μm . Fig. 2.4 shows the diagram of the straight channel. All the channels are of 5 mm long and 100 μm wide. The depths of channels vary from 20 μm to 300 μm . The surfaces of the two reservoirs have diameter of 4 mm and height of 2 mm and are initially placed at two equal elevations as shown by the dashed line. The electrodes are placed at the inlet and outlet of the channel, and an electric field of 300 V/cm is applied. H_2O is used as the working buffer with density equal to 1000 kg/m^3 , viscosity equal to 10^{-9} m^2/s and relative permittivity equal to 78.5. Zeta potential of -0.0295 V is assumed.

The theory predicts that the plug profile of EOF and parabolic profile of Poiseuille flow are linearly independent of one another, so the final resulting velocity can be viewed as the superposition of the two flows. As a result, our work on the straight channel simulation is divided into three stages. The first stage is pure EOF simulation without pressure disturbance, the second stage is pure pressure-driven flow simulation, and then the two stages of simulation are combined together to obtain the threshold height difference that causes the fluid to flow backward. On the y - z plane of the channel, a line along the z direction at the middle width is defined as chord-length. Velocities along the chord-length are studied.

Fig. 2.4 Diagram for straight channel simulation.

2.2.2.1 Numerical simulation of EOF

Two methods are employed to get the electroosmotic flow velocity: i) analytical calculations using the Helmholtz–Smoluchowski equation (Eq. (2.5)) and ii) numerical simulations performed on CFD-ACE+ (ESI Research Corporation, France) which is a

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multiple-physics software package based on the finite volume method. From the analytical calculation, EOF velocity is independent of the hydraulic diameter, which means that the average velocity does not increase with cross section. Therefore, for all channel sizes with fixed zeta potential and the electric field, the calculated values are exactly the same when the same buffer is used. According to Eq. (2.5), the calculated EOF velocity is 695 $\mu\text{m/s}$.

For numerical simulations, an iterative algorithm is implemented to solve the fluid equations until the problem converges. By post-processing, the velocities along the chord-length are plotted as a function of channel depth. Theoretically, the velocity profile is supposed to be flat, however, a concave shape is observed in the simulation results. It is also found that as the channel height increases, the velocity profile of EOF across the channel becomes more concave as shown in Fig. 2.5. This can be explained by the principle of EOF. Upon the application of an external electric field, the ionized liquid in the diffusion layer becomes mobile. Due to the viscosity of liquid, the bulk liquid is dragged to move, which means the center liquid is dragged by the side liquid near the charged surface. Thus, as channel size increases, with the same “dragging force” from the side liquid, the center fluid will experience a slower motion. The averaged velocities extracted from simulation are plotted as a function of channel depth to compare with calculated EOF velocity in Fig. 2.6.

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(a)

(b)

Fig. 2.5 Simulated EOF velocity profiles for various channel depths: (a) 50 μm deep, (b) 70 μm deep, (c) 100 μm deep.

Fig. 2.6 Comparison between the CFD simulations and the theoretical calculations of EOF velocities for various cross sections.

From the figures, two conclusions can be drawn. First, the EOF velocity is independent of the channel size since similar EOF velocities have been obtained for all channel sizes. Second, the calculated values are generally greater than the simulated ones, because, in the theoretical EOF velocity derivation, the velocity of bulk liquid is assumed to be equal to the velocity of fluid in the diffusion layer, without taking into consideration the shear force between them. Thus, the software simulation is a better representative of practical condition.

2.2.2.2 Numerical simulation of pressure-driven flow

The pressure-driven flow was also simulated using the CFD software. The average velocities along the chord-length are extracted, and written as V_p . V_p for various hydraulic diameters are plotted as a function of applied pressure as shown in Fig. 2.7. From Fig 2.7, it can be seen that the velocity increases linearly with pressure. This is in agreement with Eq. (2.6), as the effect of the pressure in these channels does depend on their cross-section. The slope of the curves increases with channel size, which implies that the pressure-driven velocity is larger for channel with bigger cross-section.

Fig. 2.7 Relationship between pressure-driven flow velocity and pressure for various cross sections. All the channel widths are fixed at 100 μm .

2.2.2.3 Threshold transport volume

The two-stage simulation enabled us to find out the threshold pressure at which the counter pressure driven flow velocity has the same magnitude with the aforementioned EOF velocity and the backflow starts. As shown in Fig. 2.8, we manage to do so by drawing a horizontal line $A-B$ at V_p equal to 695 $\mu\text{m/s}$ (EOF velocity value calculated in previous section); then through the intersection of this horizontal line and the plotted curve, a vertical line is drawn to intersect with the pressure axis. The pressure value obtained is the threshold pressure. For example, in case of $\text{Depth} = 40 \mu\text{m}$, i.e., for a $100 \mu\text{m} \times 40 \mu\text{m}$ cross sectional channel, the horizontal line $A-B$ intersects with the plotted curve at point O . Then, through point O , a vertical line $C-D$ is drawn intersecting with the pressure axis (abscissa) at 14.7 Pa. Therefore, the threshold pressure for a

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channel with a $100\ \mu\text{m} \times 40\ \mu\text{m}$ cross-section is 14.7 Pa. Finally, the threshold pressure can be used to deduce a threshold channel height using the equation $\Delta P = \rho g \Delta h$, where Δh is the height difference between the buffer reservoirs. The values of threshold pressure, height difference and transport volume for various hydraulic diameters are summarized in Table 2.1.

Fig. 2.8 Threshold pressures for various cross sections.

Table 2.1 Threshold pressures, height difference and transport volume for various cross sections.

Depth (μm)	20	40	60	80	100
$P_{\text{threshold}}$ (Pa)	61.5	14.7	8.8	6.2	4.8
$\Delta h_{\text{threshold}}$ (mm)	6.15	1.47	0.88	0.62	0.48
$Q_{\text{threshold}}$ (μl)	38.6	9.2	5.5	3.9	3

As noted from Table 2.1, the threshold height difference decreases with channel size. This implies that it is easier for the backflow to occur in a big channel. Based on the value of $Q_{\text{threshold}}$, to transport a total volume of 3 μl sample from one reservoir to another, the depth D_h of the microchannel should not be larger than 100 μm .

A complicated fluidic network is needed to realize cell counting and sorting functions. The functioning of the sorting device involves the different units for transportation, focusing and switching of injected samples. Details of focusing and switching structures design will be discussed in Chapters 3 and 4.

2.3 Fabrication and Experiment

2.3.1 PDMS fabrication process

In this project, Polydimethylsiloxane (PDMS) is chosen to fabricate the microchannels. PDMS is an excellent material for the usage of biological samples in aqueous solutions due to a number of reasons. First, features on the micro scale can be reproduced with high fidelity in PDMS by replica molding. Second, it cures at low temperatures. Third, it is nontoxic; mammalian cells can be cultured directly onto it and devices made from this material can be implanted *in vivo* [16].

Since PDMS is optically transparent down to 230 nm, it is compatible with laser induced fluorescent detection method. In addition, as PDMS chips are fabricated by replica molding, micro-lens can be easily fabricated in PDMS. However, PDMS can be easily deformed when it is subjected to pressure or exposed to organic solution, therefore, it is better to avoid organic solutions or press the PDMS chips during either the

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fabrication or the experiment. Moreover, the optical properties of the PDMS material largely depend on the fabrication process, for example, if PDMS is cured at different temperatures, the stiffness of the material will differ, resulting in different refractivity. Therefore, when fabricating micro-lens in PDMS, the parameters are set fixed to keep the consistency.

2.3.1.1 Fabrication process

The fabrication of microfluidic devices is based primarily on the techniques of soft lithography, especially rapid prototyping and replica molding [15, 61]. Soft lithography is a suite of nonphotolithography methods of replicating a pattern. Routine access to clean room is not necessary for these methods. A general fabrication process is shown in Fig. 2.9. The following sections give the detailed steps of fabricating the PDMS microfluidic channel on a glass substrate.

Fig. 2.9 Schematic of PDMS fabrication process.

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Rapid prototyping begins by creating a design for a device in a computer-aided design (CAD) program. Designs of networks of microfluidic channels (diameters $>20\text{ }\mu\text{m}$) are created in a CAD program (Freehand 8.0). High-resolution dark-field transparencies are produced (Herkules PRO image setter) from the CAD files with the inked background. To fabricate the master for molding, SU-8 50 is spin-coated onto silicon substrate at 2000 rpm for 30 secs to create the thickness of photoresist to be $50\text{ }\mu\text{m}$ deep. After coating, the substrate is soft baked on a hot plate at 65°C for 6 mins and 95°C for 20 mins to evaporate the solvent and densify the film. Next, SU-8 is exposed to UV light for 40 secs using the high-resolution transparency as mask. Following exposure, the substrate is baked again at 65°C for 2 mins and 95°C for 6 mins. Then SU-8 is developed in SU-8 developer (MicroChem). Following development, the substrate is rinsed briefly with isopropyl alcohol (IPA), then dried with a gentle stream of nitrogen. After rinsing and drying, SU-8 is hard baked at 175°C on a hot plate to further cross link the material.

Once a master is fabricated, channels in PDMS can be formed by replica molding. Replica molding is simply the casting of prepolymer against a master and generating a negative replica of the master in PDMS. A 10:1 mixture of PDMS prepolymer and curing agent (Sylgard 184) is stirred thoroughly and then degassed under vacuum to remove bubbles. The prepolymer mixture is poured onto the master and cured for one hour at 65°C . After curing, the PDMS replica is peeled from the master. Reservoirs are created by punching PDMS with a 4 mm diameter puncher.

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Molding provides a PDMS replica that contains only three out of the four walls required for enclosed channels. Sealing the replica to a flat surface provides the fourth wall. The PDMS replica is rinsed in ethanol and dried under a stream of argon; a glass slide is cleaned in heptane and ethanol and then dried. The two substrates are placed in a reactive ion etching (RIE) machine (RIE-10 NR) and subjected to the oxygen plasma for 0.3 min. The plasma is generated from room air at ambient humidity at 75 mTorr and 100 W of radio frequency power. Within 30 secs after the removal from the plasma cleaner, the substrates are brought into conformal contact whereby an irreversible seal formed spontaneously. Thus, a complete microchannel structure has been fabricated. The fabrication process, which consists of rapid prototyping, replica molding and plasma bonding, takes approximately two hours. Unmodified PDMS presents a hydrophobic surface. Channels in hydrophobic PDMS are difficult to get wet with aqueous solutions, and prone to the adsorption of other hydrophobic species and easily nucleate bubbles. The exposure to plasma oxidation renders the surface hydrophilic because of the silanol groups. Aqueous solutions can easily make these oxidized channels wet. The oxidized surface of the PDMS is unstable in air and reverts to hydrophobic in approximately 30 mins, thus the microchannels must be filled with deionized (DI) water shortly (e.g. 3 mins) after bonding to protect the surface. The water-filled PDMS channels can remain hydrophilic for a long period of three months. As a result, chips do not have to be fabricated exactly before their usage. PDMS microfluidic chip is discarded after one usage to avoid cross-contamination. The plasma bonding fabrication process is discussed in detail in Section 2.3.1.2.

2.3.1.2 Results and discussions

i) SU-8 failure analysis

For the molding fabrication technique, the realization of a precise and long lasting master is vital for making high quality microchips. Here SU-8 was chosen to fabricate the master mold because it can provide high aspect ratio imaging with near vertical side walls, film thicknesses from 1 to 200 μm with single spin coat process and superb chemical and temperature resistance. SU-8 is most commonly processed with conventional near UV (350-400 nm) radiation. However, it is not an easy task to fabricate a flawless master without experiencing fabrication failures. The entire process should be optimized for each specific application. Fig. 2.10 shows some samples of fabrication failures.

To obtain maximum reliability, the substrates should be cleaned and dried prior to applying the SU-8 resist. If dust or dirty particles are left on the substrate, hillocks will appear after development as shown in Fig. 2.10(a). The recommended cleaning procedure is to start with a solvent cleaning, or a rinse with dilute acid, followed by a DI water rinse. Substrates should be subjected to a piranha etch / clean (H_2SO_4 & H_2O_2) wherever applicable. The surface should be dehydrated at 200°C for 5 mins on a contact hot plate.

Microstructure patterns are transferred from mask to SU-8 through photo lithography. To get a sharp image, the mask must be in good alignment with the

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substrate, otherwise, thick edges will occur due to the diffraction effect as presented in Fig. 2.10(b).

(a) Hillock due to dust or dirty particle left on the substrate.

(b) The wall is too thick due to poor alignment between mask and substrate during expose process.

(c) Optical proximity error, causing the nearby SU-8 to crosslink and cannot be developed later.

(d) Cracking at the corner because ramping of temperature was carried out too quickly, preventing the release of internal stress during PEB.

Fig. 2.10 Failure analysis of SU-8-based master fabrication process.

SU-8 is optimized for near UV (350-400 nm) exposure. It is virtually transparent and insensitive above 400 nm and is highly absorbent and reactive to light with wavelengths below 350 nm. The optimal exposure dose will depend on the film thickness (thicker films require higher dosage) and process parameters. If the exposure time is too short, catastrophic adhesion failure and excessive cracking is observed; whereas if the exposure time is too long, an excessive dose may result in optical proximity errors, causing the nearby SU-8 to crosslink as shown in Fig. 2.10(c).

Following exposure, a post exposure bake (PEB) must be performed to selectively cross-link the exposed portions of the film. Optimum cross-link density is realized through careful adjustments of the exposure and PEB process conditions. More attention must be given as SU-8 is readily cross-linked and can result in a highly stressed film. Fig. 2.10(d) shows that the cracks occurred at a corner during PEB because temperature ramped too fast, so that the internal stress could not be released. To minimize stress and resist cracking, a slow temperature ramp is recommended and rapid cooling after PEB should be avoided.

ii) Plasma treatment

The plasma treatment of PDMS provides two important functions. The first is to permanently bond the PDMS replica to a substrate. PDMS comprises repeating units of $-\text{O-Si}(\text{CH}_3)_2-$. Exposing a PDMS replica to an air plasma introduces polar groups on the surface. We believe the plasma introduces silanol groups (Si-OH) at the expense of methyl groups (Si-CH_3). These silanol groups then condense with appropriate groups

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(OH, COOH, ketone) on another surface when the two layers are brought into conformal contact. For PDMS and glass, this reaction yields Si-O-Si bonds after loss of a water. These covalent bonds form the basis of a tight and irreversible seal, so that an attempt to break the seal will usually result in bulk PDMS failure. The seal withstands pressures of 30-50 psi. A plasma bonded microchip and the enlarged view are shown in Fig. 2.11.

Fig. 2.11 Microfluidic cell sorting device. (a) Photograph of a PDMS replica permanently bonded to a glass substrate. (c) Zoom-in view of the structure of the cell sorter.

During the experiment, it was found that PDMS is not always bonded to glass perfectly. To determine how the plasma parameters affect the bonding process, parameters such as radio frequency (RF) power, oxygen partial pressure, and treatment time were varied. Table 2.2 compares the permanently bonded areas on 12 samples (experimental runs) that are examined under different conditions. The results show that low RF power and short treatment times are better than high RF power and long treatment times for bonding instantly after plasma treatments, while the oxygen partial pressure does not seem to have effect on the bonding area.

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Table 2.2 Comparison of permanent bonded areas under different bonding conditions.

Sample No.	Power (W)	Pressure (mtorr)	Time (sec)	Bonded Area (%)
1	75	75	18	100
2			40	60
3		120	18	100
4			40	90
5		180	18	100
6			40	90
7	150	75	18	100
8			40	60
9		120	18	100
10			40	70
11		180	18	100
12			40	30

The second function of the plasma treatment is to modify the surface chemistry of PDMS. Unmodified PDMS presents a hydrophobic surface. Microchannels in hydrophobic PDMS are difficult to wet with aqueous solutions, and prone to the adsorption of other hydrophobic species and easily nucleate bubbles. Exposure to plasma oxidation renders the surface hydrophilic because of the presence of the silanol groups. Aqueous solutions then easily wet these oxidized channels. Here, the fluid added to

PDMS channel after plasma treatment is DI water. The pH and the specific conductance of the DI water are 5.9 and 3.1 $\mu\text{S}/\text{cm}$. The measured contact angles of the PDMS surface before and after plasma treatment were 161° and 15°, respectively. In addition, the presence of silanol groups on the walls of the channels provides ionizable ($\text{SiOH} - \text{SiO}^- + \text{H}^+$) groups that, when in contact with neutral or basic solutions, support a stronger EOF towards the cathode that could be used for sample transportation. These negatively charged channels have greater resistance to adsorption of hydrophobic and negatively charged analytes than unmodified PDMS. It was noticed that the oxidized surface of PDMS is unstable in air and reverts to being hydrophobic in about 30 mins. This problem can be avoided by keeping the oxidized PDMS in contact with a polar liquid in order to protect the surface of the PDMS.

2.3.2 Microfluidic experiment

Experiments were carried out to study the velocity profiles and transport capabilities of straight microchannels with different dimensions. The structures used for the experiments consist of a straight 5 mm-long microchannel with two 4 mm-diameter reservoirs at each end. The height of the reservoir is the same as the thickness of the PDMS layer which is 1-1.5 mm. The reservoir surfaces are at the two equal elevations initially. The depths of the microchannels vary from 20 μm to 100 μm , respectively, while the widths of all microchannels are 100 μm . The electrodes are placed at the inlet and outlet of the channel and H_2O is used as buffer liquid. The properties of H_2O have been described in the simulation section. An external 300 V/cm electric field is applied

axially along the microchannels. 300 V/cm is quite safe for living cells. Based on Harrison's report [29], cells could keep alive until 600 V/cm. The electric field was generated by an external commercial power supply (HP, USA). At this stage, we use the bulky power supply and have no intention to integrate the power supply into the microchip. Recently, Li's group [56] developed a portable microfluidic device with a reusable integrated high voltage power supply, and the device is powered by an onboard MN21 cell battery (5 mm radius, 30 mm long). Their work suggests that a miniaturized high-voltage integrated power supply could be developed for the microfluidic applications.

2.3.2.1 Experiment setup

In this study a micro particle image velocimetry (PIV) system is developed to measure the EOF in microchannels. The μ -PIV enables spatially resolved measurements of instantaneous velocity fields [57-60]. The experimental set-up is shown in Fig. 2.12. 1- μ m fluorescent micro-spheres (Molecular Probes, Inc) that have an excitation peak at 490 nm and an emission peak at 515 nm are used as seedings. The concentration of the fluorescent micro-spheres based on the interrogation volume is 7.26×10^8 particles/ml, or 0.02% in volume fraction. A micro strobe illumination system with the duration of the pulse of 250 msec is used to illuminate the seeding particles. Blue light beam passes through a beam expander to enlarge the beam diameter, and then illuminates the test section of the microchannel. A 10 $\times$ objective lens is used for magnification of the images, and the field-of-view of our experiment is 1 mm $\times$ 0.75 mm. The fluorescence signal is

recorded with an interline transfer CCD monochrome camera (Hisense MKII, Strobe double frame). The linearity of the camera response to light intensity was checked by flooding the device with different concentrations of fluorescent micro-spheres.

8 images per second are captured using the CCD monochrome camera with 640×480 pixels video format. The PIV-measurements are evaluated with standard cross-correlation methods. Each image frame is interrogated in 32×32 pixels sub images with an overlap of 50 % corresponding to intervals of 16×16 pixels. Ensemble averaging of 32 images consecutively captured for 4 sec is used to obtain the velocity measurements.

Fig. 2.12 Experimental set-up of a μ -PIV system.

The concentration of fluorescent micro-spheres based on the interrogation volume was 7.26×10^8 particles/ml, or 0.02% in volume fraction. The electrophoretic velocity of 1 μm fluorescent polystyrene particles, with a high density of carboxylic acids on their surface, is a function of the electrostatic force on the surface charge, the zeta potential

due to their charged double layers and the viscous drag. Using the permittivity of the polystyrene particles and the electric field solution, the maximum electrophoretic force due to the applied electric field (300 V/cm) was computed to be of the order of 4×10^{-15} N for the straight microchannel. A Stokes viscous drag would correspond to an electrophoretic velocity (U_{eph}) of 0.49 $\mu\text{m/s}$ so that electrophoresis is unlikely to influence our measurements.

Some elements, namely image depth of field and Brownian motion, may affect the accuracy of a micro PIV system [66]. The effects of these elements should be minimized. In the $\mu\text{-PIV}$, the image plane should be defined by the depth-of-field of the microscope objective lens. The micro PIV image plane thickness is much smaller than conventional PIV image planes that are defined by a thin laser sheet because microscope objectives usually have very thin depths-of-field. Considering diffraction and geometric affects, Inoue and Spring reported the formulation for depth-of-field as [66]:

$$\delta z = \frac{n\lambda}{NA^2} + \frac{nd_r}{MNA} \quad (2.11)$$

where n is the index of refraction of the immersion medium between the microfluidic device and the objective lens ($n = 1$ for an air), the wavelength of illuminating light $\lambda = 488$ nm, the numerical aperture $NA = 0.25$, the magnification is $M = 10$, and the CCD pixel size is $d_r = 9.0$ μm . Thus, the depth-of-field of the present micro PIV system is estimated to be $\delta z = 11.4$ μm . Since this depth-of-field is far less than the fabricated channel depth of 50 μm , we were trying to focus the light to the center plane of the microchannel by first applying a pressure driven flow. Because the flow profile of such a pressure driven flow is parabolic, the fluid in the center plane of the channel has the

maximum velocity, which makes it easy for the user to find the center plane by looking at the value of velocity. An in-depth analysis and rigorous discussions are reported for the contribution of out-of-focus particle images to the PIV correlation function.

The thermal Brownian diffusive motion may not be negligible when sub-micron particles are used for seeding since the particle diffusivity rapidly increases with decreasing particle diameter. The Brownian motion error, $\varepsilon_B = u_B / u$, where u is the characteristic velocity, is considered as having the same effect in all directions statistically. If one assumes that each particle has equal influence on the average velocity vector in the interrogation window, ε_B can be reduced by averaging the number of individual particle images within the interrogation window, A , and ensemble averaging over the number of realizations, B . The Brownian motion error for PIV recording [63] is expressed as

$$\varepsilon_B = \frac{u_B}{u} \cdot \frac{1}{\sqrt{A \times B}} \quad (2.12)$$

For example, for a characteristic velocity of 100 $\mu\text{m/s}$ from averaging of 20 image and for five pairs of particle images within a single interrogation window, the Brownian motion error is estimated to be as low as 0.6 %.

2.3.2.2 Results and discussion

Immediately after the electric field is applied, the fluid moves. At the beginning, the back flow is negligible because the liquids in the two reservoirs are at the same level. Thus, the velocity profile observed 1 sec after application of the electric field can be

considered as purely EOF. The results of the PIV vector analysis show that for purely EOF, the velocity is almost uniform in the center flow region and drops sharply near the microchannel wall. Thus the velocity in the center flow region is taken as the electroosmotic velocity V_{eof} . Fig. 2.13 plots V_{eof} under different electric fields for microchannels with channel depths of 70 μm , 80 μm and 100 μm . The results show that the electroosmotic velocity is independent of the channel size and linearly proportional to the applied electric field. This is consistent with the simulation results previously shown in Fig. 2.14.

Fig. 2.13 Experimental results for the dependence of the electroosmotic velocity on channel depth, with the intensity of the applied electric field as parameter.

Fig. 2.14 Comparison of theoretical and experimental results of electroosmotic velocity vs. applied electric field strengths.

As time goes on, the velocity profile becomes more and more concave. Fig. 2.15 shows how the velocity profile measured across the channel width varies in time for a 100 μm -wide, 50 μm -deep microchannel at an applied electric field of 300 V/cm. The profiles were taken at $t = 1$ sec, $t = 20$ sec and $t = 40$ sec respectively. Electroosmotic flow has a plug-like velocity profile initially. Due to frictional drag, the electroosmotic flow at the wall is slightly slower than the flow through the rest of the microchannel. In the fully developed electroosmotic flow, the average velocity has a value of ~ 695 $\mu\text{m/s}$. The velocity profile after 20 sec represented a superposition of the plug-like electroosmotic profile and the Poiseuille profile due to the adverse pressure gradient, which is a result from the downstream reservoir height rise with flow. By inducing

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adverse pressure gradients into the microchannel, the electroosmotic flow cannot maintain the plug-like profile and the center velocity is reduced to 559 $\mu\text{m/s}$. The velocity after 40 sec is much slower compared to the fully developed electroosmotic flow and a center fluid velocity of 418 $\mu\text{m/s}$ is observed.

Fig. 2.15 Experimental results of velocity profile with time for a microchannel with a channel depth of 50 μm .

The instantaneous flow rates at different instances are plotted for straight microchannels with depths of 26 μm and 50 μm as shown in Fig. 2.16. It shows that the 50- μm channel has higher initial flow rate. This is due to its bigger cross sectional area. However, the instantaneous flow rate of the 50- μm channel decreases faster than that of the 26- μm channel because the backward pressure has larger effect in the microchannels with bigger hydraulic diameters. Thus, fluid transport in 26- μm channel is slower but can last longer.

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Fig. 2.16 Experimental results of the instantaneous flow rate for straight microchannels with depths of $26\ \mu\text{m}$ and $50\ \mu\text{m}$ at $300\ \text{V/cm}$.

Fig. 2.17 Experimental results of the total volume transported in straight microchannels with depths of $26\ \mu\text{m}$ and $50\ \mu\text{m}$ at $300\ \text{V/cm}$.

Figure 2.17 plots the total transported sample volumes calculated by integrating the instantaneous flow rate in time. It can be seen that the 50 μm microchannel managed to transport a total volume of 0.305 μl and the back flow occurs 240 secs after starting the transportation. In contrast, the 26 μm microchannel transports 0.33 μl within 425 secs, which is much slower compared to the 50 μm microchannel, but back-flow occurs much later. This observation confirms that the occurrence of the back-flow is due to the adverse pressure gradients and is much more significant for larger microchannels. Thus, there is a trade-off between the flow rate and the stability against the adverse pressure-driven flow.

The experiments proved the linear dependence of electroosmotic velocity on an imposed hydrostatic counter pressure as discussed above, however, a big discrepancy is observed between the simulated and experimental results of total volume transported. The maximum volume of fluid in each reservoir is about 12 μL . However, at the end of the experiment, the inlet reservoir was empty even though the highest total volume transported was only 0.33 μL . We suspect that the fluid loss was due to evaporation and electrolysis. Evaporation (the change of liquid into vapors at temperatures below the liquid's boiling point) occurs as the molecules near the liquid surface escape from the liquid into the atmosphere. Evaporation is faster at higher temperature, lower pressure and with forced convection. Electrolysis is the electrochemical process in which an electrolyte is decomposed by a current passing through it. In our case, the current passing through water decomposed water to hydrogen gas at the cathode and oxygen gas at the anode. These two elements may lead to 90% loss of the fluid. Evaporation can be

decreased by putting cover lids on reservoirs and electrolysis can be reduced by applying smaller electric fields or using a buffer with a lower conductivity.

2.4 Summary

In conclusion, based on the performance specifications of the cell sorter, a novel microfluidic cell sorting chip which includes a microfluidic system for cell manipulation and an optical system for cell detection is proposed. EOF is exploited to transport sample and buffers. Electrokinetic focusing and switching structures are designed to confine sample stream and select targeted cells. To avoid adverse pressure effect in microchannel, the depth of the channel should not exceed 100 μm . The optical system comprises of a laser, a micro lens array and a photodetector which is capable to provide 488 nm excitation wavelength and detect 515 nm emission wavelength. The fabrication of the cell sorting system employs the rapid-prototyping “photolithography” technique using PDMS. Since PDMS surfaces are inherently hydrophobic, oxygen plasma bonding is exploited. The channel is terminated by reservoirs at which electrodes are introduced to generate the electroosmotic flow. Experiments are performed in straight microchannels made of PDMS with channel depths varying from 20 μm to 100 μm . The micro PIV system is used to measure the EOF velocity in microchannels and the results from the experiment are within expectation. Instantaneous flow rate and total transported volume for $100 \times 26 \mu\text{m}^2$ and $100 \times 50 \mu\text{m}^2$ straight channels are plotted. It is observed that fluid transport in 50 μm channel is faster but time lasted is shorter. For both channels the flow velocity decreases with time and back flow eventually occurs but back flow effect is

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larger for bigger microchannels. Therefore, there is always a trade-off between flow rate and stability against adverse pressure-driven flow.

It can be seen from the experimental results that the pressure effect can be significantly minimized by reducing the cross-section size of the microchannel, but the volume flow rate is sacrificed in this method, which ends up with smaller throughput. There are different methods to reduce the pressure effect, such as replacing the punched reservoirs by peripheral bottles or implementing special structures such as Tesla valve, which can also be used to overcome the problem of back flow.

CHAPTER 3 THE ELECTROKINETIC FOCUSING EFFECT AND MICROCHANNEL DESIGN

The objective of this chapter is to optimize the design of the microfluidic focusing structure. At the beginning of this chapter, an equivalent circuit model is built up for the Electrokinetic focusing structure. Then different structures are simulated by varying two important parameters, applied voltage ratio and channel width ratio. Finally the simulation results are verified by experimental results.

3.1 The Electrokinetic focusing Effect

3.1.1 Function of electrokinetic focusing

Electrokinetic focusing uses electrokinetic transport to spatially confine a sample [67]. Fig. 3.1 shows the schematic of focusing effect. Arrows depict the direction of fluid transport. The sample is injected from the top of the vertical channel in a continuous fashion. Fluid from the two side channels, focusing 1 and 2, confine the sample stream. The side flow squeezes or electrokinetically focuses the inlet flow into a thin stream that exits the intersection sheathed in buffer fluid. The focusing width can be controlled by adjusting the relative voltages driving the side and the inlet flows [68-71].

Fig. 3.1 Schematic of the focusing effect.

Basically, the focusing structure serves two functions. First, this design guarantees high detection efficiency in the sorting device by laterally confining the sample solution to a cross section that can be largely covered by the tiny detection

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volume [38]. At the intersection, the sample in the main channel is confined electrokinetically to a narrow stream. This enables a single cell to be placed and to flow along the center line of the microchannel, as shown in Fig. 3.1. By this technique, the cells reach the detection point successively in line, like beads on a chain, and a complete particle counting and analysis is guaranteed. Thus, electrokinetic focusing eliminates the need for extremely narrow channels that are difficult to handle and suffer from frequent clogging. Additionally, in this design, more sensitive measurements can also be made.

Second, the electrokinetic focusing structure is capable of separating two or more attached cells by introducing different velocities at the intersection. A cell sorting system is able to count and detect cells or particles only if they travel past the sensing point in a single cell suspension. But in practice, cells may attach together due to Van der Waals force, electrostatic attraction or chemical binding. Fig. 3.2 presents the way in which two attached cells can be separated in the focusing structure. At the beginning, cell *a* (in black) and cell *b* (in white) are traveling at the same speed because the EOF profile is flat. In Fig. 3.2 (b), the velocity profile around the intersection varies very sharply due to the squeeze of sample solution by two side flows. Since the flow is still laminar, two interfaces are formed between inlet and side flows. As cell *a* and cell *b* travel down to the intersection, they are subjected to different velocities due to their different radial positions, resulting in a shear force between them. Next, if this shear force is sufficient to overcome the bonding force, cell *a* and cell *b* are going to be separated as shown in Fig. 3.2 (c). After they pass through the intersection area, cells will reach a steady velocity

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again which is much faster than the velocity before the intersection. The distance between the two cells becomes bigger which allows more sensitive measurement.

Fig. 3.2 Schematic diagram illustrating the separation of two attached cells using an electrokinetic focusing structure.

3.1.2 Equivalent circuit model

Many aspects of hydrodynamic focusing are described by a simple circuit model [72]. Similarly, we postulate that electrokinetic focusing can also be modeled with a simple electrical circuit. The fluid flow can be mapped to a network of resistors under two conditions. First, the flow must be laminar. Second, the width of the intersection must be negligibly small compared to the length of the channels. Both of these conditions are satisfied in the devices described in this report. The volume flow rate maps to the electric current. The relationship between fluid flux and electric current is an electrokinetic analog to Ohm's law from which the appropriate resistance can be calculated from the channel geometry as shown in Fig. 3.3.

The inlet branch of the circuit is driven by voltage V_i , the sides by αV_i and the voltage at the intersection of four channels is represented as V_x . The outlet branch terminates at ground. The resistances of the side, inlet and outlet arms of the circuit are R , γR , and σR , respectively, with the parameters γ and σ reflecting differences in channel geometry. For simplicity, in this study, we assume $\sigma = \gamma$, i.e., the inlet and the outlet channels have same size. The flow velocities at side, inlet and outlet branches are v_s , v_i , v_o , respectively. W_f is the width of the focused inlet stream and W_s is the width of the squeezed focusing stream. The following Table 3.1 gives some important definition for the model.

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(a)

(b)

Fig. 3.3 Equivalent circuit model. (a) Electrokinetic focusing switching. (b) Equivalent electric circuit.

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Table 3.1 The parameters mapping between microchannel and its circuit model.

Microchannel		Circuit Model	
<i>Parameter</i>	<i>Description</i>	<i>Parameter</i>	<i>Description</i>
$W_i = W/\gamma$	Width of inlet channel	$R_i = \gamma R$	Resistance of inlet channel
$W_o = W/\sigma$	Width of outlet channel	$R_o = \sigma R$	Resistance of outlet channel
$W_F = W$	Width of side channel	$R_F = R$	Resistance of side channel
$W_i \times v_i = W_f \times v_o$	Flow rate of inlet channel	I_i	Current of inlet branch
$W_F \times v_s = W_s \times v_o$	Flow rate of side channel	I_s	Current of side branch
$W_o \times v_o$	Flow rate of outlet channel	I_o	Current of outlet branch

The *Focusing Ratio* (β) is defined as the ratio of the focused inlet stream width to the outlet channel width, which can be expressed as

$$\beta = \frac{W_f}{W_o} = \frac{I_i}{I_o} \quad (3.1)$$

β shows the focusing ability of the system: the greater the value, the thinner the middle stream in outlet channel.

The *Applied Voltage Ratio* (α) is defined as the ratio of voltage applied at the side channel to the voltage applied at the inlet channel, given by

$$\alpha = \frac{V_{side}}{V_{inlet}} \quad (3.2)$$

For the same applied inlet voltage, the smaller the value of α , the lower the electrical power is needed, which in turn minimizes the Joule heating.

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The *Channel Width Ratio* (γ) is the ratio of the width of the side channel to the width of the inlet channel. This is one of the parameters that was varied in the simulations, and it will be shown later. γ is defined as

$$\gamma = \frac{W_{side}}{W_{inlet}} = \frac{W_{side}}{W_{outlet}} \quad (3.3)$$

The *Sample field strength* is defined as the electric field strength in the sample channel, and correspondingly, the *focusing field strength* is the electric field strength in the focusing channels.

The focusing region, over which the width of the inlet flow varies rapidly, extends about one channel width from the inlet opening. Once focused, the width of the stream remains constant unless it is broadened by diffusion into the side flow or there is an increase in the width of the channel. The width of the focused stream does not depend on the magnitude of the applied voltages, but rather on the ratio of the side voltage (V_s) to the inlet voltage (V_i): $\alpha = V_s / V_i$. Focusing is observed for different α . The focused stream narrows as α increases.

To achieve a certain focusing ratio β , it is necessary to find the best combination of channel width ratio γ and applied voltage ratio α . Several equations are generated based the circuit model. According to Kirchoff's current law, the output current is expressed as

$$I_o = I_i + 2I_s \quad (3.4)$$

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Eq. (3.4) can be rewritten as

$$\frac{V_x}{\sigma R} = \frac{V_i - V_x}{\gamma R} + \frac{2(\alpha V_i - V_x)}{R} \quad (3.5)$$

From Eq. (3.5), relationship between V_x and V_i can be derived as

$$V_x = \frac{\sigma + 2\gamma\alpha\sigma}{\gamma + \sigma + 2\gamma\sigma} \cdot V_i \quad (3.6)$$

Substituting Eqs. (3.1) and (3.6) into Eqs. (3.5), the applied voltage ratio can be expressed as

$$\alpha = \frac{1 + 2\sigma - \beta}{2\gamma\beta + 2\sigma} \quad (3.7)$$

and focusing ratio can be written as

$$\beta = \frac{W_f}{W_c} = \frac{(1 + 2\sigma - 2\sigma\alpha)}{(1 + 2\gamma\alpha)} \quad (3.8)$$

where W_c is the width of the outlet channel. The focusing ratio W_f/W_c at a given value of α is the key performance indicator for focusing structure, and the circuit model of the fluid flow outlines a straightforward means of improving it. Eq. (3.8) indicates that tighter focusing can be achieved by increasing the channel width ratio γ and the applied voltage ratio α . The equations also imply that the applied voltage ratio is determined by both the focusing ratio and the channel width ratio. Therefore, for a given focusing width, one can find out the suitable value of channel width ratio γ to get the smallest possible applied voltage ratio α .

3.2 Design and Simulation of Focusing Structures

3.2.1 Structure of focusing channel

The design of an electrokinetic focusing structure highly depends on its application. In this project, the cell sorting chip is mainly used for blood cell counting and sorting, especially for leukocytes. Generally, the size of leukocytes is within the range of 11 μm to 13 μm . To ensure effective focusing, we want to design a focusing structure with which a 10 μm focusing width can be achieved while the power consumption is as small as possible.

With this objective, a cross-shaped channel structure is designed, as shown in Fig. 3.4, closed by walls from above and below. The process considered is electroosmotic driven flow from inlet I to outlet O. Transport from the two side channels, F1 and F2, confines the sample stream. The side flow electrokinetically focuses the inlet flow into a thin stream that exits the intersection sheathed in buffer fluid. The focusing width is mainly controlled by adjusting the two parameters, *applied voltage ratio* (α) and *channel width ratio* (γ), which are the major considerations during design process. Widths of the inlet and outlet channels are fixed at 50 μm , while width of the focus channels is designed to have two different values. For design A, the width of the focusing channel is 50 μm , i.e., $\gamma = 1$; for design B, the width is 100 μm , or $\gamma = 2$. The lengths of all the channels are designed to be 5 mm.

Fig. 3.4 Design of focusing structure: Design A with $\gamma = 1$ and Design B with $\gamma = 2$.

Three main objectives have been set while running the simulation of the focusing structure:

- 1) To test and verify the circuit model as proposed in Section 3.1.2 as this model has only been proven to be true for hydrodynamic focusing.
- 2) To utilize the fluid dynamics simulation so that the optimized geometry can be obtained in the design of the focusing structure based on the circuit model.
- 3) To investigate the performance limitations of the designed structure.

The simulation models the designed two-dimensional cross channel geometry shown in Fig. 3.4. The focusing width is mainly controlled by adjusting the values of *applied*

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voltage ratio (α) and *channel width ratio* (γ). In the simulation, to investigate the effect of applied voltage ratio α , the electric potential at the inlet is fixed at 100 V, whereas the electric potentials at two ends of focusing channels are varied from 83 V, 100 V to 120 V, which is corresponding to $\alpha = 0.83, 1$ or 1.2 respectively. In order to study the effect of channel width ratio γ , the width of inlet and outlet channels are fixed at 50 μm , while the width of the two focusing channels have two values 50 μm or 100 μm , i.e., $\gamma = 1$ or 2 . Steady state streamlines, electrostatic fields and fluid flow velocities at the intersection are examined in details.

The buffer solution used in simulation is H_2O with density of 1000 kg/m^3 , kinematic viscosity is $10^{-6} \text{ m}^2/\text{s}$ and relative permittivity is 78.5. These values give rise to a Reynolds number of order 1. A Debye thickness of $1 \times 10^{-9} \text{ m}$ and a zeta potential of -0.0295 V are applied to all the walls. The values are kept constant during the entire simulation.

3.2.2 The effect of the voltage ratio

The effect of the applied voltage ratio α is examined by simulating the two designed structures at different focusing voltages. For Design A shown in Fig. 3.4, the inlet, the outlet and the focusing channels have equal width of 50 μm , i.e., $\gamma = 1$. The operation of the two-dimensional focusing is shown in Figs. 3.5 to 3.7. For these figures, the potential at the inlet (I) is held constant while the voltages at the two ends of the

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focusing channels ($F1$ & $F2$) are allowed to vary from 83 V to 100 V to 120 V, i.e., $\alpha = 0.83, 1$ or 1.2 . The simulation results are summarized in Table 3.2.

Table 3.2 Summary of simulated results for $\gamma = 1$.

Applied Voltage Ratio α	Sample Field Strength (V/cm)	Focusing Field Strength (V/cm)	Outlet Field Strength (V/cm)	Sample Velocity ($\mu\text{m/s}$)	Focusing Velocity ($\mu\text{m/s}$)	Outlet Velocity ($\mu\text{m/s}$)	Focused Stream Width (μm)	Focusing Ratio $1/\beta$
0.83	70	36	142	161.7	83.16	326.6	25	2
1	50	50	150	115	115	345	16.7	3
1.2	30	70	170	69.3	161.7	392.7	8.8	5.7

In Fig. 3.5, the sample stream widths are plotted for three different sets of potentials applied to the two ends of focusing channels. The light grey presents the sample stream and the black presents the focusing stream. The circuit model is verified by these simulation results. The relationship between the focusing ratio β and the applied voltage ratio α is derived in Section 3.1.2. From Eq. (3.8), for $\alpha = 0.83$ and $\sigma = \gamma = 1$, $\beta = 1/2$; for $\alpha = 1$ and $\sigma = \gamma = 1$, $\beta = 1/3$; for $\alpha = 1.2$ and $\sigma = \gamma = 1$, $\beta = 1/5.7$. The calculated values show excellent agreement with the simulated values, which means that the circuit model can accurately describe the electrokinetic dynamics in microchannels.

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Fig. 3.5 Simulation of streamlines when $\gamma = 1$: (a) $\alpha = 0.83$, $W_f = 25 \mu\text{m}$, $\beta = 1/2$; (b) $\alpha = 1$, $W_f = 16.7 \mu\text{m}$, $\beta = 1/3$; (c) $\alpha = 1.2$, $W_f = 8.8 \mu\text{m}$, $\beta = 1/5.7$.

Electric potential distributions along the microchannels are shown in Fig. 3.6. The black lines inside the microchannels are isoelectric curves, i.e., the voltage between each two adjacent isoelectric lines is the same. Thus, the density of isoelectric lines presents the rate of change of electric potential, i.e., the electric field strength. The values of sample field strengths and focusing field strengths are labeled accordingly. As the potential at the focusing reservoirs increases, the sample field strength decreases, and the focusing field strength increases resulting in a tighter focus of the sample stream. Essentially, as the field strength in the focusing channels increases, the degree of focusing increases.

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Fig. 3.6 Simulation of electric potential along microchannels when $\gamma = 1$: (a) $\alpha = 0.83$, (b) $\alpha = 1$, (c) $\alpha = 1.2$.

Figure 3.7 presents the flow velocities when three different sets of voltages are applied at the ends of two focusing channels. The velocities at both focusing channels

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and at the outlet channel increase with the potential, however, the velocity at the inlet channel decreases when the potential increases. Compared to the values of electric field strengths, the flow velocities are found to be directly proportional to the electric field strengths. Additionally, from Fig. 3.7 it is observed that the focusing ratio equals the inverse of the velocity ratio. This can be explained by using the law of mass conservation. Since inflow must be equal to outflow, i.e., $W_i \times v_i = W_f \times v_o$, it is obvious that $W_f / W_c = v_i / v_o$. This conclusion is a fundamental principle that can be used to analyze the experimental results later. We will discuss this in detail in Section 3.3.

Fig. 3.7 Simulation of flow velocities when $\gamma = 1$: (a) $\alpha = 0.83$, (b) $\alpha = 1$, (c) $\alpha = 1.2$.

For Design B, the sample channel has a width of 50 μm and the focusing channels have equal width of 100 μm , i.e., $\gamma = 2$. Similar to Design A, the potential at the inlet (I) reservoir is held constant with the voltages at the two focusing reservoirs (F1 & F2) vary

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from 83 V to 100 V to 120 V, i.e., $\alpha = 0.83$ or 1 or 1.2. The simulation results are summarized in Table 3.3.

Table 3.3 Summary of simulated results for $\gamma = 2$.

Applied Voltage Ratio α	Sample Field Strength (V/cm)	Focusing Field Strength (V/cm)	Outlet Field Strength (V/cm)	Sample Velocity ($\mu\text{m/s}$)	Focusing Velocity ($\mu\text{m/s}$)	Outlet Velocity ($\mu\text{m/s}$)	Focused Stream Width (μm)	Focusing Ratio $1/\beta$
0.83	56	22	144	129.4	50.8	332.6	20	2.57
1	32.9	32.9	164.5	76	76	380	10	5
1.2	6.2	46.2	193	14.3	106.7	445.8	1.72	29

In Fig. 3.8, the sample stream is plotted for three different sets of the potentials applied to the focusing reservoirs 1 and 2. The light grey color presents the sample stream and the black color presents focusing stream. From Figs. 3.8 (a) – (c) it can be seen that after the intersection, the width of the focused sample stream becomes smaller, meaning that a better focusing effect is achieved with increasing the applied focusing voltage. The circuit model can be modified again by these simulation results. From Eq. (3.8), for $\alpha = 0.83$ and $\sigma = \gamma = 2$, $\beta = 1/2.57$; for $\alpha = 1$ and $\sigma = \gamma = 2$, $\beta = 1/5$; for $\alpha = 1.2$ and $\sigma = \gamma = 2$, $\beta = 1/29$. The calculated values agree well with the simulated values, which means that the circuit model can be used to describe the electrokinetic dynamics in the microchannels.

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Fig. 3.8 Simulation of streamlines when $\gamma = 2$: (a) $\alpha = 0.83$, $W_f = 20 \mu\text{m}$, $\beta = 1/2.57$; (b) $\alpha = 1$, $W_f = 10 \mu\text{m}$, $\beta = 1/5$; (c) $\alpha = 1.2$, $W_f = 1.72 \mu\text{m}$, $\beta = 1/29$.

In Fig. 3.9, the potential distributions along the microchannels are shown. The sample field strengths and focusing field strengths are labeled accordingly. Similarly, as the potential at the focusing reservoirs increase, the sample field strength decreases, and the focusing field strength increases resulting in a tighter focus of the sample stream.

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Fig. 3.9 Simulation of electric potential along microchannels when $\gamma = 2$: (a) $\alpha = 0.83$, (b) $\alpha = 1$, (c) $\alpha = 1.2$.

Fig. 3.10 presents the flow velocities of the microfluidics when three different sets of voltages are applied at two ends of focusing channels. With the increase in potential, the velocity at the inlet channel decreases, while the velocities at the focusing channels increase. It can be seen from Table 3.3 that the flow velocities are directly proportional to the electric field strengths.

Fig. 3.10 Simulation of flow velocities when $\gamma = 2$: (a) $\alpha = 0.83$, (b) $\alpha = 1$, (c) $\alpha = 1.2$.

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As discussed above, increasing the applied voltage ratio results in a tighter focus for both structures, but there are upper and lower limits for the possible values of the applied voltage ratio. Actually, focusing can only be observed over a range of values for the applied focus ratio α . Below a lower limit α_{min} , the electric potential at the intersection exceeds the potential applied to the focusing reservoir and fluid from the inlet channel flows out laterally, along the side channels. Above an upper limit α_{max} , the electric potential at the intersection exceeds the potential applied to the sample reservoir and the focused stream is pinched off and fluid from the side channels flows out at the inlet channel. Both α_{min} and α_{max} are independent of the overall potential scale, but are dependent upon the details of channel geometry. The limiting values are functions of the resistance in each circuit arm, which are defined as:

$$\alpha_{min} = \frac{\sigma}{(\sigma + \gamma)} \quad (3.9)$$

$$\alpha_{max} = \frac{(1 + 2\sigma)}{2\sigma} \quad (3.10)$$

For Design A, $\alpha_{min} = 1/2$ and $\alpha_{max} = 3/2$, which are corresponding to $\beta_{min} = 1$ and $\beta_{max} = \text{infinity}$. Fig. 3.11 (a) simulates the case when $\alpha = 0.4 < \alpha_{min}$, and sample leaks to focusing channel. Fig. 3.11 (b) shows the case when $\alpha = 1.6 > \alpha_{max}$, i.e., sample transport through the focusing chamber stops and the direction of transport in the sample channel reverses. For Design B, the limit of applied voltage ratio is expressed by $\alpha_{min} = 1/2$ and $\alpha_{max} = 5/4$, which are corresponding to $\beta_{min} = 1$ and $\beta_{max} = \infty$.

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Fig. 3.11 Simulation results of velocity field in microchannels when $\gamma = 1$: (a) $\alpha = 0.4$, (b) $\alpha = 1.6$.

3.2.3 The effect of channel width ratio

The two designed structures with different applied focusing voltages have been simulated in the last section. To study the effect of the channel width ratio γ on the stream width, the field strength and the flow velocity, a comparison between the performances of the two structures with the same applied voltage ratio is made.

Fig. 3.12 Comparison of focusing ratio vs. applied voltage ratio for $\gamma = 1$ and $\gamma = 2$.

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Figure 3.12 compares the focusing ratio at three different applied focusing voltages for both structures. It can be concluded that when applying the same focusing voltage, Design B can achieve better focusing effect with a narrower focused width than Design A. The difference is more distinct with the increase in the applied voltage. As shown in Table 3.3, the focusing field strength increases steadily with voltages at the focusing channel, while the inlet field strength drops dramatically from 56 V/cm to 6.2 V/cm. This indicates that the inlet field strength in Design B is more sensitive to the change in the applied voltage than in Design A.

Comparing Figs. 3.7 and 3.10, it can be noticed that the velocity vectors in Design B appear to be more chaotic at the intersection area and it takes longer distance to reach steady state than in Design A. This is due to the bigger width difference between the inlet channel and the focusing channel for Design B. Hence, the sample stream is subjected to a larger force from the focusing stream which is favorable in this case because there is a higher chance for attached cells to be separated.

From the above discussions, it is found that for the same applied voltage ratio, the performance is better for the focusing structure with a larger channel width ratio γ . This also implies that, in order to achieve the same focusing ratio, the design with larger channel width ratio requires less voltage at the two focusing reservoirs provided that the same voltage is applied at the inlet. Therefore, it is concluded that, in order to obtain the same focusing effect, Design B is more desirable since less applied voltage means less Joule heating and less power consumption.

3.3 Experimental Results and Discussions

To test the reliability of the simulation and to ensure that the circuit model can be implemented in practice, experiments were carried out to qualitatively reconfirm the focusing effect. Two PDMS microchips with the same dimensions used for simulation were fabricated by soft lithography. The potential at the inlet (I) was held constant at 100 V and the voltages applied at the two focusing channels (F1 & F2) are allowed to vary from 83 V to 100 V to 120 V, i.e., $\alpha = 0.83$ or 1 or 1.2. The buffer used for the experiment is H₂O. The properties of H₂O have been described in the simulation section. Flow velocities are measured by micro PIV system as described in Chapter 2.

3.3.1 Effect of voltage ratio

The effect of the applied voltage ratio α is verified by measuring flow velocities for both structures at three different focusing voltages. Fig. 3.13 presents the measured flow velocities of the microfluidics when three different sets of voltages are applied at two focusing reservoirs for design A. For demonstration purpose, Fig. 3.13 (d) shows the focusing effect in a CCD image taken by the microPIV under the condition of Fig. 3.13 (c). With the increase in potential at the focusing reservoirs, the velocity at sample channel decreases, while the velocities at both focusing channel and outgoing channels increase. Due to the fact that the micro PIV system can only give us velocities inside microchannels, it is relatively hard to measure the focused sample width directly. Since the focusing ratio is equal to the inverse value of the velocity ratio, the focusing ratio is calculated according to the previously introduced equation: $W_f / W_c = v_i / v_o$. This is regarded as the fundamental tool with which one can compare the experimental results

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with the simulated ones. The values of the focusing ratio β for these three conditions are 1/2.1, 1/3.4 and 1/5.8, respectively.

Fig. 3.13 Experimental results of flow velocities: (a) $\alpha = 0.83$, $W_f = 25 \mu\text{m}$, $\beta = 1/2$; (b) $\alpha = 1$, $W_f = 16.7 \mu\text{m}$, $\beta = 1/3.4$; (c) $\alpha = 1.2$, $W_f = 10 \mu\text{m}$, $\beta = 1/5.8$; (d) CCD image showing focusing effect under the condition of case (c).

Figure 3.14 presents the measured flow velocities of the microfluidics when three different sets of voltages are applied at the two ends of focusing channels for Design B. As before, with the increase in the potential at the focusing reservoirs, the velocity at the

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inlet channel decreases, while the velocities at the focusing channels increase. In Figs. 3.14 (a) – (c), the velocities at the sample channels are 110.4 $\mu\text{m/s}$, 64.7 $\mu\text{m/s}$ and 14.3 $\mu\text{m/s}$ respectively, and the velocities at the outlet channel are 332.5 $\mu\text{m/s}$, 383 $\mu\text{m/s}$ and 446.6 $\mu\text{m/s}$ respectively. The velocities agree well with the previous simulation results listed in Table 3.2 and also more chaotic patterns are observed in the intersection area. The values of the focusing ratio β for these three conditions are 1/3, 1/5.9 and 1/31.3, respectively. The micro PIV system exhibits excellent fidelity in increasing the microfluidics flow in the microchannels, and it can precisely measure the velocity, as low as 14 $\mu\text{m/s}$. As discussed above, the increase in the applied voltage ratio results in a tighter focus for both structures.

(a)

(b)

Fig. 3.14 Experimental results of flow velocities: (a) $\alpha = 0.83$, $W_f = 25 \mu\text{m}$, $\beta = 1/3$; (b) $\alpha = 1$, $W_f = 8.5 \mu\text{m}$, $\beta = 1/5.9$; (c) $\alpha = 1.2$, $W_f = 1.6 \mu\text{m}$, $\beta = 1/31.3$.

3.3.2 Effect of channel width ratio

To study the effect of the channel width ratio γ on the stream width, field strength and flow velocity, the performances of the two structures were compared. The simulation and experimental results of focus ratio are plotted with respect of the applied voltage ratio α in Fig. 3.15 for Design A, and Fig. 3.16 plots simulated and experimental results of focus ratio as a function of the applied voltage ratio for Design B. It can be seen that the experimental and simulated results are comparable for both diagrams. It can be concluded that when the same focusing voltage is applied, the structures with larger γ can always

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achieve better focusing effect with a narrower focusing width. The difference is more distinct at a larger applied voltage.

Fig. 3.15 Focusing ratio $1/\beta$ vs. applied voltage ratio α for $\gamma = 1$.

Fig. 3.16 Focusing ratio $1/\beta$ vs. applied voltage ratio α for $\gamma = 2$.

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The curve shown in Fig. 3.16 is steeper compared to that of Fig. 3.12, which means that the structure with larger γ is more sensitive to the change in the applied voltage ratio α . A small change in the applied focusing ratio $1/\beta$ can cause a bigger change in the focusing ratio for Design B than Design A and this is more obvious with the increase in the focusing voltage. Hence, it is more difficult to precisely control the focusing width during the experiment in Design B when α is getting higher. As a result, large applied voltage ratios should be avoided during the experiment.

There is a little discrepancy between the simulation and experiment results. This can be explained by three factors such as pressure disturbance, fabrication imperfection and electrode position:

- (i) *Pressure disturbance.* During the experiment, all the sample streams and focusing streams flow to the outlet reservoir. After a while, the fluid height difference builds up and, hence, backward pressure will coexist with the forward electroosmotic force. The presence of a parabolic, pressure-driven flow must be subtracted from the even radial profile of the axial velocity generated by EOF such that generally experimental results are relatively smaller than simulated values.
- (ii) *Fabrication imperfection.* Due to some equipment constraints, such as low resolution transparency mask, low precisions of mask aligner and human elements during fabrication procedures, it is relatively hard to fabricate exactly symmetrical structures. This

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explains why flows from two sides of the focusing channels are not always equal.

- (iii) *Electrode position.* The electric field is applied across the microchannels by inserting thin platinum wires into big reservoirs. The position of the platinum wires may vary a lot each time when the experiments are conducted. This will affect the magnitude of the generated electric field required to generate the EOF.

3.4 Summary

As mentioned, the incorporation of transversal spatial confinement of the sample allows for more sensitive measurements. Two focusing structures are designed by varying the width of the focusing channel. Both the simulation and experimental results prove that the circuit model can accurately represent the network of focusing structures. Previously, microchannels could only be described by differential equations, in which many parameters are involved, and this complication greatly reduced the ease of designing complex microchannel structures. Through a series of simulations and experiments, it was found that the focusing ratio is mainly determined by two variables, the channel width ratio and the applied voltage ratio. For the same structure, the higher the applied voltage ratio, the narrower the focused sample stream. With the same focusing voltages, the larger the channel width ratio, the better the focusing effect. Therefore for our application of blood cell sorting, Design B is recommended. A

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potential of 100 V can be applied to the inlet and the focusing reservoirs to achieve a focus ratio of 5, corresponding to a focusing width of 10 μm . For other applications, a different combination of values for the channel width ratio and applied focusing ratio can be selected in order to achieve the desired focusing ratio. Finally, it was found that there exists an optimal range of values for the applied voltage ratio, and a high value for the applied voltage ratio is not recommended because it is hard to precisely control the focusing ratio when α is close to α_{max} .

CHAPTER 4 THE ELECTROKINETIC SWITCHING EFFECT AND MICROCHANNEL DESIGN

The objective of this chapter is to study the most favorable design of the microfluidic switch structure. Firstly, the principle of electrokinetic switching is explored. Then, an equivalent circuit model is used to identify switching conditions under which the samples are directed into the specified outlet channels. Thereafter, Y-junction and T-junction structures are designed and simulated. The effect of switching angles on two parameters, namely switching accuracy and switching speed, are studied and the performances of switching structures are evaluated. Lastly, theoretical and experimental results of several different microfluidic switching structures are compared.

4.1 The Electrokinetic Switching Effect

4.1.1 Function of electrokinetic switching

The electrokinetic switching effect is a valveless switching process. When a potential difference is applied across a microfluidic channel, an electroosmotic flow is induced. Beads, cells or other particles in the electrolytic solutions are being carried along with electroosmotic flow. In the case of an electroosmotic flow induced by a high applied voltage (electrical field strength up to a few hundred V/cm) between miniature electrodes inserted into the channel input and output reservoirs, the direction of the flow can simply be regulated by electrically switching the respective electrodes inserted at the ends of the different output channels [67]. Cells or particles in a transporting liquid stream are sorted by precisely steering the liquid flow as shown in Fig. 4.1. The dark grey color represents electrolytic solution. The white and dark circles present two types of cells, Cell A and Cell B. There are two reservoirs, the collection reservoir and the waste reservoir for each type of cell. By an appropriate manipulation of the applied electrical potentials at the different collection reservoirs, the cell flow can be directed to a specific outlet channel connected to the collection reservoirs. If 0 V is applied at Reservoir A and Reservoir B is left open, electrolyte solution is flowing to Reservoir A and also A-type cell is carried to Reservoir A. When one B-type cell comes to the junction, 0 V is applied to Reservoir B and Reservoir A is left open so that flow direction of electrolyte solution is switched to reservoir B and B-type cell is carried to Reservoir B. At the same time, the electroosmotic flow of the output channel toward Reservoir A can be completely blocked and A-type cells in the bottom leg remain static. The switching of fluid flow is almost as

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instantaneous as the switching of the potentials. Electroosmotic flow is thus a good and easy way to manipulate particles and create valveless switches within any microfluidic device. Since the response of the electroosmotic flow to the direction of the applied field is instantaneous, electrical manipulation usually provides much shorter switching times and thus, theoretically yields much higher sorting speeds.

(a) Sample is switched to Reservoir

(b) Sample is switched to Reservoir B.

Fig. 4.1 Schematic of the electrokinetic switching effect.

4.1.2 Equivalent circuit model

For the electrokinetic cell sorter, normally the switching structure consists of three arms, one for input sample transportation and the other two for collecting targeted cells and waste. A high voltage potential is applied to one electrode at the input. The ground potential and the open circuit state are switched between the other two electrodes at the collection and waste wells according to the detection signal.

Fig. 4.2 Circuit model of electrokinetic switching.

As in the cross-shaped focusing structure considered previously, Kirchhoff's law can also provide an estimation of the overall electric field strength for switching structure [73, 74]. The arms of the switching structure can be considered as three wires with identical resistance (assumed to have identical dimensions) as shown in Fig. 4.2. The volume flow rate will correspond to the electric current. The inlet branch of the circuit is driven by voltage V_i and the voltage at the intersection of three channels is represented by V_j . The collection and waste branches terminate at V_c and V_w respectively. The resistances

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of the inlet, collection and waste arms of the circuit are all R . The relationship between the fluid flux and the electric current is an electrokinetic analog to Ohm's law which can be expressed as:

$$I_c R = V_j - V_c \quad (4.1)$$

$$I_c R = V_j - V_c \quad (4.2)$$

$$I_w R = V_j - V_w \quad (4.3)$$

According to Kirchhoff's law, the current from the input well I_i equals the combined currents going into the collection I_c and the waste wells I_w at the junction, which can be written as

$$I_c + I_w = I_i \quad (4.4)$$

Substituting Eqs.(4.1), (4.2) and (4.3) into Eq. (4.4), we have

$$(V_j - V_c) + (V_j - V_w) = V_i - V_j \quad (4.5)$$

Eq. (4.5) can be rewritten as

$$V_i + V_c + V_w = 3V_j \quad (4.6)$$

If the waste channel is set to be “floating”, then $V_w = V_j$. Ideally, there is no current flowing into the “floating” waste channel and hence no electrokinetic flow. Samples coming from the inlet all flow to the collection channel.

In any sorting logic, the detection of each cell first determines whether or not the criteria are met; when these criteria are met, a logic signal is generated to trigger sorting. For our microfluidic cell sorter, the standard “forward” sorting algorithm is used which consists of running the cells from the input channel to the waste channel until a cell's

fluorescence is above a preset threshold, and at this point the voltages are temporarily changed to divert the cell to the collection channel [75]. Detection occurs just a few micrometers before the junction and sorting is immediately performed in continuous flow. This may allow for more accurate and synchronous sorting which can be critical for time-course measurements or any downstream analysis. With electrokinetic flow, throughput is limited by intrinsic switching time of the device and the highest voltage that can be applied to the sorter.

4.2 Design and Simulation of Switching Structures

4.2.1 Structure of switching channel

There are a number of switching structures reported in the literature, but most of the work is just “proof-of-concept” without any special designing rules [27, 43, 67]. In this project, the effect of structures on switching is investigated and design rules which allow one to develop optimized structures are established.

The performance of a switching structure can be evaluated using two important parameters, *switching speed* and *switching accuracy*. Switching speed refers to the speed of fluid switched from one direction to another. It directly determines the throughput of the whole system. Switching accuracy refers to the rate of successful guidance of the targeted cells to the predetermined reservoir. Theoretically, if a cell is supposed to go to the collection reservoir, the fluid will be switched towards that reservoir and

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electroosmotic flow towards the waste reservoir is totally blocked. However, as shown in Fig. 4.1, switching errors due to the leakage of fluid cannot be avoided in practice. The smaller the leakage, the higher the switching accuracy. For this application, the switching structure should be capable of sorting two different types of cells. In this cell sorting system, the sample is first pre-focused by the electrokinetic focusing structure and is then guided into the required outlet port via switching structure. Two kinds of three-arm structures intersecting at a junction are designed and shown in Fig. 4.3 (a) and (b). The structures are known as “Y-junction switching” and “T-junction switching”. To obtain the optimum switching effect, simulations were carried out to determine the angle θ between the two outlet channels, which provides the best switching results.

Fig. 4.3 Design of switching structures: (a) Y-junction, and (b) T-junction.

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To study the effect of the angle between two outlet channels on the switching time and the switching accuracy, both switching structures are simulated at different angles. There are three reservoirs for each structure. “I” is the inlet for sample injection. “C” is the collection reservoir and “W” is the waste reservoir depending on what kind of cell/particle they collect. In the simulation, the electric potential at the inlet is fixed at 100 V. By an appropriate switching of the applied electrical potentials at different collection reservoirs between the ground state and floating state, the flow can be directed to the specific outlet channel connected to the collection reservoir or waste reservoir.

As discussed above, the performance of the switching structure is evaluated based on two aspects. The first one is the switching accuracy which is related to the leakage of EOF to the undesired outlet channel, and the second one is the switching speed which is reflected by the time needed to switch the flow from one direction to another. Steady state simulations were conducted to perform the leakage study, and the leakage distances were measured for each structure according to the velocity along the center line. Transient state simulations were also carried out to find the switching time, which was measured based on the velocity of the intersection point.

The buffer solution used in simulation is H₂O, with a density equal to 1000 Kg/m³, a kinematic viscosity of 10⁻⁶ m²/s and relative permittivity of 78.5. These values give rise to a Reynolds number of order 1, implying a very steady laminar flow. Debye thickness of 10⁻⁹ m and a zeta potential of -0.0295 V are applied to all the walls. The values are kept constant during the entire simulation.

4.2.2 Y-junction switching

4.2.2.1 Switching accuracy

Since switching accuracy can be reflected by the amount of leakage, the leakage distance was measured for Y-junction switching structures with different angles using steady state simulation. During the simulation, the electric potential at input reservoir “I” is fixed at 100 V, the voltage at collection reservoir “C” is set at 0 V and the waste reservoir “W” is set at a floating potential. As a result, the sample solution is defaulted to flow to collection reservoir “C”. The definition of leakage distance is explained in Fig. 4.4. In Fig. 4.4 (a) the dark grey areas represent zero velocity. The sample solution is directed into collection channel “C”, and the fluid flow into the waste channel “W” is supposed to be zero. However, due to the inertia of the fluid, there is some velocity, and therefore fluid flow, in channel “W” near the junction which is represented by the light grey color. To determine the leakage distance, the line $M-N$ is drawn along the center line of Channel C, and the velocity along this line is plotted as shown in Fig. 4.4 (b). The critical velocity was chosen to be 10% of the maximum velocity, which in this case equals 16 $\mu\text{m/s}$, and the distance between the point M and the position at which the velocity reaches 16 $\mu\text{m/s}$ is defined as leakage distance. By performing the same analysis for different values of the angle θ in step of 30° , we manage to establish the relationship between the leakage distance and the switching angle θ as shown in Fig. 4.5.

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Fig. 4.4 Definition of the leakage distance.

Fig. 4.5 Relationship between the leakage distance and the switching angle.

Apparently the leakage distance decreases with the increase in the switching angle θ . This can be clearly explained. When the angle between two output channels is small, the inertia from inlet channel to the undesired channel is large, resulting in a large leakage distance. Take for instance the cases when $\theta = 90^\circ$ and $\theta = 180^\circ$. Figs 4.6 and 4.7 present the simulation results of streamlines and velocity vectors for these two angles. When $\theta = 90^\circ$, the magnitude of the resolved inlet channel velocity vector in the outlet channel directions is large, hence the inertia in the outlet channel direction is also very large. In contrast, for $\theta = 180^\circ$, the direction of the inlet velocity is perpendicular to the velocity in the outlet channel, so there is no resolved inlet channel velocity vector, i.e., no inertia in the outlet channel directions at all.

Fig. 4.6 Streamlines of a Y-junction switching structure: (a) $\theta = 90^\circ$, (b) $\theta = 180^\circ$.

Fig. 4.7 Simulation of velocity vectors: (a) $\theta = 90^\circ$, (b) $\theta = 180^\circ$.

4.2.2.2 Switching speed

To study the effect of angle θ on the switching speed, a series of transient simulations were carried out at different angles. The initial condition is shown in Fig. 4.8 (a). The electric potential at the input reservoir “I” is fixed at 100 V, the voltage at collection Reservoir “C” is set to 0 V and the waste reservoir “W” is set at a floating potential. Thus, initially the sample solution is defaulted to flow to collection Reservoir “C”. By switching the floating potential to reservoir “C” and the ground state to Reservoir “W”, the fluid will change its path from $I \rightarrow C$ to $I \rightarrow W$ as shown in Fig. 4.8

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(b). To measure the switching time, the velocity at the intersecting point O as a function of time is plotted as shown in Fig. 4.8 (c). It is sensible to see an increase in velocity magnitude, as during the switching period, the state of the fluid in the waste channel transforms from static to dynamic. The time interval necessary for the fluid velocity to reach the final steady value, as measured from the moment when the potential was applied, is defined as the switching time. The smaller the value, the faster the switching speed.

Fig. 4.8 Definition of the switching time.

Fig. 4.9 Relationship between the switching time and the switching angle.

By performing the same analysis for different values of the angle θ in steps of 30° , the relationship between the switching time and switching angle θ was established, as shown in Fig. 4.9. The graph reveals that the switching time increases with the switching angle value. This is because as the switching angle increases, the magnitude of the velocity vector to be changed from one output channel to another is larger and, as a result, the switching time is longer.

Based on the above analysis, an important conclusion can be drawn. There is always a trade off between the leakage effect and the switching speed. If a smaller switching angle is chosen, such as 60° , the switching speed is faster but a larger leakage is observed, inducing lower accuracy in cell sorting. If a larger switching angle is selected, for example 160° , the leakage problems can be reduced, however, the switching speed is slower. For the application of blood cell sorting, both switching accuracy and switching speed are important. Thus, the angle θ should be neither too large nor too small. In this case, θ is chosen to be 90° .

4.2.3 T-junction switching

As summarized in Section 4.2.3, for Y-junction switching structure, the leakage phenomena is less serious as the angle between two output channels increases but at the same time the switching time becomes longer. Therefore, the angle θ between two output channels should not be too big or too small and 90° is a good choice that compromises the above mentioned contradictory requirements. Because the Y-junction switching structure is symmetrical, it is suitable to sort two types of cells present in almost equal amounts in the initial sample. However, in case that one type of cells is sparsely distributed in the sample solution, the side switching structure as shown in Fig. 4.2 (b) is believed to have a better performance. To further illustrate this concept, a side switching structure with 90° switching angle and a Y-junction switching structure with 90° switching angle are compared in this section.

Similar to the previous Y-junction switching structure analysis, both steady state and transient simulations were carried out in this case as well. For steady state, two situations were simulated in order to study leakage phenomena in a T-junction switching structure. This is due to the fact that T-junction switching structure is not symmetrical. As shown in Fig. 4.10, for situation (a), the fluid turns from inlet Reservoir “I” to waste Reservoir “W” and for situation (b), the sample fluid goes straight from inlet Reservoir “I” to collection Reservoir “C”. For the transient simulations, two situations were also simulated to find out the switching times, as shown in Figs 4.11 and 4.12. In Fig. 4.11, the fluid initially flows from “I” to “C” and then the direction is switched to I→W by appropriate manipulation of the electric potentials at the two output reservoirs. The steady state simulation results of situation (b) were used as initial condition in the

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transient simulation. In Fig. 4.12, the direction of the sample solution is switched from $I \rightarrow W$ to $I \rightarrow C$. Again, the steady state simulation results of situation (a) were used as initial condition in its transient simulations.

Fig. 4.10 Leakage phenomena study for the T-junction switching structure using steady state simulation: (a) Velocity plot along "I" to "C" when sample flows from "I" to "W", (b) Velocity plot along "O" to "W" when sample flows from "I" to "C".

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(c) Velocity plot at point O during the switching process

Fig. 4.11 Switching time study for the T-junction switching structure using transit state simulation. The direction of fluid is switched from $I \rightarrow C$ to $I \rightarrow W$.

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(c) Velocity plot at point O during the switching process

Fig. 4.12 Switching time study for the T-junction switching structure using transit state simulation. The direction of fluid is switched from $I \rightarrow W$ to $I \rightarrow C$.

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A comparison is made between the T-junction and Y-junction switching structures in terms of leakage distance and switching time which is summarized in Table 4.1. For the T-junction switching, the leakage distance is smaller when fluid flows directly from top to bottom. This is because inlet Channel I is perpendicular to the waste Channel W, so there is no resolved inlet channel velocity vector, i.e. no inertia in the direction of the collection channel. The value of the leakage distance of a Y-junction structure is intermediary between those for the two T-junction switching situations. The switching times for the all the three situations are quite comparable since the switching angle between the outlets are fixed at 90°.

If the targeted cells are rare in the sample solution, the switching accuracy is the first factor to be considered and the side switching structure is recommended in this case. Most of the time, sample flows to the waste reservoir and only when rare cells are detected, the fluid will be switched to the collection reservoir. With bottom set to collection reservoir and right set to waste reservoir, the switching accuracy is expected to be the highest since the leakage distance is the smallest at this condition.

Table 4.1 Comparison of T-junction and Y-junction switching structures.

Structure description	Leakage distance (μm)	Switching time (ms)
T-junction Switching (a)	38	7
T-junction Switching (b)	58	6.7
Y-junction Switching (90°)	52	8

4.3 Experimental Results and Discussions

Experiments were conducted to verify the simulation results. Three PDMS microchips were fabricated: two of them had Y-junction switching structures with switching angles of 90° and 180° , and the third microchip had the T-junction switching structure with a switching angle of 90° . All the dimensions were set to be the same as those used in the previous design. The potential at the inlet Reservoir I was held constant at 100 V and the voltages applied at the collection and the waste reservoirs were switched between 0 V and floating potential to achieve proper switching. The buffer used for experiment is deionized H_2O . The properties of H_2O have been described in the simulation section. Flow velocities were measured by micro PIV system. Leakage distances are determined using the definition explained in the simulation section.

The effects of the switching angle on the switching accuracy are verified by measuring the flow velocities for the Y-junction structures with switching angles $\theta = 90^\circ$ and 180° , as shown in Fig. 4.13. The sample solution flows from inlet to the collection reservoir by default. For $\theta = 90^\circ$, the velocities at the inlet, the collection and the waste channels are $257 \mu\text{m/s}$, $229 \mu\text{m/s}$ and $21.8 \mu\text{m/s}$, respectively. In contrast, for $\theta = 180^\circ$, the velocities at the inlet, the collection and the waste channels are $265.1 \mu\text{m/s}$, $254.7 \mu\text{m/s}$ and $9.6 \mu\text{m/s}$ respectively. The velocity of the waste channel entrance for $\theta = 90^\circ$ is bigger than that for $\theta = 180^\circ$, implying a larger leakage distance for $\theta = 90^\circ$.

Fig. 4.13 Measured flow velocities for a Y-junction structure: (a) $\theta = 90^\circ$, (b) $\theta = 180^\circ$.

The switching effect in T-junction switching structure was also measured by micro PIV. Two situations listed in Fig. 4.10 were tested and the results are shown in Fig. 4.14. When the fluid goes straight from inlet to the collection reservoir, the velocities at inlet, collection and waste channels are 259.1 $\mu\text{m/s}$, 247.7 $\mu\text{m/s}$ and 11.2 $\mu\text{m/s}$, respectively. For the other situation, when the fluid turns from inlet to waste reservoir, the velocities at inlet, collection and waste channels are 206.2 $\mu\text{m/s}$, 230.8 $\mu\text{m/s}$ and 30.3 $\mu\text{m/s}$ respectively. The leakage for the second situation is larger due to a larger inertia of the fluid flow downwards. Fig. 4.15 demonstrated the sorting events corresponding to case (b) in Fig. 4.14, where dark grey color beads were switched to “W”.

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Fig. 4.14 Measured flow velocities for the T-junction switching structure: (a) Sample flows from "I" to "C", (b) Sample flows from "I" to "W".

Fig. 4.15 Image of sorting events. Beads are sorted into “W”.

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The switching times were also measured for both structures. However, the accuracy of the measured results is limited by the micro PIV system. The minimum time between every frame is 250 msec and, as a result, the measured switching time can only be a multiple of 250. Hence, the simulated switching times are not going to be compared with the experiment results. Table 4.2 summarizes the simulated and experimental results for the leakage distance.

Table 4.2 Comparison of simulation and experimental results of leakage distances.

Structure Description	Leakage Distance (μm)	Leakage Distance (μm)
	(Simulation)	(Experiment)
Y-junction ($\theta=90^\circ$)	53	55.2
Y-junction ($\theta=180^\circ$)	25	25.4
T-junction (situation a)	38	35.1
T-junction (situation b)	58	58.5

It can be seen that there is always a trade off between the switching accuracy and the switching speed. It can be concluded that as the switching angle increases, the switching accuracy is higher while the switching time is longer. The design of the switching structure largely depends on the application. If accuracy is the first priority, a large switching angle is desired. If throughput is more important, a smaller angle is preferred. The Y-junction structure is more suitable when the amount of the two types of

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cells is more or less the same, while a T-junction structure will give better performance for the application of rare cell sorting. For the objective of blood cell sorting in this project, since white blood cells are not rare cells in blood, a Y-junction switching structure with a 90° switching angle is recommended.

4.3 Summary

Electrokinetic switching is an easy way to manipulate particles and create valveless switches within any microfluidic device. Since the response of electroosmotic flow to the direction of the applied field is almost instantaneous, electrical manipulation usually provides much shorter switching times. In this work, a circuit model is used to control the applied voltage in such a way that the sample is directed into the specified outlet channels. Three arm Y-junction switching structures with different angles are designed and simulated. The effects of the switching angles are studied and it is found that as the switching angle increases, the switching accuracy is higher and the switching time is longer. The theoretical results are verified by experiments. Several switching microfluidic chips are fabricated and tested and the results of leakage distance are quite comparable with simulation. The optimum design of the microfluidic switching is greatly determined by its application. For the objective of blood cell sorting in this project, a Y-junction switching structure with 90° switching angle is highly recommended with simulated leakage distance of 53 μm and switching time of 8 msec.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

The objective of this chapter is to conclude the major findings of this project. Important results achieved in previous chapters are highlighted and recommendations for future work are given at the end of the chapter.

5.1 Conclusions

In this project, a microfabricated fluorescent-activated cell sorter with novel integrated and functional structures has been designed, simulated, fabricated and tested. The cell sorter comprises of two major parts, a microfluidic system for cell manipulation and a micro optical system for cell detection. Electroosmosis is used as the driving mechanism to transport, focus and switch cells.

The basic straight microchannels, complex focusing and switching structures have been extensively studied to optimize the design of the cell sorter. For the straight microchannels, the results of numerical simulations of EOF and opposing pressure induced flow in microchannels was presented and experiments were carried out using micro particle imaging velocimetry (PIV) system for verification. The result showed the linear dependence of electroosmotic velocity on an induced hydrostatic pressure. A total volume of 0.305 μl was transported in the 50 μm microchannel and the back flow occurred after a 240 secs transportation. For comparison, the 26 μm microchannel transported 0.33 μl within 425 secs, which is much slower compared to the 50 μm microchannel, but back flow occurred much later. Thus, there is a trade-off between the flow rate and the stability against the adverse pressure-driven back flow.

The structures for electrokinetic focusing of sample in flow cytometry and cell sorting were designed. To find the optimized geometry, different configurations with varying channel widths were simulated and tested. Both simulation and experimental

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results proved that a simple circuit model can accurately represent the network of the focusing structures, which greatly reduces the complexity of microchannel design. The focusing ratio is found to be determined by two variables, the channel width ratio and the applied voltage ratio. For the same structure, the higher the applied voltage ratio, the narrower the focused sample stream width. With the same focusing voltages, the larger the channel width ratio, the better the focusing effect. Electrokinetic focusing of sample stream to the width of 10 μm has been achieved for the application of blood cell sorting with a channel width ratio of 2 and focusing ratio of 5.

Three arm Y-junction and T-junction switching structures with different angles were designed and simulated. The effects of switching angles were investigated and it was found that, as switching angle increases, the switching accuracy becomes higher while switching time will be longer. For the objective of sorting blood cells in this project, a Y-junction switching structure with 90° switching angle is highly recommended with a simulated leakage distance of 53 μm and a switching time of 8 msec.

The integrated microfluidic cell sorter clearly offers several advantages over the conventional FACS machines. The structure is simple and it can be fabricated easily using standard microfabrication methods, and can be monolithically integrated with other microfluidic structures. Because of its simple fabrication process and inexpensive materials, the microfluidic cell sorting device can be disposed to eliminate any cross-contamination from previous runs. In contrast to the conventional aerosol droplet sorters, the entire cell sorting chip is self-contained, allowing for relatively safe sorting of

biohazard materials. The integrated cell sorter provides an inexpensive, robust, and flexible way to sort and manipulate single cells. Innovative sorting schemes can be implemented on the device to perform time-course measurements on a single cell for kinetic studies.

5.2 Recommendations

Having identified the value of the research efforts carried out thus far, more works are scheduled in the research plan to improve the performance of microfluidic cell sorting system. There are four challenges ahead: to improve the sorting speed, to counter the hydrostatic pressure effect, to test real cell samples and to integrate more functions in order to make the chip a more complete analysis system.

Although a microfluidic cell sorting system provides an inexpensive and innovative way to sort cells, the most challenging task is to design a sorter comparable to the conventional FACS machines in terms of throughput and recovery. The sample throughput of the microfluidic cell sorter is orders of magnitudes lower than the offered by a commercial FACS. One of the promising ways to increase the overall throughput is to multiplex the cell sorting channels and allow for simultaneous measurements of cells in different compartments. This is the major target of future work to make the microfluidic cell sorter a real commercial product.

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Electrokinetically driven flow has been used to transport fluid in microchannels. However, during the experiments, the adverse hydrostatic pressure induced flow is found, which is mainly caused by unequal levels in solution reservoirs and it counter affects the electroosmotic flow. As mentioned in Chapter 2, we have tried to minimize the pressure effect by reducing the cross-section size of the microchannel. Although this method significantly diminishes the pressure effect, adverse pressure-induced flow is still observed if the running time of the microchip is longer than half an hour. In addition, the volume flow rate is sacrificed by reducing the cross-section size, which ends up with smaller throughput. Methods other than reducing size, such as replacing the punched reservoirs by peripheral bottles or implementing special structures such as Tesla valve, can be considered to counter this problem.

In this study, optimized microfluidic structures have been designed based on the physics of microfluidics, but so far the chips were only tested using fluorescent microbeads. Since the microfluidic chip was developed for cell sorting applications, to further advance the research, in future it is necessary to conduct cell transportation and switching using living cells.

The integration of complete analyses systems “on chip” is one of the great potentials of microfabricated devices. Sample dispensing and other chemical or enzymatic reactions, such as cell lysis or polymerase chain reaction, can also be carried out downstream immediately after the cell has been sorted. This will enable single-cell studies to be achieved in a more precise and automated fashion. More fluidic

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functionalities, such as rotary pumps and incubation, can be integrated in the future. We anticipate that an integrated lab-on-a-chip will be realized in the near future, where cell sorting is one of the steps towards a complete analysis system.

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AUTHOR'S PUBLICATIONS

Journal Papers

1. Y. Sun, C. S. Lim, A. Q. Liu, T. C. Ayi and P. H. Yap, "Study of induced hydrostatic pressure and switching effect in electroosmotically driven microfluidic cell sorting system", *Sensors and Actuators A Physical* (Accepted).

Conference Papers

1. Y. Sun, A. Q. Liu, P. H. Yap and T. C. Ayi, "An Integrated Biophotonic and Microfluidic Chip for CD4 Cell Sorting Applications", *Proceedings of the 9th International Conference on Miniaturized Chemical and Biochemical Analysis Systems (μ TAS)*, Boston, Massachusetts, USA, October 9 - 13, 2005, Volume 1, pp. 536-538.
2. Y. Sun, A. Q. Liu, P. H. Yap and T. C. Ayi, "A Self-contained Microfluidic Cell Sorting System for HIV Diagnosis", *Proceedings of the 13th IEEE International Conference on Solid-State Sensors Actuators and Microsystems (Transducers)*, Seoul, Korea, June 5-9, 2005, Volume 2, pp. 1708-1771.
3. Y. Sun, L. C. Ng, S. K. Chua, X. M. Zhang, P. Droge, T. C. Ayi, P. H. Yap and A. Q. Liu, "A high-accuracy cell sorter using electroosmotically-driven microfluidic system", *Proceedings of Asia-Pacific Conference of Transducers and*

Micro-Nano Technology (APCOT), Sapporo, Japan, July 4-8, 2004, Volume 3, pp. 267-271.

Patent

1. Ai Qun Liu, Yi Sun, Xiao Jun Liang, Xu Ming Zhang and Peter Droge, “Microfluidic cell sorter system”, ITTO Ref no. PAT014/04/014/04, August 2004 (US patent filed).

Awards

1. Bronze Prize of the 5th Young Inventor Awards, Asian Wall Street Journal, “A biophotonic chip for cancer diagnosis” in 2004.