Microfluidic devices for cell separation and sample concentration

Ning, Liu

2018

Ning, L. (2018). Microfluidic devices for cell separation and sample concentration. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/73351>

<https://doi.org/10.32657/10356/73351>

NANYANG
TECHNOLOGICAL
UNIVERSITY

Microfluidic Devices for Cell Separation and Sample Concentration

Ning Liu

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2017

Microfluidic Devices for Cell Separation and Sample Concentration

Ning Liu

Division of Physics and Applied Physics

School of Physical and Mathematical Sciences

A thesis submitted to the Nanyang Technological University

in fulfilment of the requirement for the degree of

Doctor of Philosophy

2017

For my parents

And

my girlfriend Li Ji

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude to my supervisor, Prof. Wen Siang Lew for his patient instruction and comprehensive academic training offered to me. His generous support and continual encouragement tremendously benefited me throughout my doctoral research. I am impressed by his diligent, hard-working spirit and enthusiasm in his work, all of which motivate me to pursue the academic achievement. Without his kind advice and help, I could not image that I can achieve timely completion of my PhD work.

Next, it is my pleasure to thank our group members for their selfless help in my research work.

It is also my pleasure to thank Prof. Jun Shan Liu at Dalian University of Technology for advice on microfabrication work.

In addition, I would like to express my appreciation to all my collaborators and friends, who offered my generous help during my PhD studies, especially Dr. Hou Han wei, Dr. Michinao Hashimoto, Dr. David Brown, Dr. Hong Jie An, Dr. Nishanth V. Menon, Dr. Dinh-Tuan Phan, Dr. Jing Xu, Dr. Jinhong Guo, Dr. Jun Zhang, Ms Hui min Tay, Mr Petchakup Chayakorn.

Last but not least, I am deeply indebted to my parents Yu qi Liu and Zi ying Liu, my sister Peng and Jing for their unconditional love. I would like to express my sincere gratitude to my girlfriend Li Ji for her unwavering emotional support, inspiring and believing in me. She has been my rock in the past years.

List of Publication

Journal Papers

1. **Ning Liu**, Dinh-Tuan, Phan, Wen Siang Lew, An Enclosed Paper Microfluidic Chip as A Sample Pre-concentrator Based on Ion Concentration Polarization, *IEEE Transactions on Biomedical Circuits and Systems*, (accepted)
2. **Ning Liu**, Jing Xu, Hong Jie An, Dinh-Tuan Phan, Michinao Hashimoto, Wen Siang Lew, Direct Spraying Method for Fabrication of Paper-based Microfluidic Devices, *Journal of Micromechanics and Microengineering*, (accepted)
3. De Wei Wong, Wei Liang Gan, **Ning Liu**, Wen Siang Lew, Magneto-actuated cell apoptosis by biaxial pulsed magnetic field, *Scientific Reports*, (under revision)
4. Qi Ying Wong, **Ning Liu**, Cheng-Gee Koh, Hoi-Yeung Li, Wen Siang Lew, Isolation of magnetically tagged cancer cells through an integrated magnetofluidic device, *Microfluidics and Nanofluidics*, (2016) 20: 139

Conference Presentations

1. De Wei Wong, Wei Liang Gan, **Ning Liu**, Wen Siang Lew, In vitro magneto-mechanical cancer cell destruction efficacy under biaxial DC pulsed magnetic field, *INTERMAG 2017*, Dublin, Ireland
2. **Ning Liu**, Hui Min Tay, Han Wei Han, Wen Sian Lew, Magnetic Separation of Inertially Focused Cells/Particles, *BioMEMS 2017*, Boston, USA
3. De Wei Wong, **Ning Liu**, Chor Boon Tan, Wei Liang Gan, Indra Purnama, Wen Siang Lew, Multi-vortex magnetic nanoparticles: A twin-functionalized agent for magnetomechanical cancer-cell destruction and hyperthermia, *The 20th International Conference on Magnetism 2015*, Barcelona, Spain

4. De Wei Wong, Yong Yang, Wei Liang Gan, **Ning Liu**, Indra Purnama, Chandra Sekhar, San Yee Wong, Jun Ding, Wen Siang Lew, Magnetic nanoparticles for magnetomechanical cell destruction and magnetic hyperthermia agents, ***INTERMAG 2015***, Beijing, PR China

Patent

1. Han Wei Hou, **Ning Liu**, Wen Siang Lew, A continuous-flow size and affinity-based microfluidics cell sorter, Technology Disclosure, NTU ref TD/189/17

Table of Contents

List of Figures	VIII
Abstract	XVI
Chapter 1	1
Introduction	1
1.1 Background and Motivation	1
1.2 Thesis Scope and Structure	2
Chapter 2	4
Literature Review	4
2.1 Microfluidics	4
2.1.1 Fabrication methods of microfluidic devices	5
2.1.2 Applications of microfluidics	6
2.2 Microfluidic cell separation	8
2.3 Active cell separation in microfluidics	11
2.3.1 Electric force-based separation	12
2.3.2 Magnetic force-based separation	14
2.3.3 Optical force-based separation	20
2.3.4 Acoustic force-based separation	21
2.4 Passive cell separation in microfluidics	23
2.4.1 Hydrodynamic Filtration	23
2.4.2 Pinched flow fractionation (PFF)	24
2.4.3 Deterministic lateral displacement (DLD)	26
2.4.4 Affinity-based methods	28
2.4.5 Other passive methods	29

2.5 Hybrid separation methods.....	30
2.5.1 Dielectrophoresis-assisted hybrid methods.....	30
2.5.2 Optophoresis-assisted hybrid methods	31
2.5.3 Acoustophoresis-assisted hybrid methods	32
2.5.4 Magnetophoresis-assisted hybrid methods	32
2.5.5 Other hybrid methods	34
2.6 Paper-based microfluidics	35
2.7 Paper-based microfluidics for sample preconcentration	40
Chapter 3	47
Magnetofluidic Device for Cancer Cells Separation	47
3.1 Background	47
3.1.1 Circulating tumor cells.....	47
3.1.2 Microfluidic approaches for CTCs separation.....	48
3.1.3 Microelectromagnetic separation.....	51
3.1.4 Motivation.....	52
3.2 Design principle	53
3.3 Numerical study	54
3.4 Experimental procedures.....	59
3.4.1 Device fabrication.....	59
3.4.2 Cell culture.....	63
3.4.3 Measurement setup	63
3.5 Results and discussions.....	64
3.5.1 Magnetic Particle Tagging of HeLa Cells.....	66
3.5.2 Sorting of Magnetically Tagged HeLa Cells.....	67
3.6 Summary	69

Chapter 4	71
Size and Affinity-based Multiplexed Cell Sorter	71
4.1 Background	71
4.1.1 Inertial microfluidics	71
4.1.2 Inertial effects for cell separation	74
4.1.3 Motivation	77
4.2 Design principle	78
4.3 Experimental procedures	81
4.3.1 Microchannel fabrication	81
4.3.2 Micromagnets fabrication	83
4.3.3 Sample preparation	85
4.3.4 Immunofluorescence staining and characterization	86
4.3.5 Device characterization	86
4.4 Results and discussion	87
4.5 Summary	93
Chapter 5	94
An Enclosed Paper Microfluidic Chip as A Sample Pre-concentrator Based on Ion Concentration Polarization	94
5.1 Background	94
5.2 Experimental procedures	94
5.2.1 Design principle	94
5.2.2 Device fabrication and operation	97
5.3 Results and discussions	99
5.3.1 Parafilm sealing method	99
5.3.2 Mechanical property test	102

5.3.3 Fluid flow measurement in enclosed and open channels.....	104
5.3.4 ICP demonstration on enclosed μ PADs	106
5.3.5 Mitigated evaporation influence	113
5.4 Summary	114
Chapter 6.....	116
Direct Spraying Method for Fabrication of Paper-based Microfluidic Sample Preconcentrator	116
6.1 Background	116
6.2 Experimental procedures	116
6.2.1 Direct spraying hydrophobic patterning	119
6.2.2 Fabrication and operation of leak-free paper-based ICP preconcentrator	122
6.2.3 Fabrication of paper-based mSC.....	125
6.3 Results and discussions	127
6.3.1 Direct spray hydrophobic patterning	127
6.3.2 Leak-free paper-based ICP preconcentrator for fluorescence sensing.....	131
6.3.3 Paper-based microsupercapacitors	133
6.4 Summary	138
Chapter 7	139
Summary and Outlook	139
7.1 Original contribution of this thesis.....	139
7.2 Future work	140
References.....	144

List of Figures

- Figure 2.1.** A broad classification of microfluidic cell separation methods. Microfluidic cell separation can be primarily categorized as active methods, passive methods and hybrid methods.11
- Figure 2.2.** Working principle of continuous flow dielectrophoresis cell sorting. The electrodes are used to generate electric field. The cells with different surface charges subjected to the electric field experience different DEP forces, result in the isolation of target cells from initial cell mixture.....12
- Figure 2.3.** Schematic illustration of magnetic force-based microfluidic cell sorter. A magnetic field is applied perpendicular to the flow direction. The immunomagnetically tagged cells are attracted by the magnetic source and move laterally across laminar flow.....18
- Figure 2.4.** Schematic illustration of working principle of a SSAWs-based cell separation device. The SSAWs are produced by interdigital transducers. Cells with different sizes experience different acoustic forces and follow distinct trajectories in the microchannel.....22
- Figure 2.5.** Working principle of pinched flow fractionation (PFF). The PFF device consists of pinched segment and broadened region. The particles with different sizes are focused in the pinched segment. The alignment differences of various particles are amplified by the broadened region, leading to apparent separation of the particles according to their sizes.....25
- Figure 2.6.** Schematic illustrating the working principle of cell separation by deterministic lateral displacement using periodic arrays of cylindrical posts. The posts are asymmetrically arranged in the flow direction to induce particles/cells with different sizes to follow different flow paths, resulting in lateral displacement and thus separation of particles/cells by size.....27
- Figure 3.1.** Microfluidic CTCs separation methods. CTCs isolation methods can be primarily categorized into two classes: (A) Affinity-based methods, which utilize the biochemistry properties

of CTCs, and (B) biochemistry properties-based methods, which take advantage of biophysical properties of CTCs.....49

Figure 3.2. Schematic of the of the device design consisting a microchannel and a microstripline as two major functional components. The width of the microstripline is 100 μm . The two parallel wires configuration can enhance the electromagnetic field.53

Figure 3.3. Magnetic flux density distribution in the (a) x , (b) y and (c) z components for the current values $0.4\text{A} \leq I \leq 1.0\text{A}$ along a reference line of measurement at the centre of the microstripline. (d) Magnetic field gradient distribution of the y -component in the double microstripline structure with current at 0.4A. (e) Surface temperature distribution along the microstripline for current values ranging $0.4\text{A} \leq I \leq 0.9\text{A}$. (f) Temperature change across the microstripline after a period of 5 minutes for current values ranging $0.4\text{A} \leq I \leq 0.9\text{A}$56

Figure 3.4. Procedures of magnetofluidic chip fabrication, including SU-8 mold fabrication, microstripline fabrication and the bonding process.....60

Figure 3.5. Schematic illustrating the detailed steps of the microstripline fabrication.....61

Figure 3.6. The microscopic image of microstripline. The width of a wire is 100 μm and the space between two wires is 100 μm61

Figure 3.7. The microscopic image of microfluidic channel and microstripline. The microstripline is positioned beneath the microchannel. There is a little offset between the channel centreline and the microstripline to induce the cell's deflection bias the upper outlet.62

Figure 3.8. (a) Schematic of Experimental setup of the magnetofluidic device on top of a dielectric Peltier cooling chip fitted with cooling fins for cooling down of the chip. (b) Simulations of magnetic particle flow in a magnetofluidic microchip for current values ranging $0.4\text{A} \leq I \leq 0.9\text{A}$,

partial deflection occurs at 0.6A and complete deflection occurs at 0.9A. The flow is governed by the Navier-Stokes equation and the beads flow is forced to the central line using the hydrodynamic focusing approach.63

Figure 3.9. Sorting of magnetic particles from glycerol mixture. (a) When no current was applied, the magnetic particles flow to Outlet 2 and (b) upon the activation of a minimum current of 0.5A, deflection starts to show. (c) Sorting efficiency relationship with controlled current. At 1.0A, 91% of the particles were deflected.....64

Figure 3.10. High binding efficiency of the Ep-CAM antibodies conjugated with 8 μm magnetic beads to HeLa cells was observed on (a) bright field (b) fluorescent and (c) confocal microscope. Multiple magnetic beads could be found attached to the cell.....66

Figure 3.11. (a) Hela cells tagged with anti Ep-CAM antibody-conjugated 8 μm magnetic beads. (b) Optical microscope image of the deflection of the tagged HeLa cells (indicated by the red circles) when 0.9A electric current was applied.....68

Figure 4.1. Integrated size and affinity-based cell sorter. (A) Design layout of the 2-inlet, 3-outlet cell sorter consisting of 1) size-based Dean Flow Fractionation (DFF), 2) serpentine channel for cell alignment using inertial focusing, and 3) magnetic separation channel with micromagnets. (B) Schematic illustration of cell trajectories at different stages of the device. (i) At the first stage, larger monocytes/neutrophils were fractionated by DFF into the top channel while smaller lymphocytes, RBCs and excess magnetic beads are removed as waste. Randomly distributed monocytes (ii) align along the centreline into a single stream at the end of the serpentine channel (iii) due to inertial focusing. This enables efficient deflection and collection of immunomagnetically labelled monocytes into the lower outlet (O2) while unlabeled cells are sorted into outlet 3 (O3).....79

Figure 4.2. Two units consisting of small and large curvature. For the large curvature, the center of the two curves is offset by 160 μm . The width of small and large turns is 160 μm and 350 μm , respectively.....80

Figure 4.3. Schematic of the PDMS microchannel fabrication process. A double-casting process assisted by Deep Reactive Ion Etching (DRIE) was adopted for the PDMS microchannel fabrication.....82

Figure 4.4. Fabrication of micromagnets. Microscopic images at (A) 4× and (B) 10× magnification illustrating magnetic powder loading process. The magnetic beads were first manually injected into the microstructure and could not fill up the smaller tooth structures. After centrifugation, the microstructures were completely filled by the beads.....83

Figure 4.5 Isolation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque84

Figure 4.6. Device characterization using monocytes. Sorted monocytes were randomly distributed at (A) stage 1 bifurcation of DFF, and (B) the start part of serpentine channel. (C) Monocytes focused inertially into a single stream at the end of serpentine channel.87

Figure 4.7. Phase contrast images indicating monocytes immunomagnetically labelled with 1.5 μm magnetic beads.....88

Figure 4.8. Average composite images indicating monocyte flow positions (stage 3) at (A) the start of the micromagnets array, (B) the end of the micromagnets array, and (C) the bifurcation prior outlet 2 and outlet 3. (D) Magnified bright field image at the bifurcation at stage 3. Red arrows indicate the monocytes labelled with magnetic beads.89

Figure 4.9. Immune cell separation using DFF. High recovery of monocytes in outlet 3 (A), and lymphocytes in outlet 1 (B) were achieved for all flow rates tested.90

Figure 5.1. (a) Schematic illustration of the device fabrication process. (b) The photograph of an enclosed paper-based pre-concentrator. Two gaps were cut on the two wings, leaving the

microchannel as the only path connecting the two reservoirs. Two electrode windows were located in the two reservoirs, respectively.95

Figure 5.2. (a) Close view of the μ PAD with two electrodes fixed by a 3D printed holder. (b) Schematic illustration of ICP induced by external electric field. The Nafion permselective film only allow cations to pass through. Ion depletion region forms at the anodic side of Nafion, repelling negatively charged analytes toward the anode.97

Figure 5.3. Cross sectional view of the Parafilm and paper. (a) a filter paper and parafilm laminated together before heating; (b) a filter paper penetrated by Parafilm from bottom side; (c) a filter sandwiched by two layers Parafilm after heating.100

Figure 5.4. Comparison of cutting performances on (a) bare filter paper and (b) Parafilm-paper composite. Tears and ruptures occurs very often when filter paper was used as substrate for craft cutting, this can be attributed to the rougher texture of filter paper comparing with printing paper. For the Parafilm-paper composite, since one side was covered with smooth Parafilm, the cutting can be performed smoothly without tears and ruptures.101

Figure 5.5. Mechanical strength test of different paper substrates. Due to the super-low mechanical strength of wet filter paper, the test was only performed on bare filter paper in dry state. Each force value is averaged over $N = 4$ measurements. The error bars represent one standard deviation.102

Figure 5.6. The representative time sequential images show the propagation of red dye front in enclosed channel (a) and open channel (b) with time. 20 μ L red color food dye was dripped in the circular reservoir at the bottom of a straight channel. Under the function of capillary force, the red dye moved along the paper channel.104

Figure 5.7. Flow comparison of red dye in enclosed channel and open channel. The results indicate a slightly slower flow velocity in the case of enclosed channel as compared to open channel within 400 s. The flow front of red dye in open channel stopped at 400 s, indicating the red dye has dried

in the open channel due to evaporation. Each distance value is averaged over $N=4$ measurements, and the error bars represent one standard deviation.105

Figure 5.8. Formation of depletion region and concentration plug in sample loading region of an enclosed μ PAD. The sample loading region has a nozzle-like shape to produce a better concentration effect. (a) Time sequential images of 5 μ M FITC in the sample loading region under an applied voltage of 50V. The time when the power supply started to provide voltage was set as start time point. (b) The corresponding fluorescence intensity profiles at selected time points are plotted. The maximum concentration of FITC fluorescent dye reaches more than 100-fold at 250s.....107

Figure 5.9. Movement of pre-concentration plug in an enclosed straight channel downstream of the sample loading region. (a) Time sequential images and (b) corresponding fluorescence intensity profiles of the pre-concentration plug are recorded at selected time points. The boundaries of the paper channels are indicated by the dashed lines. The concentration enhancement reached its maximum at 150 s and maintained at its maximum level for more than 300 s.111

Figure 5.10. ICP performance on the pre-wetted enclosed μ PAD that has been left in atmosphere for 2 hours: (a) time sequential images and (b) corresponding fluorescence intensities of pre-concentration plug at different time points. The time point of applying external voltage was set as $t = 0$112

Figure 6.1. A schematic overview of the process described in this paper. Direct hydrophobic spaying method can create hydrophobic-hydrophilic patterns on paper substrate. These patterns can be further modified for broad applications. After Parafilm embedding, the device can be used as sample preconcentrator based on ICP effect (Part II). In addition, we fabricated electrodes using this spaying method (Part III). The electrodes hold a number of application potentialities, such as microsupercapacitors.117

Figure 6.2. (A) Schematic illustration of the mask-assisted hydrophobic spraying process. (i) Paper substrate was adopted without any pretreatment. (ii) Paper substrate (filter paper or

membrane filter) was covered by a mask. (iii) Hydrophobic substance was evenly sprayed onto the substrate. (iv) The desired patterns were obtained after disassembling the mask from the paper substrate. (B) The sandwiched structure with the implementation of magnetic force (not to scale).119

Figure 6.3. Non-metal masks were applied to fabricate fluidic patterns by using direct spraying method. The masks were made out of inexpensive paperboard. The feasibility of the fabricated fluidic device was demonstrated by the time sequential images of blue dye flowing through the channels.120

Figure 6.4. (A) A cross-sectional micrograph of a Parafilm embedded filter paper. The total thickness of Parafilm and filter paper changed as a function of (B) temperature and (C) heating time. Each thickness value is averaged over N=5 measurements.122

Figure 6.5. Demonstration of ICP phenomenon in the leak-free μ PAD. (A) Schematic illustration of the paper-based ICP preconcentrator. A hybrid substrate was fabricated through embedding Parafilm into filter paper. The hydrophobic barrier was constructed through direct hydrophobic spraying on the hybrid substrate. (B) Representative images of fluorescent tracer at selected time points. The dashed lines indicate the boundaries of hydrophilic channel. (C) Fluorescence intensity profiles corresponding to above time sequential images.123

Figure 6.6. Schematic fabrication of paper-based mSC based on hydrophobic spraying: (i) Interdigital electrodes pattern was fabricated through spraying hydrophobic coating on membrane filter. (ii) Vacuum filtration technique was utilized to deposit conductive MWCNTs onto the uncoated part of the membrane filter (inset: before vacuum suction, the membrane filter was submerged by carbon solution (left), interdigital electrodes appeared on the membrane filter after vacuum suction (right)). (iii) The cartoon of as-fabricated planar carbon electrodes on paper substrate. (iv) Drop-casting of PVA/H₂SO₄ gel electrolyte onto the planar electrodes on paper substrate. (v) Attachment of copper tape to cathodic and anodic electrodes for electrical connection.125

Figure 6.7. Surface characterization of paper substrates. SEM images of Whatman[®] grade 1 filter paper (A) before and (B) after hydrophobic coating; (C) AFM 3D morphology of filter paper after hydrophobic coating; (D) water contact angle measurement on filter paper after hydrophobic coating.127

Figure 6.8. The microscopic views of paper-based microfluidic channels observed by optical microscope at 4× magnification. (A) The boundary between hydrophobic coating (top) and hydrophilic paper channel soaked with blue food dye (bottom). (B) A hydrophilic channel defined by hydrophobic coating.129

Figure 6.9. Performance of the fabricated micro-supercapacitors. (A) The photograph of as-fabricated mSC. (B) Cyclic voltammetry (CV) curves of mSCs at different scan rates from 5 to 500 mV/s. (C) Galvano static charge-discharge (GCD) curves of mSC at various charge/discharge rates. (D) Rate capabilities of mSCs.....135

Abstract

Microfluidic platforms for cell separation and downstream bio-sample analysis provide powerful tools for biomedical fundamental research and practical applications. Therefore, the overall goal of my dissertation is to develop such integrative platforms using novel techniques.

We first develop a magnetofluidic device using microelectromagnets for magnetic field generation and applied it for cancer cell separation. The cell separation efficiency can reach up to 79 % using 1A electric current. This tunable device would be a promising platform to separate many types of cells. Next, we propose a hybrid size and affinity-based multiplexed cell sorter, consisting spiral channel, inertial alignment segment and magnetic separation part. This is the first platform integrating spiral inertial microfluidics and magnetophoretic separation technique in one single chip. Using blood cell sample, a good separation performance (>90% efficiency) is achieved. This novel hybrid platform offers a promising alternative to many existing cell separation technologies.

Sample concentration technology is another critical aspect of biomedical innovation. Utilizing our developed fabrication techniques, we establish paper-based microfluidic platforms using ion concentration polarization effect for bio-sample concentration. The sample concentration performances are comparable with other reported results. Our paper-based platforms and fabrication techniques hold great promise for creating simple and inexpensive paper-based assays, which can be used for practical point-of-care (POC) diagnostics.

Chapter 1

Introduction

1.1 Background and Motivation

Abundant information about an individual's health exist in various cells and biomolecules in human body. Cell separation and sample concentration are two essential techniques to extract such information. On one hand, the ability of selective isolation of desired cells from a heterogeneous mixture is the prerequisite for subsequent cellular analysis. For example, separation of circulating tumor cells (CTCs) from peripheral blood stream can help doctors to perform accurate diagnosis and monitor treatment efficacy [1]. On the other hand, detecting biomedical analytes with ultralow concentration in biological fluids also plays an important role in biomedicine development. For instance, high sensitive drug molecules detection in biological is very useful for fast clinical and forensic diagnostics [2].

Due to its numerous unique features, microfluidics has been widely accepted as an excellent tool for cell separation and biosample concentration. In terms of cell separation, the development of microfluidic cell-sorting methodologies has experienced explosive progress in the past decades, including active, passive and hybrid methods. And for sample concentration, numerous microfluidic platforms have been proposed toward practical applications. However, there are still many problems such as low throughput, high operation complexity and complicated fabrication, suffered by existing microfluidic bio-sample preparation processes.

To contribute to the field of microfluidic bio-sample preparation, we develop novel microfluidic platforms for cell separation and sample concentration by using advanced techniques.

1.2 Thesis Scope and Structure

The aim of this research is to develop advanced technologies for cell separation and sample concentration from experimental aspect. The specific objectives includes: (i) develop a tunable microfluidic device using electromagnets for cancer cell separation with separation efficiency $>70\%$; (ii) integrate inertial force separation with magnetic separation technologies on a single chip for multiplexed blood cell sorting with high separation efficiency ($>70\%$) and high processing throughput ($>100 \mu\text{l}/\text{min}$); (iii) develop bio-sample concentration μPADs with new fabrication technologies.

At the beginning of the dissertation, **Chapter 1** is a short introduction of the research.

Chapter 2 gives the literature review.

Chapter 3 presents a microelectromagnets-based cell sorter for cancer cell separation. In this chapter, the potential application of this cell sorter for circulating tumor cells (CTCs) separation is firstly introduced. Following this, the fabrication process of this microelectromagnet is presented. To understand the magnetic field distribution in the vicinity of the microelectromagnet, numerical analysis using COMSOL software is provided. To demonstrate the practical feasibility of this device, cell separation using Hela cell is displayed. This work has been published on *Microfluidics and Nanofluidics*.

Chapter 4 reports a size and affinity-based microfluidic cell sorter. Following the introduction of the research background, the device fabrication process is displayed. Experimental results is obtained using monocytes and whole blood as separation samples. At last, the success of high efficient separation of monocytes using this device is presented.

Chapter 5 presents a paper-based microfluidic device for sample concentration. To demonstrate the device characterizations, the mechanical strength of different devices and the flow velocities

on enclosed and open devices is compared. Following these comparisons, the pre-concentration performances of the paper-based device are presented and discussed. This work has been accepted by *IEEE Transactions on Biomedical Circuits and Systems*.

Chapter 6 presents a paper-based sample concentrator fabricated by spraying method. The leak-free sample pre-concentrator is based on ion concentration polarization (ICP) effect. Additionally, a paper-based supercapacitor is fabricated based on the same spraying method. The supercapacitor hold potential for building integrative self-powered sample concentrator. This work has been accepted by *Journal of Micromechanics and Microengineering*.

Finally, **Chapter 7** summarizes the major contribution of this work and explains the future research direction.

Chapter 2

Literature Review

2.1 Microfluidics

Microfluidics is a multidisciplinary field that deals with fluids at the submillimeter length scale. Microfluidics is an enabling technology with the capability of scaling down traditional biochemical laboratories to a miniaturized integrative platform, namely, lab-on-a-chip (LOC), or micro total analysis systems (μ TAS). Microfluidic platform presents unique benefit of integrating multiple functional and possesses capability of manipulating fluid and biological entities at small length scale. In addition, microfluidic devices provide unprecedented opportunities for investigating some phenomenon can only occur at micrometer scale [3].

The first microfluidics prototypes emerged in 1970s at Stanford University as a mini-sized gas chromatography (GC) system [4] and at IBM as an ink-jet printer [5]. The original microanalytical methods, such as gas-phase chromatography (GPC), high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE), worked in capillary format and demonstrated high sensitivity at very low cost of samples. This success encouraged people to develop more versatile analytical devices with miniaturized footprints and to explore more application areas. With the development of molecular biology and biochemistry, the demand for handling and process small amount of aqueous sample, such as DNA and proteins, also demanded miniaturized devices and technologies. The support from US Department of Defense, aiming to develop microfluidic systems to detect chemical and biological weapons, is another stimulus for the fast growth of microfluidics. In the early 1990s, Manz *et al.* firstly introduced the concept of micro total analysis systems (μ TAS), or

Lab-on-a-chip (LOC), with the aim to integrated seamlessly all functional sections into one single device with highly shrunk physical size [6, 7].

There are several reasons making microfluidics attractive for both academic researchers and industrial groups: 1) the sample and reagent consumption, as well as minimized waste production are dramatically reduced on the miniaturized devices; 2) with the advances of fabrication technologies, the fabrication cost of the devices are continuously reduced; 3) microfluidics encompass structures with feature size in the range of 1~1000 μm , enabling small sample handling; 4) the analysis time required are reduced on microfluidic platform; 5) portable and versatile devices can be realized; 6) microfluidic technology enables the possibility of integration of many laboratory functional sections into a single system with small footprint.

2.1.1 Fabrication methods of microfluidic devices

The fabrication of microfluidics devices originally rooted in the basic manufacture process of semiconductor industry. The first generation of microfluidic devices fabricated on hard substrates, such as silicon and glass, which are compatible with established microelectronics fabrication methods. Although glass is preferred material because the early microfluidics largely works on electrophoretic effects, it requires expensive fabrication (*e.g.* photolithography, dry or wet etching) to form micro-structures. Silicon is an opaque material, making it is incompatible with popular microscopes [8].

To reduce the device cost and simplify the manufacture process, polymers have been employed as alternative materials for microfluidic devices fabrication. In contrary to glass and silicon, polymers are inexpensive, facilitating the manufacturing of low-cost and disposable biomedical devices. From the viewpoint of fabrication, the requirement for polymer-based devices fabrication are much lower than silicon and glass machining, which usually involve clean room, expensive

equipment and hazardous etching reagent. Based on the fabrication mechanism, polymer-based fabrication can be categorized as replication methods and rapid prototyping methods. Soft-lithography is the prevailing replication method that was introduced by Whitesides and his colleagues in the late 1990s [9, 10]. The introduction of soft lithography brought a significant advancement to the Lab-on-a-chip research community. It dramatically simplified microfluidic devices fabrication process and reduced the expense. Nowadays, soft-lithography has become the most prevailing method accepted by microfluidics community. The utilization of elastomer materials and related fabrication process enable more researcher can make non-expensive microfluidic devices by themselves. Compared to replication fabrication, rapid prototyping is a straightforward method without the need for mold. The common rapid prototyping methods include laser ablation, computer numerical controlled (CNC) milling, 3D printing, etc.

Recently, paper-based microfluidics have attracted much attention in biomedical research community. As a result, many novel corresponding fabrication methods have emerged. Since these fabrications are based on paper material, most of the fabrication processes are distinct from the methods mentioned above. The fabrication techniques of paper-based microfluidics are introduced in 1.6.

2.1.2 Applications of microfluidics

In the past decades, microfluidics has deeply influenced many subjects and been applied for a vast range of research and application purposes.

Genetic analysis

Microfluidic devices have been widely for high-performance genetic analysis. Traditional genetic analysis process suffers from intrinsic drawbacks, such as inevitable sample dilution and expensive reagents loss, high risk of sample contamination. Microfluidic genetic platforms can

integrate multiple genetic processing steps into one single device. These devices can greatly reduce the total sample and reagent consumption while achieve high-throughput with high assay speed and increased sensitivity. Moreover, through seamless connection of different steps, the integrated microfluidic platform can highly diminish the sample contamination risk [11].

Drug discovery and organ-on-a-chip

With the emergence of various microfluidic devices, the applications of microfluidics are changing the face of drug discovery. To bridge the gap between biochemical assays and animal testing, cell culture is performed to provide a complicated biological environment for candidate drug compound assay. However, traditional cell-based assays have some severe disadvantages. For example, the cells may lose their phenotypic features in traditional cell culture environment. As an alternative, microfluidics has been used to control the cellular microenvironment for cell culture, which leads to the development of organ-on-chips as biomimetic microsystems to model the environment complexity of human organs (*e.g.* brain, lung, liver, kidney). These models can be used for cell development to predict drug efficiency [12].

Single cell manipulation and analysis

Conventional cellular investigations were based on statistical analysis that performed on large cell population. However, the cellular heterogeneity can induce the loss of some valuable information during analyzing cells in bulk. Nowadays, single cell studies are becoming essential for many applications and fundamental research. The extracted information from the biomolecular, such as protein and DNA in single cell play significant role to understand fundamental cellular mechanism. Consequently, the single cells manipulation and analysis are becoming crucial approaches for this demand. Microfluidics provides a sophisticated micro-scale environment for cellular investigation. The confined experiment units in microfluidic enable precise cell

manipulation and detection, which is very useful for revealing the single cell behavior. Single cell trapping is the prerequisite to single cell analysis. Microfluidic devices provide effective and ideal technique solutions for single cell trapping with high spatiotemporal precision [13].

Environmental monitoring

The tremendous growth in industrial, biochemical, pharmaceutical, and medical fields has induced the increasing environmental protection concern. Hence, effective environmental monitoring techniques are in high demand currently. However, a lot of conventional analytical methods are not suitable for this demand due to intrinsic shortcomings such as bulky dimension, costly setup, complicated operation process. In some circumstances, the monitoring requires long time in-field measurement with highly integrative and inexpensive systems. For this reason, microfluidic technologies are emerging as effective tools for environmental monitoring. Microfluidics offers some prominent advantages to this application by improving detection sensitivity, reducing testing time, and allow in-field monitoring. Microfluidics can be used for separation and preconcentration target analytes from complex pollutant with high accuracy. Numerous detection method can be used by microfluidics, such as electrochemical detection, surface-enhanced Raman scattering, mass spectrometry [14].

2.2 Microfluidic cell separation

Cell is regarded as the fundamental unit of biological organisms. Although each cell shows apparent similarity with others in the same cell line, single cell analysis implies that these cells display different properties from many aspects. The cell-to-cell differences in RNA transcripts and protein expression is informative for biomedical research, diseases diagnostics and drug screening. Therefore, effective cell separation is of significance in many biological assays to obtain desired

cells within a heterogeneous population. For example, the ability of sorting individual cells is the first step of single cell sequencing, which extracts the sequence information from single cells, allowing the subsequent investigation of tumor evolution and chromosomal variation. Cell sorting also is the prerequisite in many diagnostic processes, such as the prognosis using circulating tumor cells (CTC) separated from peripheral blood of cancer patients.

Conventional cell sorting techniques include fluorescence-activated cell sorting (FACS), magnetic-activated cell sorting (MACS), laser capture microdissection (LCM), and manual cell picking [15]. FACS is the first commercial cell sorter introduced in the late of 1960s [16]. It can perform multi-parametric analyses of single cells based on size, granularity, and fluorescence. However, FACS requires a large number of cells for analysis, which is a prominent disadvantage when dealing with rare cells. In addition, the high pressure during FACS operation may stimulate cell mutation or reduce cell viability. MACS utilizes magnetic force to attract target cells, which are immunomagnetically labeled by magnetic beads, from a mixed cells population [17]. This technique has been widely used as a standard cell separation method. However, this technique can only separate cells according to the presence of specific cell surface proteins and performed in batch mode. Laser capture microdissection (LCM) provides an accurate and efficient method to isolate single cell from tissue or a large cells population [18, 19]. The cells of interest are first visualized using a microscope and a laser beam that coupled with microscope is focused on the tissue to cut the target cells out and separate them from adjacent cells. However, this separation method is laborious and requires well trained professionals. The manual cell picking method usually relies on a micromanipulator consisting of an inverted microscope and a micro-pipette mounted on a motorized stage. After determining the target cells via microscope, the operator can aspirate the cells using micropipette [20]. Although manual cell picking is a simple method, like

LCM, it is laborious and the throughput is very low.

Over the past decade, with the advance of microfabrication technologies, various microfluidic technologies have been realized for cell manipulation and analysis. Accordingly, a number of microfluidic expertise have presented comprehensive reviews on this topic [1, 21-43]. Continuous flow microfluidic technologies provide a compelling alternative for cell separation. Comparing with above mentioned conventional cell separation technologies, continuous flow microfluidic cell separation technology has a number of unique advantages: 1) owing to the miniaturized nature of microfluidic channel, microfluidics offers great control capability with high spatiotemporal precision for cellular handling on micrometer-scale, which is far beyond the ability of conventional bulk-scale methods; 2) since the cells can be continuously introduced into microfluidic devices and be continuously harvested from the end of the device, the cells separation process can be integrated with upstream and downstream cell manipulation and analysis processes. This integration not only reduces entire cells analysis time, but also prevents cell contamination. Moreover, cell functionalities can be well preserved in the integrative device; 3) given to its capability of processing small volume samples, microfluidic separation technology dramatically reduces sample cost and the invasiveness to patients for sample extraction; 4) thanks to the miniaturized device footprint and low fabrication cost, the microfluidic separation systems hold the potential of massive parallelization for high-throughput processing; 5) since numerous external fields (*e.g.* electric field, magnetic field, acoustic field) can be coupled with microfluidic devices, as well as countless structure designs have been implemented on microfluidic platforms, multiparameter cell separation can be achieved by taking advantages of these existing techniques.

According to working mechanism, continuous flow microfluidic cell separation schemes can be roughly categorized as active methods and passive methods. Active methods utilize external fields

to interfere the movement of cells in laminar flow to isolate the target cells from complex background. On the contrary, passive separation schemes relies on the interaction between cells, laminar flow and structures of microchannel. The two methods will be viewed with more detail in 2.3 and 2.4, respectively. Furthermore, a number of hybrid methods have emerged recently to combine the advantages of both active and passive methods for high throughput cell separation. The hybrid separation strategies will be discussed in 2.5.

Figure 2.1 A broad classification of microfluidic cell separation methods. Microfluidic cell separation can be primarily categorized as active methods, passive methods and hybrid methods.

2.3 Active cell separation in microfluidics

Active microfluidic cell separation exploits external fields exerting forces on target cells. Based

on the intrinsic properties of cells, such as electrical conductivity, optical polarizability, magnetic property, various force fields can be applied for cell separation.

2.3.1 Electric force-based separation

Electric force-based scheme is one of the first strategies used for microfluidic cell separation. It is suitable for cell separation due to the fact that a high electric field can be generated within the small dimension of microchannel. Dielectrophoresis (DEP) and electrophoresis (EP) are the mostly used electrokinetic effects for cell separation on microfluidic platforms.

Dielectrophoresis

In dielectrophoresis cell sorting device, an alternating current (AC) or direct current (DC) field is applied to polarize the cells. Due to the induced electric dipole moment across the cell, the electric field can displace the cells either toward or opposite the region. If the permeability of a cell is higher than that of the liquid medium, the cell will be attracted toward the location of the peak field. This phenomenon is called positive dielectrophoresis (pDEP). Otherwise, the cell will be repelled away from the peak field region, which is termed as negative dielectrophoresis (nDEP). Comparing with pDEP, where the strong electric field is a severe threaten for cells, the nDEP is favorable to preserve the functionality and viability of cells. Since the displacement effect depends on cell size and the electric permeabilities of cell and medium, DEP can be used for cell separation according to size and dielectric properties. And thanks to the fact that DEP separation relies on the cell intrinsic properties, this method is considered as a preferable strategy for label-free cell separation [44].

Figure 2.2. Working principle of continuous flow dielectrophoresis cell sorting. The electrodes are used to generate electric field. The cells with different surface charges subjected to the electric field experience different DEP forces, result in the isolation of target cells from initial cell mixture.

The time-average dielectrophoretic force acting on a spherical particle is given by [45, 46]:

$$F_{\text{DEP}} = 2\pi \epsilon_m r^3 f_{\text{CM}} \nabla E^2 \quad (2-1)$$

where E is the electric field, ϵ_m represents the absolute permittivity of the suspending medium, r is the radius of the cell, f_{CM} is the Clausius-Mossotti (CM) factor as given below:

$$f_{\text{CM}} = (\epsilon_p - \epsilon_m) / (\epsilon_m - 2 \epsilon_m) \quad (2-2)$$

where ϵ_p and ϵ_m denotes the permittivity of particle and medium, respectively. If $\epsilon_p > \epsilon_m$, the CM is positive, implying the DEP force shares the same direction with the electric field gradient. In this case, the DEP is termed as positive DEP. Otherwise, the DEP force is in the opposite direction of the electric field gradient. The DEP force is then called negative DEP.

Electrophoresis (EP)

Electrophoresis is a well-studied technique for charged particle separation and delivery. The working principle of EP is based on the Columbic force experienced by target cells due to their surface charge. Unlike DEP, where the electric field in the microchannel must be non-uniform, the

field in EP device is a uniform field. Due to the presence of charged lipid headgroups on cell surface, a cell can be taken as a negatively charge entity. Under the electrophoretic force, the charged cell migrates to the direction of positive electrode. The force experienced by the cell is proportional to its charge. Guo *et al.* [47] developed an electrophoretic cell sorting device through separating single droplet, which contains several cells, in continuous flow. Departing from conventional electrical charging droplet, which was charged through its interaction with electrode surface, their droplet generated by flow-focusing method showed “self-charged” behavior. Under the function of electric force, which was generated by a pair of well-arranged electrodes, a single droplet can be isolated from the mainstream to a side branch channel. In this device, they can sort single droplet within 15 ms using a square electric pulse with 6 ms width and 800 V amplitude.

2.3.2 Magnetic force-based separation

In the past few years, magneto-microfluidics has seen tremendous progress. This can be attributed to the technology advances and the prominent advantages of combining microfluidics with magnetism [48]. MEMS techniques have been applied for the fabrication of miniaturised magnets, enabling the integration of magnets and microfluidics at micrometre scale. The functionalized magnetic particles have been off-the-shelf only in recent years, providing more choices to the researchers. Compared to electric-force based separation method, the magnetic interaction does not affected by surface charges, pH, or ionic concentrations. In addition, magnetic field-induced particle manipulation is free of undesirable fluid heating, which always occurs in electric, acoustic and optical field actuated microfluidic devices [49].

The magnetic force acting on a magnetic particle in fluid can be determined by the magnetic energy density

$$e_m = -\mathbf{M} \cdot \mathbf{B} \quad (2-3)$$

$$\mathbf{f} = -\nabla e_m = \nabla(\mathbf{M} \cdot \mathbf{B}) = (\mathbf{M} \cdot \nabla)\mathbf{B} + (\mathbf{B} \cdot \nabla)\mathbf{M} + \mathbf{M} \times \nabla \times \mathbf{B} + \mathbf{B} \times \nabla \times \mathbf{M} \quad (2-4)$$

Since there is no electric current, $\nabla \times \mathbf{B} = 0$, $\nabla \times \mathbf{M} = 0$. Hence, the magnetic force can be written as

$$\mathbf{f} = (\mathbf{M} \cdot \nabla)\mathbf{B} + (\mathbf{B} \cdot \nabla)\mathbf{M} = \mathbf{f}_B + \mathbf{f}_M \quad (2-5)$$

Here, $\mathbf{B}$ and $\mathbf{M}$ are the magnetic flux density and magnetization. The magnetic field gradient $(\nabla\mathbf{B})$ gives rise to magnetic gradient force

$$\mathbf{f}_B = (\mathbf{M} \cdot \nabla)\mathbf{B} \quad (2-6)$$

and the magnetization gradient force

$$\mathbf{f}_M = (\mathbf{B} \cdot \nabla)\mathbf{M} \quad (2-7)$$

is generated by a gradient of magnetization $(\nabla\mathbf{M})$ [50].

For a magnetic particle suspended in a diamagnetic fluid in the presence of a uniform magnetic field, the magnetic force imparted on the particle stems from the magnetization mismatch between the magnetic particle and the diamagnetic medium. The mismatch leads to a magnetization gradient and hence a magnetization gradient force. The magnetic force is given by Rosensweig [51],

$$\mathbf{f} = -\frac{1}{2}V_m\mu_0\nabla\chi|H|^2 \quad (2-8)$$

where V_m is the volume of each magnetic particle, χ is the susceptibility of the diamagnetic medium, $\mu_0 = 4\pi \times 10^{-7} \text{NA}^{-2}$ is the permeability of the free space and H is the strength of the external uniform magnetic field. The magnetic force only acts on the interface where the discontinuity in magnetic permeability takes place [52]. Here, χ_p and χ_f are the susceptibility of magnetic particle and diamagnetic medium, respectively. For a magnetic particle ($\chi_p > 0$) in a diamagnetic medium ($\chi_f < 0$), the term $\Delta\chi = \chi_p - \chi_m$ is positive. Therefore, the particle can be attracted to the magnetic field [48].

In previous reports, various strategies have been exploited to magnetic field for particles and cells manipulation on magnetofluidic devices. Most of the strategies applied non-uniform magnetic fields. The application of non-uniform fields in magnetofluidics has been well studied and become a mature technology. However, it suffers from many limitations. For instance, (i) large magnets are required to generate high magnetic field, leading to the integration difficulty of the Lab-on-a-Chip (LoC) system; (ii) the magnetic field distribution in the microchannel is very sensitive to the position of the magnets; (iii) particle manipulation on LoC platform at lower magnetic fields is challenging [53].

Recently, utilizing uniform magnetic field in magnetofluidics have attracted researchers' attention. The uniform magnetic field possesses many prominent advantages for magnetofluidics, such as: (i) it does not require complex device design and fabrication for the integration of magnetic source with microchannel; (ii) since the uniform magnetic field-based magnetofluidics exploit the intrinsic susceptibility of particles or cells, it does not require the complex binding of magnetic particles to the non-magnetic entities; (iii) the uniform magnetic field can be provided by a bulky electromagnet, enabling wireless, programmable and remote control of the magnetic field. Because of these merits, uniform magnetic field on Lab-on-a-Chip (LoC) platforms has been used to investigate ferrohydrodynamic instabilities [54, 55], ferrofluid droplets [52, 53, 56-59], bacteria trapping [49], magnetofluidic mixing [60], and ferrofluid spreading [61].

Due to the simple configuration and implementation, uniform magnetic field can be used in magnetofluidic spreading (MFS), which is useful for various applications, such as micromixers and micro-chemical reactors. Wang *et al.* [61] studied the MFS in a three-stream flow system under the influence of a uniform magnetic field. In the system, a water based ferrofluid core was cladded by a diamagnetic buffer fluid. Because of the susceptibility mismatch between the ferrofluid and

the diamagnetic fluid, the magnetic field lines bent towards the ferrofluid, resulting in magnetic field gradients, and hence the magnetic forces on the ferrofluid. They also experimentally and numerically investigated various parameters that affected MFS within a uniform magnetic field, and concluded that the spreading can be mainly attributed to the cross-sectional convection induced by magnetic force [62].

The study of MFS is useful for the understanding of uniform magnetic field induced micromagnetofluid instability, which is advantageous for LoC-based mixing applications. Based on the studies mentioned above, researchers from the same group further investigated the instability-induced mixing of ferrofluids in uniform magnetic fields. In their three-stream fluid system, they demonstrated that a uniform magnetic field of only a few millitesla can give rise to substantial instability and mixing [63]. Moreover, they exploited the uniform magnetic field to trap non-magnetic bacteria in ferrofluid. They placed a non-magnetic elliptical pillar island in microchannel filled with ferrofluid. When an external uniform magnetic field was applied, the magnetic field in the microchannel was distorted by the island due to the susceptibility difference between the island and the ferrofluid. This distortion gave rise to gradient of magnetic field, leading to magnetic force. Therefore, the magnetic particles and bacteria accumulated at different region of the island, resulting in separation and trapping [49].

The manipulation of liquid droplet is of great interest for the field of droplet-based microfluidics. Researchers from Nguyen's group and Ramanujan's group have systematically investigated the effects of uniform magnetic field on ferromagnetic droplet and related applications [52, 53, 56-59]. Nguyen *et al.* numerically and experimentally investigated the ferrofluid droplet formation process [52, 56] and non-linear deformation [57] with the presence of uniform magnetic field. To facilitate the contact-free, wireless and programmable manipulation of droplet for LoC

applications, Ramanujan *et al.* employed uniform magnetic fields to induce the merging of ferrofluid droplets at various flow rate ratios. They found that the droplet merging were influenced by the droplet velocity and magnetic field strength. This study is significant for many LoC applications, such as wireless controlled droplet merging, mixing, Janus particle synthesis and biosensing [53].

Janus particles have drawn great attention due to their attractive combinations of properties, such as hydrophilic-hydrophobic, magnetic-plasmonic [64, 65]. However, controlled synthesis of Janus particles is a challenging task. Ramanujan *et al.* developed a high flow rate method to synthesize magnetic Janus particles (MJP) through the integration of droplet micro-magnetofluidics with hybrid magnetic fields. The hybrid magnetic fields consisted of uniform magnetic field and non-uniform magnetic field. The uniform magnetic field induced the formation of magnetic particle chains in the droplet. The magnetic phase inside droplet was further concentrated by the non-uniform magnetic field. Following the magnetic concentration, synthesis of Janus particles was conducted by photopolymerization [59].

Figure 2.3. Schematic illustration of magnetic force-based microfluidic cell sorter. A magnetic field is applied perpendicular to the flow direction. The immunomagnetically tagged cells are attracted by the magnetic source and move laterally across laminar flow.

Simultaneous sorting of multiple cells with high purity, recovery and throughput is in high demand. However, conventional magnetic-force based cell sorting can only separate cells according to the presence or absence of magnetization. To bridge this gap, Adams *et al.* developed a multitarget magnetic activated cell sorter, which can simultaneously sort up to three classes of cells at high purity. An external neodymium-iron-boron (NeFeB) permanent magnet and microfabricated nickel patterns were used as magnetic field sources. By exploiting permanent magnets, the cells were attracted to the bottom of the microchannel and subjected to the working region of short-range magnetic field. The two ferromagnetic strips were arranged with certain angles with respect flow direction, aiming to direct cells flow to designed outlets. Using this device, they demonstrated the capability of simultaneous sorting multiple cells types with 90% purity and >500-fold enrichment at a high throughput of 10^9 cells per hour [66].

2.3.3 Optical force-based separation

In 1970, Ashkin first reported the observation of optical force on micro-sized matters [67]. In the past decades, optical force has been intensively applied for micro-objects manipulation, including emerging particles separations on microfluidic platforms. Optical methods utilize highly focused light beam to exert optical forces on targets of interest. The Gaussian intensity profile of a laser beam can produce optical scattering and gradient forces on cells due to the mismatch in refractive index between cell and liquid medium. The scattering forces can be used to push cells away from their original trajectories, while gradient force holds the ability of attracting cells to the region of maximal optical strength. This method can separate cells based on size and refractive index. Moreover, many light parameters, such as wavelength, power density and trap pattern, can be modulated to achieve high selective separation. The advantages of using optical methods lies in its ability of remaining cell integrity, and precisely manipulating cells in three dimensions.

Wang *et. al* presented a microfluidic cell sorter integrated with optical tweezers for high accuracy cell sorting. They first took advantage of the laminar flow nature to focus the cells of interest at specific region. Then, under the assistance of an image processing technique, the target cell was recognized from the cell population. Following this detection, the optical traps were positioned on the target cell and move the cell to desired region. This method is distinctive for its high recovery rate and purity [68].

Interferometric patterns of light can create 3-dimensional optical lattices. The influence of optical lattice on particle is dependent on the relative polarizability of the particle. Exploiting the interaction between optical force and particles, MacDonald *et al.* developed a passive optical cell sorting method. They created a 3-D optical lattice at a junction of microchannel. When a mixture of particles passed through the lattice, the trajectories of selected particles were shifted due to the

high optical force, leading to these particles migrate to desired outlet. While other particles, which had low sensitivity to the optical potential, were not affected by the presence of the optical lattice. This optical lattice can separate particles by size and by the refractive index [69].

2.3.4 Acoustic force-based separation

The acoustic force generated in a microfluidic channel can provide precise spatiotemporal control and non-contact ability for cell handling. The separation of particles or cells can be performed in label-free mode, based on size and the acoustic contrast factor relative to the medium. Under a certain amplitude, the ultrasonic force will not bring any physiological damage to cells. So far, the bulk standing waves and standing surface acoustic waves (SSAWs) are the prevalent fashions used for microfluidic cell sorting.

Bulk standing waves

A bulk Acoustic streaming can be induced when the microchannel is excited by ultrasound to a resonance mode, in which the applied wavelength matches the dimension of the microchannel [35]. In terms of one-dimensional plane acoustic standing wave, the acoustic radiation force experienced by a particle subjected in the acoustic field is given by the following equation [70]:

$$F_{ax} = 4\pi a^3 E_{ac} k \sin(2kz) \Phi \quad (2-9)$$

where E_{ac} is the acoustic energy density, a denotes the particle radius, z is the distance from pressure antinode in the wave propagation axis, k is the wavenumber, and Φ is the acoustic contrast factor as defined as below:

$$\Phi = \frac{\rho_p + \frac{2}{3}(\rho_p - \rho_0)}{2\rho_p + \rho_0} - \frac{1}{3} \frac{\rho_0 c_0^2}{\rho_p c_p^2} \quad (2-10)$$

Where, ρ_p and ρ_0 are density of particle and fluid medium, respectively, c_p and c_0 are sound speed in particle materials and fluid, respectively.

Johansson *et al.* first reported the application of bulk acoustic waves for cell separation. A miniaturized piezoelectric transducer was integrated at the bottom of microchannel excited at 10MHz to generate acoustic standing wave acting on the fluid-fluid density interface. Conventional acoustic microsystem applied acoustic radiation force directly on particles, and the maximum displacement is limited to a quarter of a wavelength. However, through applying acoustic force on a density interface, they achieved up to 700 μm sideways displacement of 10 μm beads in microchannel [71].

Standing surface acoustic waves (SSAWs)

Different from bulk acoustic standing waves, SSAWs utilize interdigital transducers (IDTs), which is fabricated in the device substrate, to generate standing wave along the bottom of the microchannel. In conventional acoustic separation devices, the standing acoustic wave direction was parallel with the flow direction. With this scheme, the separation distance was very limited, resulting low separation efficiency and resolution. To overcome this shortcoming, the researchers from Huang's lab devised a microfluidic cell sorter utilizing titled-angle configuration, where the IDTs were placed with a titled angle with respect to the microchannel. Therefore, unlike the conventional fashion, the SSAW induced nodal lines were slanted at a particular angle to the flow direction. As the result of the competition between the acoustic radiation force and the laminar drag force, the migration distance of the particles in the microchannel was amplified several times comparing with that in conventional SSAW platforms. The increased particle migration resulted in enhanced separation resolution. In the SSAWs device, the separation of MCF-7 breast cancer cells from leukocytes was demonstration and a high separation purity of 84% was achieved [72].

Figure 2.4. Schematic illustration of working principle of a SSAWs-based cell separation device. The SSAWs are produced by interdigital transducers. Cells with different sizes experience different acoustic forces and follow distinct trajectories in the microchannel.

2.4 Passive cell separation in microfluidics

Unlike active methods that utilize external forces to drive cells separation, passive microfluidic cell separation relies on sophisticated microstructure arrangement and hydrodynamic effects, or the alliance of both strategies to achieve cells separation. Passive cell separation methods can be categorized into some subgroups.

2.4.1 Hydrodynamic Filtration

For a particle flowing in a microchannel, its center position cannot be present in a certain distance from channel wall, where the distance is equal to the particle radius. Based on this hydrodynamic phenomenon in laminar flow, Yamada *et al.* presented the concept of

‘hydrodynamic filtration’ [73]. The microfluidic device consists of a main channel and many small branch channels connected with the main channel. A particle mixture is introduced from the left side of the microchannel. Owing to the small dimension of the branch channel, only small portion of liquid flow out from the main stream at each branch point. Due to the fact mentioned above, the particles would not flow into the branch channels when the relative flow rates in the branch channels are small, but slightly shift toward the sidewalls. With the accumulation of flow loss in the main stream, the relative flow rates in the downstream branch channels increases, leading to the exit of the particles through the branch channels. Through precise design of the microfluidic channel, this method can achieve selective concentration of specific cells or other biological entities. To demonstrate the validity of this method, they performed leukocytes enrichment and achieved ~29-fold concentration enhancement in relation to erythrocytes. To verify the potentiality of this separation method used for clinical settings and biomedical research, the same group also demonstrated size-dependent liver cell separation utilizing hydrodynamic filtration with high throughput $>2 \times 10^5$ cells/min [74].

2.4.2 Pinched flow fractionation (PFF)

Pinched flow fractionation makes use of laminar flow characteristics in microchannel to continuously separate particles. In PFF device, a sample fluid and a sheath fluid are pumped into the microchannel simultaneously from two different inlets. The sample containing separation targets are focused by the sheath fluid in the “pinched segment”, which is a critical part on PFF device. A particle in laminar flow has the tendency to flow along the streamline passing through its center of mass. In the “pinched segment”, larger and smaller particles flow along different streamlines based on their sizes. When the streamlines enter the broaden segment, which connected with the pinched segment, this difference is significantly amplified by the streamlines spreading,

leading to the particles separation according to their sizes.

The first PPF microfluidic device was proposed by Yamada *et al.* [75], which has a symmetrical configuration in the broaden segment. They investigated the influence of the flow rate and channel shapes on the separation performance and expressed the particle effluent position Y in the broadened segment by the following equation:

$$Y = (W_p - D_p/2)W_b/W_p \quad (2-11)$$

where W_p and W_b represent the width of pinched and the broadened segments, respectively, D_p denotes the particle diameter. The separation resolutions depend on particle size difference, microchannel sidewall roughness as well as aspect ratio of microchannel. Through accurately controlling the ratio of sample to sheath flow, a same PPF device can be used for separation of cells of varying sizes.

Some modifications have been proposed to improve the PPF separation performance. For example, Takagi *et al.* [76] introduced an asymmetric PPF device with branched outlets were arranged asymmetrically about the pinched segment. This device used shorter and/or broader branch outlet to reduce the flow resistance, therefore most liquid flowed into the shorter/broader outlet. This arrangement resulted in asymmetrical distribution of particles in all outlets, which amplified the separation effect. To enhance sorting efficiency, Morijiri *et al.* [77] combined centrifugation effect with PPF for particle separation. The centrifugal force increased the separation resolution based on density. By adding a PDMS membrane valve in the outlet, Sai *et al.* [78] developed a tunable PPF sorter, which can control flow rate through the outlets by switching the valve on and off, leading to the variation of effluent position of particle. Compared with traditional PPF, the tunable PPF can significantly improve the particles separation accuracy.

Although PPF provides an easy cell separation method, this technique has to be operated in low

flow rates as the motion of particles can be affected by the inertial due to high flow rates, resulting in disturb of sorting efficiency.

Figure 2.5. Working principle of pinched flow fractionation (PFF). The PFF device consists of pinched segment and broadened region. The particles with different sizes are focused in the pinched segment. The alignment differences of various particles are amplified by the broadened region, leading to apparent separation of the particles according to their sizes.

2.4.3 Deterministic lateral displacement (DLD)

Huang *et al.* introduced the first Deterministic lateral displacement (DLD) microfluidics that takes advantage of asymmetric bifurcation for continuous particles or cells separation [79]. DLD is a steric separation approach exploits pillar array in microchannel to separate cells based on cell size. The periodic pillar arrays were arranged at certain angle with respect to flow direction. The space between two cylindrical pillars was set larger than the size of cell. When the cells flow in the microchannel, they interact with the physical obstacle and the symmetry of cell trajectory was

broken due to the presence of the array angle, leading to the lateral displacement of cell position. When the cells pass through the entire pillar arrays, all these minor displacements accumulated and resulted a macroscopic cell separation. Huang *et al.* claimed separation resolution can be down to 20 nm. They also demonstrated the feasibility of this approach by fractionating blood cells. Through changing the pillar to pillar distance, blood cells can be separated into different outlets in high throughput. The DLD method can be used for continuous separation of a wide range of cells sizes. However, the major drawback of this technique lies in the presence of massive obstructions that easily induce cell clogging and deformability.

The intrinsic advantages, such as high throughput, makes DLD method an attractive for various applications. The isolation of circulating tumor cells plays an important role in cancer prognoses and fundamental cancer metastasis study. Particularly, the efficient capturing of multicellular aggregates of CTCs (CTC clusters) is equally important as that of individual CTCs. The presence of CTC cluster in blood are correlated with survival rates decrease in cancer patients. However, the majority of current microfluidic CTC separation techniques are not capable for CTC cluster separation. To bridge this gap, researchers from Toner's group introduced an integrated DLD device for CTC clusters separation [80]. This device consists of two stage DLD separation part. The first stage was designed to remove isolate large CTC cluster from whole blood based on "standard" DLD size separation principle. The second stage consisting of an array of pillar with asymmetric shape was used to separate small CTC clusters come from the undeflected product of Stage 1. The asymmetric pillars increased small cluster capture efficiency by 64% compared with standard cylindrical pillars. Sorting of other biological entities, such as sleeping parasites in blood [81], exosomes [82], and deformable objects [83], also have been reported by using DLD methods with some necessary device modifications .

Figure 2.6. Schematic illustrating the working principle of cell separation by deterministic lateral displacement using periodic arrays of cylindrical posts. The posts are asymmetrically arranged in the flow direction to induce particles/cells with different sizes to follow different flow paths, resulting in lateral displacement and thus separation of particles/cells by size.

2.4.4 Affinity-based methods

Affinity-based microfluidic separation refers to the separation technique that relies on the specific interaction between target cells and some parts of the microfluidic device, which are immobilized with complementary molecules. The typical model is using specific antibody to modify the wall, bottom microchannel, or microposts located in the microchannel. When a heterogeneous cell population flows through the microchannel, the target cells will be tethered to the wall, bottom or microposts due to the specific interaction between the antibody and the complementary antigen expressed on the cell surface.

Researcher from Toner's group developed an affinity-based cell sorter for circulating tumor cells (CTC) separation [84]. They fabricated the microdevice on silicon wafer, which contains equilateral triangular array of microposts. To increase the interaction frequency between the

microposts and cells, the repeated patterns of the microposts arrays were vertically shifted for every three rows. The microposts were functionalized with anti-epithelial-cell-adhesion-molecule (EpCAM) antibodies, which held the specificity for CTC capture. The whole blood sample containing CTCs was pumped into the device. And most of the CTCs can be captured by the microposts through optimizing flow velocity and shear force.

Although advantages, the complex micropost array limited the scale up of the separation throughput. In terms of affinity-based separation, the frequency of the interaction between cells and biomolecular-coated microchannel surface is critical. High interaction frequency indicates high capture possibility. In order to further increase the interaction frequency whilst simplify device structure, the same group designed a herringbone-chip, which can generate microvortices to enhance the cell-antibody interaction, for high-throughput CTC separation [85].

2.4.5 Other passive methods

Besides the methods discussed above, many other passive methods have been reported. Microfabricated filters with precisely defined microscale structures can be used for size-based cell separation. The filters include four different formats: membrane, weir, pillar and cross-flow [24, 86]. Membrane-type filtration has been exploited for CTCs capture [87]. Weir-type filtration has been used to extract cell-free plasma for diagnosis [88]. Pillar-type filtration used spatially separated micropost array to exclude cells which are larger than critical microposts spacing [89]. Cross-flow filtration works under similar principle. However, the filters are positioned perpendicular to main stream flow to avoid clogging.

Zweifach-Fung effect, also known as bifurcation law, have been utilized for microfluidic cell separation. In this effect, a particle tends to follow the high flow rate channel at a bifurcation point. This phenomenon has been successfully applied to separate plasma from blood sample with up to

45% initial hematocrit [90].

Conventionally, the Reynolds numbers of microfluidic systems fall in the range of 1-100. Therefore, the inertial effects in microfluidic systems are usually neglected. However, some intriguing inertial effects recently have drawn much attention and been put into practical application [91]. The inertial forces in microfluidics can be used for particle focusing and size-based cell separation [92-95]. Numerous reports on the applications of inertial effects emerged in the past years. As one of the microfluidic devices presented in this thesis involves application of inertial effects, the detailed discussion about this phenomenon will be presented in **Chapter 4**.

2.5 Hybrid separation methods

Passive separation methods utilize intrinsic hydrodynamic phenomena to manipulate targets in microfluidic channels. However, the fixed layout of passive devices limited their range of operation for different samples. Active separation methods can precisely manipulate cells. However, the flow rates are usually very slow in active devices. Hybrid separation strategies combining active and passive methods have emerged to address the shortcomings and make use the advantages of both methods.

2.5.1 Dielectrophoresis-assisted hybrid methods

Dielectrophoresis (DEP) can combine with various secondary separation methods, including passive and active approaches. DLD separation method can be used for high resolution separation. However, the fixed micropost size reduced the flexibility of this device. To address this problem, Chang *et al.* presented a virtual DLD concept by using spot electrodes to replace the mechanical post array. Through adjusting the frequency and amplitude of AC electric field, the DEP-DLD microfluidic sorter can be used to separate particles with different sizes.

Zhang *et al.* proposed an integrated microfluidic device by coupling DEP with inertial force. They fabricated interdigitated electrodes to generate n-DEP force, which was used to modify the inertial focusing patterns in a serpentine microchannel. Using the tuned inertial force, they can levitate modify focused particle pattern in three-dimensional.

As an active separation method, DEP can be coupled with another active method to increase separation efficiency. Soh *et al.* demonstrated Dielectrophoretic-Magnetic Activated Cell Sorter (iDMACS)

2.5.2 Optophoresis-assisted hybrid methods

Optophoresis (OP) is considered as a non-contact and biocompatible cell separation method. This separation method holds the advantage of high separation resolution in three dimensions. A major issue of using OP for cell separation is the low throughput. To bridge this gap, some hybrid strategies have been introduced through combining the advantages of OP with other methods.

Due to the simple structure of Pinched flow fractionation (PFF) method, optophoresis can be easily coupled into PFF microchannel. As stated above, at the boundary of pinched area and broadened area, larger particles experience a force pointing to the center of the channel, while smaller particles are directed by a force toward the sidewall. The differences lead to slight separation in the pinched segment, which can be significantly amplified in the broadened region. Lee *et al.* presented an optophoresis-assisted PFF separation method, which exploited optical scattering forces to modulate the particle position in the pinched segment. This position modulation was further enhanced when the particles flowed into the broadened region, resulting in increased separation distance. This separation scheme was verified using three different beads with various diameters and up to ~15-fold separation distance enhancement was obtained [96].

2.5.3 Acoustophoresis-assisted hybrid methods

The first concern of using acoustophoresis (AP) is the low-throughput issue. The marriage of AP with other separation techniques can overcome this shortcoming. Although, reports on this combination is rare, some successful example have been presented recently.

Adams *et al.* devised an integrative microfluidic device consisting of acoustic and magnetic separation modules for multiparameter separation. This device integrated label-free acoustophoresis and affinity-based magnetophoresis separation in a serial manner. The injected multicomponent sample was first subjected to acoustic radiation force, followed by magnetic force in a same microchannel. Under the function of acoustic force, the target particles, including label-free particles and magnetically labelled particles, were separated from non-target particles. In the subsequent magnetic separation region, the magnetically labelled particles were further separated from the label-free particles [97].

More recently, researchers from Duck University developed a highly integrative microfluidic device, which was composed of three modules, for CTCs separation and downstream analysis. They used acoustic standing waves in the first module to focus the cells in a single flow stream, facilitating subsequent separation. In the second module, the focused cells were separated by magnetic force, where the target cells were deflected to the third module and captured by microwells for analysis [98].

2.5.4 Magnetophoresis-assisted hybrid methods

Magnetophoresis (MP) is one of the mostly studied cell separation methods. Due to its simple working principle and high operation flexibility, this method can combine with many other separation methods to achieve high efficient cell separation. In the past decade, numerous integrated cell separation technology combining with magnetophoresis have been reported.

Conventional batch processing method for CTCs separation suffers low yields and purity. With the advance of microfabrication techniques, the emerging microfluidic devices offer the enhanced abilities of handling cells with high accuracy. Researchers from Toner's group. recently reported an inertial focusing-assisted integrated CTC cell sorter, called CTC-iChip, which coupled size-based DLD separation and affinity-based magnetophoresis. This device consists of three sequential modules: size-based DLD separation module, inertial focusing module and affinity-based magnetophoresis module. In the DLD module, the CTCs and white blood cells were separated from red blood cells and platelets due to size differences. The separated CTCs and white blood cells were then aligned in a serpentine channel under the function of inertial force. The cell alignment enabled the cell deflection using minimum magnetic force. In the magnetophoresis module, the immunomagnetically labeled CTCs were deflected by the magnetic force and separated from white blood cells [99].

Similarly, Jiang *et al.* also reported a CTCs sorting device using DLD as the first module to separate CTCs from whole blood. Following an automatic purifying module, an affinity-based capturing dish coated with rat-tail collagen was used to capture the isolated and purified cancer cells. A positive capture rate of 83.3% of CTCs from blood of metastatic cancer patients was obtained on this device [100].

Yamada *et al.* presented a microfluidic platform integrating hydrodynamic filtration (HDF) and magnetophoresis for multiparameter cell separation. This device consists of a main channel interfaced with multiple branch channels, and permanent magnets positioned at the rear of the flow stream. Based on the concept of HDF, cells were sorted into different lane at the end of the main channel. In the presence of magnetic field, the magnetically labeled cells were attracted by the magnetic force and flowed into target outlets. Using human lymphocyte cells as demonstration,

they obtained >90% separation purity [101].

Due to its intrinsic simplicity, flexibility and versatility, the combination of magnetophoresis centrifugal separation has been developed [102, 103]. For example, Kirby *et al.* first introduced magnetophoretic separation onto a centrifugal microfluidic platform. The separation was governed by different parameters and forces, such as disc rotation speed, particle diameter, geometrical arrange of microchamber. The centrifugal disk was rotated by a spin-stand motor to induce push the particles into focusing channel, which was exposed to the magnetic field generated by on-chip permanent magnets. At the end of focusing channel the magnetic particles was deflected toward to magnets and separated from non-magnetic particles [102].

2.5.5 Other hybrid methods

Most of existing microfluidic devices rely on external pump or power source to drive sample flow in microchannel. Huh *et al.* developed a hybrid microfluidic sorter exploiting gravity force and hydrodynamic separation. Sample and buffer were introduced into a focusing channel that is positioned parallel to gravity. Under the function of gravity force, the sample and buffer flowed downward and turned 90° to a broadened channel, where the differential sedimentation of particles was induced by gravity force. Due to the intrinsic property of laminar flow, the gravity induced particle separation was further amplified by hydrodynamic force when the streamlines continuously expanded [104].

Hsu *et al.* reported a microvortex manipulator (MVM) that utilized the interplay of helical flow, buoyant, and gravity force for particle separation. They found that the helical flow induced by channel surface patterns owned the ability to focus particles at certain positions. After passing through these patterns, particles were remarkably focused to single or multiple streamlines. Based

on this fact, a density-based separation method was developed and used to separate two bead populations whose density difference was as small as 0.1 g cm^{-3} [105].

White blood cells play an important role in innate immunity. Effective separation of white blood cells from whole blood specimen is critical for diagnostic testing and biomedical study. However, successful examples of high efficient such separation on microfluidic devices had been rarely reported. Herein, Li *et al.* devised a hybrid microfluidic cell sorter exploiting crossflow filtration and membrane filtration to achieve WBC separation from unprocessed whole blood. The device consists of top and bottom microchannels which were separated by the microfiltration membrane. The crossflow pushed the red blood cells pass across the microfiltration membrane to the bottom channel, while the WBCs remained in the top channel due to their larger sizes. This device achieved high recovery rate ($27.4 \pm 4.9\%$), high cell purity ($93.5 \pm 0.5\%$), as well as high throughput (1 mL h^{-1}) [106].

2.6 Paper-based microfluidics

Low-cost and flexible microdevices are attracting much attention for the applications in biomedical engineering, energy generation and storage, consumer electronics and industry sensors. In the past decades, we have witnessed great progress in fabrication techniques of such devices. Using readily available materials and simple manufacturing procedures is a requirement for fabrication of economic, flexible devices. To that end, papers and other porous substrates have offered remarkable advantages in cost and availability.

The first paper based bioassay can date back to 1957, where glucose level in urine was detected by a strip assay paper [107]. The well-known example is the dipstick pregnancy test developed in

1980s. Microfluidic paper-based analytical devices (μ PADs) was first introduced by Martinez *et al.* in 2007 as a promising technology for point-of-care diagnostics [108]. The device was patterned by photolithography technique for urine and protein testing using colorimetric method. Recently, the development of microfluidic μ PADs has witnessed explosive growth due to its great potential applications in a number of fields [108-119], such as biomedical research, environmental monitoring, and point-of-care diagnostics. Comparing with traditional microfluidic analytical devices which use silicon, glass and polymer as structural materials, the μ PADs, fabricated on paper substrates, offer a much more affordable choice for research and practical applications. Using paper as substrates offers many advantages: 1) paper is abundant, inexpensive and disposable, making the paper-based devices can potentially be mass-produced; 2) the operation on the paper substrate can be simple and robust; 3) Paper holds the ability to process large sample volumes in a planar format; 4) the intrinsic capillary property of paper can be used for liquid sample delivery, without necessitating external pumps, which will dramatically reduce the device footprint and operation complexity; 5) paper is compatible with many fabrication methods.

The basic format of paper-based microfluidic devices is the hydrophobic-hydrophilic networks. The one-dimensional capillary flow occurs in cellulose paper can be described by Washburn equation [120]:

$$L = \sqrt{\frac{(\gamma r \cos\theta)}{2\eta}} t \quad (2-12)$$

where L is the penetration distance, t represents the time of penetration, r denotes the average radius of micropore, η and γ are the viscosity and surface tension, respectively. The contact angle between liquid and solid is represented by θ . This equation indicates that liquid penetration will not occur when contact angle is larger than 90° (*i.e.* hydrophobic surface).

In the past years, many techniques have been reported for Paper-based microfluidic devices fabrication, the major methods include photolithography, wax printing, laser cutting, craft plotter cutting.

The key step in fabricating μ PADs is to construct hydrophobic barriers on a paper substrate. To achieve this goal, various strategies have been introduced in the past years. Current fabrication methods for making μ PADs have been well-reviewed elsewhere [109, 115, 118, 121, 122]. In short, photolithography and wax printing are the most commonly used strategies. Each of these methods has its own advantages and limitations. Photolithography is the first technique used to fabricate μ PADs, where required consumables and facilities are generally expensive. Wax printing exploits commercialized wax printer to print wax on paper substrate, then utilizes hotplate to melt the wax to spread the wax into the paper [123]. Spreading of the wax is difficult to control during heating. Other fabrication methods were also reported, such as laser ablation [124], programmable paper cutting [125], flexography printing [126] and Parafilm embossing [127].

Photolithography The critical step of fabricating μ PADs is to create hydrophobic barriers confining hydrophilic paper channel for directional liquid delivery and confinement. Photolithography is the first technology used for paper-based microfluidic devices fabrication, where photo resist was employed to form hydrophobic barrier. However, the involvement of complex fabrication process, costly equipment, organic solvent and photoresist compromised the affordable merit of μ PADs.

Wax printing To further simplify the fabrication method, researchers from US and China developed several techniques using wax as hydrophobic material [123, 128]. Wax printing is a more advantageous strategy used for μ PADs fabrication. It comprises of two major steps: (i)

printing wax patterns on paper substrate; (2) melting the wax to penetrate into the paper to form hydrophobic barriers [123]. In contrast to photolithography, this method is cheaper and environment friendly due to the exemption of costly clean room facility and organic solvent.

Inkjet printing Abe *et al.* developed a A paper substrate was soaked with polystyrene toluene solution. Once the toluene solvent was printed onto the coating, the hydrophilic part was etched out [129]. Similar with wax printing, the desired patterns can be easily designed on computer and rapidly printed on paper substrates. This technique can also pattern biomolecules and analytical indicators into certain parts of the paper substrates to create biochemistry sensing device.

Plasma etching A similar concept as inkjet printing was proposed by Li *et al.* [130] using plasma to etch hydrophobized paper to create hydrophilic channel and reaction zones. Alkylketene-dimer (AKD) was used to transfer the paper to be hydrophobic by chemical reaction. Metal masks were used protect the hydrophobic barriers and expose the desired channels. In plasma treatment, the exposure areas were oxidized by the plasma while other parts remained hydrophobic.

Craft cutting Different from patterning hydrophobic barriers mentioned above, creating physical boundaries is another feasible approach to fabricate μ PADs. Fenton *et al.* utilized a programmable *x-y* knife plotter to cut various patterns on paper substrates. To increase the device mechanical strength, transparent polymer films were used to sandwich the paper strips [107]. The cutting method holds many apparent advantages. First, high fabrication reproducibility can be obtained using this method. In addition, the desired pattern can be rapidly fabricated in single step. Moreover, the fabrication cost is very low and does not required complex facilities except a computer controlled craft cutter, which is inexpensive.

Laser cutting Laser fabrication technology has been applied in a number of industrial fields. Base on the its high power or intensity, laser can cut many materials, such as metal, leather, glass

and ceramic. Similar as craft cutting, laser can also be used to create physical boundaries to define hydrophilic patterns on paper. Some groups reported the applications of laser as a knife to cut paper substrates for μ PADs [124, 131]. Following the predesigned pattern, the laser head that was controlled by computer generated high light powder to ablate the paper, resulting physical boundaries along the desired patterns.

Besides the mainstream methods mentioned above, a number of interesting methods have been introduced recently. For instance, Yu *et al.* reported a fabrication method using Parafilm embossing method to form hydrophobic barriers. The predesigned patterns were printed on a transparent film and transfer to a Parafilm, which was covered by a layer of UV (ultraviolet) photosensitive ink, after UV exposure by a 12 W UV lamp. Following this step, the Parafilm was immersed into alkaline solution for development. Then a filter paper was laminated with the Parafilm bearing with patterns and placed on a hot plate for $\sim$ 2-5 min. After heating treatment, the melted Parafilm fully penetrated into the filter paper forming hydrophobic barriers, while the photoresist patterns prevented the penetration in desired areas, forming hydrophilic channels and reaction zones [127].

Direct spraying hydrophobic materials on porous substrates provides yet another low-cost, simple route to create hydrophobic barriers. However, reports about its application for fabrication of microdevices are limited to date. Matthew *et al.* presented a simple method for fabricating gradient generator on glass slide using hydrophobic spray [132]. Firstly, they masked a rectangular region on a glass slide using a strip of tape. Then they applied hydrophobic spray on the glass slide surface. After 2 days drying, the tape mask was removed to expose hydrophilic stripe surrounded by hydrophobic boundary. Liu *et al.* exploited hydrophobic spray method for fabricating plasma separator [133]. They sprayed a commercial water repellent product on a 3D-printed substrate to form hydrophobic

barriers. Thara *et al.* first introduced a concept of spraying method for fabrication of paper-based microfluidic devices [134]. They used a laser-cut iron mask to pattern hydrophobic regions and protect the remaining hydrophilic region in the paper. Then commercial lacquer was manually painted around the mask to make hydrophobic barrier. While these demonstrations have shown the potentials of direct spraying for low-cost, simple and fast patterning of materials, further improvements shall be needed for practical applications.

2.7 Paper-based microfluidics for sample preconcentration

Paper-based biomedical assays have been indicated as a promising technique for point-of-care diagnostics. However, the poor limit of detection (LOD) ability of these devices is often criticized. In most diagnostic circumstances, such as early diagnosis of cancer, the protein markers are usually present at very low concentrations. This fact poses a real challenge for paper-based biomedical assays and limits their applications for diagnostic applications. To address this issue, a variety of strategies have been proposed to improve detection limits of paper-based assays [135-140], among which electrokinetic methods provide an effective and simple solution.

The first application of electric field-assisted paper assay was the electrophoresis implemented on paper strip. Electrophoresis refers to the differential movements of charged molecules or ions with different charge and mass in an electric field. Electrophoresis has been widely used many fields of biomedical sciences, such as protein separation, DNA analysis. In 1950s, Kunkel and Tiselius invented a paper-based electrophoresis for protein separation. The use filter paper strip as substrate, which was sandwiched by two glass plates to prevent evaporation. The two ends of the paper strips were immersed into two electrode vessels to absorb liquid. The feasibility of this method has been verified by various of serum protein separations [141]. Recently, electrophoresis

has been coupled with μ PADs to expand its application. Ge *et al.* first introduced electrophoretic separation into μ PAD with an electro-generated chemiluminescence (ECL) detector. This paper-based electrophoretic separation device was featured with low-cost, low-power requirement and high portability [142]. Chen *et al.* demonstrated electrophoretic separation in a Y-shaped channel powered by 3D fluidic batteries [143]. However, the high voltage involved in the above devices undermined their practical applications possibility. To reduce the voltage required for electrophoresis in paper device, Luo *et al.* presented a μ PAD coupled with electrophoretic function for protein separation, requiring only ~ 10 V electric voltage. The device was fabricated in 3D format using origami method and fast separation of fluorescent molecules and serum proteins can be achieved in 5 min [144].

In the past decade, electrokinetic effects have been exploited in μ PADs for sample preconcentration. Isotachopheresis (ITP), electrophoresis (EP) and ion concentration polarization (ICP) are the two major electrokinetic preconcentration and extraction techniques achieved in paper platforms.

ITP is a non-linear electrokinetic analytical technique that is widely used for sample preconcentration and separation in capillaries and microfluidics. In ITP, the ionized sample migrates between the leading electrolyte (LE), which has a high mobility, and a trailing electrolyte (TE), which has a low mobility. Due to the electrophoretic effect, the target analytes gradually accumulate around the LE and TE boundaries, leading to a dramatic increase of analyte concentration in a thin band. To improve the detection limit of paper-based immunoassay, Moghadam *et al.* developed an integrative paper-based microfluidic device using isotachopheresis effect. In their sample pre-concentrator made of nitrocellulose membrane strip, they achieved up to 900-fold sample enhancement within 5 min. And the extraction factor achieved was more than 80%

in a large sample volume ($>100\ \mu\text{L}$) [145]. In another lateral flow assay, they achieved two orders of magnitude sample enhancement using isotachophoresis effect [146]. Rosenfeld and Bercovici presented a paper-based microfluidic device for sample focusing using isotachophoretic effect. They used a shallow channel for sample delivery and concentration. Managing Joule heating is a challenging task when combining electric field with μPADs . A shallow channel offered high ratio of surface to volume, enabling fast heat dissipation. Therefore, this device can dramatically reduce evaporation influence at high temperature. Using this device, around 1000-fold enhancement in peak concentration was achieved in several minutes [147].

ICP is an electrokinetic phenomenon that ion concentration was polarized across the interface of microchannel and nanochannel. Ion selective membranes are usually exploited to induce ICP in microfluidic devices. Very recently, ICP has been accepted as a promising sample pre-concentration technique and was coupled with μPADs for POC applications. The first coupling of ICP with μPADs was introduced by Gong *et al.* to achieve target sample concentration and directional transport [148]. They fabricate microfluidic channel on chromatography paper using wax printing method and deposited Nafion nanomembrane in the paper channel. External electric voltage was applied across the channel to induce ICP in the channel, which created a depletion region and pushed the negatively charged fluorescent tracer to move toward anode side. This paper strip achieved 22-fold concentration and up to 88% transport efficiency of a fluorescent tracer. Later on, they extended this application for paper-based DNA analysis. Using the similar paper-based devices mentioned above, they preconcentrated hepatitis B virus (HBV) DNA in human serum with LOD of 150 copies/mL, verifying the capability of this device for early diagnosis of HBV. In addition, the results of fertility assessment obtained on this device correlated well with those obtained using standard method. This device demonstrated its potential for rapid and

inexpensive DNS analysis for global health applications [149]. Based on ion concentration, Han *et al.* presented paper-based microfluidic biomolecule preconcentrator. Maintaining steady flow in paper channel is critical to achieve high preconcentration effect. Therefore, an absorbent pad was connected with one end of the paper channel to keep steady lateral flow, which can continuously deliver analytes to the preconcentration region. Using fluorescein dyes as sample, they achieved up to 1000-fold sample enhancement [150]. Yeh *et al.* fabricated a shallow paper channel using wax printing for sample preconcentration. They printed wax from both sides of paper substrate and inserted the substrate into a temperature-controlled laminator to carefully control the heating effect, allowing the wax penetration to be accurately adjusted. The shallow allowed applying high driving voltage across the channel to induce ICP effect. The concentration factor was enhanced from original 130-fold in full-thickness channel to 944-fold by the shallow channel [151].

2.8 Enclosed μ PADs

Microfluidic paper-based analytical devices (μ PADs), first proposed by Whitesides and colleagues [108] have received growing attention due to their excellent features, such as affordable, disposable and ease of fabrication. These paper-based assays are excellent candidate techniques for POC diagnostics [118, 121]. Despite these, there are still a lot of room to improve the performance of the existing μ PADs. Due to the traditional fabrication characteristics, most of the channels of μ PADs are exposed to the atmosphere. This open structure results in some drawbacks: 1) the sample in the open channel are easy to be contaminated; 2) the μ PADs suffer great liquid loss due to evaporation; 3) in some operations, the paper devices must be suspended in air to avoid contacting with any other solid surfaces to reduce contamination risk, however, this would increase the operation complications [152].

To address these issues, sealing techniques are required to enclose and protect the paper devices and several packing strategies have been proposed so far [153]. For example, Martinez *et al.* [152] introduced a fully enclosed μ PAD which was sealed by hydrophobic toner. They printed toner on both sides of a μ PAD using a color laser printer to create hydrophobic layers, which could effectively protect the reagents stored on the paper chip. They also used toner as adhesive layer and stack two single-ply channels on top of each other to construct an enclosed configuration, which can improve liquid sample delivery speed [154]. Fenton *et al.* [107] presented a method for paper-based lateral-flow test strips fabrication. They sandwiched porous nitrocellulose materials between vinyl cover tape and polyester plastic films to construct test strips. The packed device held the ability of reducing evaporation and protecting reagents from contamination. Fan *et al.* [155] developed laminated paper-based analytical devices (LPAD) by sandwiching paper strips between two polyester films. The bottom film was used to protect the strips and provide mechanical backing, and the sample could be loaded from the window located at the top film. To prepare the paper strip, they fixed chromatography paper to an adhesive carrier sheet and cut the paper using a cutting plotter. Renault *et al.* [156] described a method to fabricate hemichannels and fully enclosed channels. They printed wax pattern first on the top side of filter paper, and then turned over the printed paper and manually fed into the printer for printing on its bottom side. An interesting method that using triboelectric charges to seal and control the flow in paper-based analytical devices was introduced by Silva *et al.* [157]. They used poly(ethylene terephthalate) (PET) film to seal a paper channel and rubbed the PET surface against acrylic or Teflon surfaces to generate triboelectric charges, leading to fluid delivery delay and evaporation decrease. However, the triboelectric charges cannot main for very long time. Hence, the device can minimize fluid evaporation only within short period.

Although advantageous, these reported sealing strategies have some drawbacks of their own. The adhesion of tape to paper substrate will severely deteriorate when the channels are wet. The printed toner provides good sealing effect but is not able to provide mechanical support for the paper channels. Many sealing methods are based on wax printing, however, spreading of the wax is difficult to control during heating, and incomplete melting of wax could lead to sample leakage. In addition, some sealing methods involve laborious fabrication or operation process.

Another challenge in μ PADs applications is to improve their sensitivity. Since that detection sensitivity is critical for POC applications, numerous approaches have been developed to tackle this challenge, such as field-amplified sample stacking (FASS) [135, 136], isotachopheresis (ITP) [137, 138], and temperature gradient focusing [139, 140]. Very recently, ion concentration polarization (ICP) technique has been introduced onto μ PADs as a sample pre-concentration approach with the aim to increase detection sensitivity [158, 159] or to integrate with a droplet microfluidic platform for bioassay [160, 161]. ICP is a fundamental electrokinetic effect that occurs at the interface of ion-selective nanostructures (*i.e.* nanochannels, Nafion junction formed by resin, or off-the-shelf membrane [162]) under the function of external electric field [163]. Based on ICP technique, Gong *et al.* [149] presented direct DNA analysis on paper-based microfluidic devices, where they concentrated and detected DNA samples on paper-based ICP devices and achieved comparable performance to the standard clinical equipment. Yeh *et al.* [151] improved ICP concentration performance by reducing paper channel depth, which resulted in decreased electroosmotic flow (EOF) speed. Phan *et al.* [125] demonstrated a 60-fold ICP pre-concentration enhancement on a paper microfluidic device cut by electric craft cutter. To maintain a steady fluid flow, Han *et al.* [150] integrated an absorbent pad on their paper ICP device and achieved up to 1000-fold pre-concentration factor.

Chapter 3

Magnetofluidic Device for Cancer Cells Separation

3.1 Background

3.1.1 Circulating tumor cells

Cancer has claimed the lives of many thus leading to increase interest in cancer diagnostics and anti-cancer strategy. Cancer cells can spread from the primary tumor to secondary sites via the bloodstream in the form of Circulating Tumor Cells (CTC) [164] in a process known as metastasis. Metastasis related disease, instead of direct effects of the primary tumor, is responsible for approximately 70% death of cancer patients [165]. A tumor can release millions of tumor cells into the circulation system in one day. Even though most of the cells cannot survive, the tiny minority of the cells are lethal to life. The first identification of CTCs can date back to 1869, when an Australian physician named Thomas Ashworth first observed CTCs in the blood of a man suffered metastatic cancer. CTCs can act an informative biomarker for the investigation of metastasis process, which is composed of series of complex events. However, the rare presence of CTCs in bloodstream poses a hinder for CTCs study, particularly for CTCs capture. The available CTCs can be derived from an average male patient are less than 200 per milliliter. In contrast, the ordinary white blood cells account for approximately 7 million/mL, and red blood cells account for approximately 5 billion/mL. Therefore, to isolate those exceedingly rare cancer cells from a background of millions of blood cells is a challenging task.

3.1.2 Microfluidic approaches for CTCs separation

Although CTCs have been identified for more than 100 years, reliable and reproducible technologies for CTCs capture and detection emerged only in recent years. Numerous research programs aim to identify the processes involved in metastasis. However, methods to effectively remove CTC from the circulatory system are limited [166]. As such, the detection and eradication of CTC from the circulation is an important complementary anti-cancer therapeutic strategy.

Microfluidics naturally offers an effective solution for CTCs capturing based on some unique advantages. First, on microfluidic platform, both physical and biological parameters of cells can be exploited for CTCs separation. In addition, comparing with bulk-mode separation methods, microfluidic approaches can handle the cells in more gentle manner, enabling the preservation of cell functionalities. Third, microfluidics provides an opportunity to integrate separation function with downstream cell assays, opening an avenue for the development of highly integrative, versatile and portable POC facilities. Recent progress of microfluidic CTCs separation can be found in some comprehensive reviews [27, 29-31, 38].

Microfluidic CTCs isolation technologies relies either on CTCs' biochemical properties (the biomarker expressed on cancer cell surface), or on their biophysical characteristics (*e.g.* cell size, density, surface electric charges). The two methods can be termed as affinity-based label-free isolation, respectively. No matter using which strategy, the method should meet the three imperative criteria: 1) high recovery, 2) high purity separation and 3) high throughput.

CTCs have relative larger size than those of blood cells. Specifically, the diameter of a CTC cells usually falls in the range 13-25 μm , while the diameter of a blood cell varies from 8-11 μm . This physical property can be used as biomarker to separate CTCs from blood cells [38]. Based on cell size differences, filtration methods and hydrodynamic methods have been developed.

The filtration methods employed membrane (with micropores ranging from 7-8 μm), micro-pillars, and microfabricated pores to exclude the CTCs from complex blood cells background. Although this method is straightforward and low-cost, clogging often occurs in the microstructures, leading to low throughput. Moreover, a small portion of CTCs show small diameters, causing the loss of CTCs during capture. The hydrodynamic methods make use of various passive techniques for CTCs separation, for example, deterministic lateral displacement, and dean flow fractionation. Dielectrophoresis has also been used for CTCs separation based on cell surface charges. However, the selectivity and throughput of this method is not satisfying [167].

Affinity-based isolation takes advantages of the affinity of antigen to its complementary antibody. Antigens expressed on CTCs surfaces can be targeted by specific antibodies, which was immobilized on solid surfaces before. This principle has been adopted in CellSearch and CTC-chip. In microfluidic devices, antibodies were immobilized on the surfaces of microstructures (*e.g.* channel walls, microposts) in microchannel. When CTCs pass through these microstructures, the antigens (and hence the CTCs) stick to their surfaces through antigen-antibody interaction. The CTC-chip is the first technology that exploited affinity principle for CTCs separation [84]. Afterward, a number of devices have been introduced using based on the affinity method, such as herringbone chip [85], graphene oxide chip [168].

Immuno-magnetic capture based on affinity principle is a popular strategy for CTCs isolation. In this method, magnetic particles functionalized with antibodies are used to grab target cells. Then the external field can exert force on the particles (and hence the cells) to separate them from heterogeneous cells mixture. The MACS (magnetic activate cell sorter) mentioned before is one example of this technology, which works in bulk manner. The integration of magnetic manipulation with microfluidics, which can be called magnetofluidics, has effectively expedited

the development of cell separation [41, 50].

Figure 3.1. Microfluidic CTCs separation methods. CTCs isolation methods can be primarily categorized into two classes: (A) Affinity-based methods, which utilize the biochemistry properties of CTCs, and (B) biochemistry properties-based methods, which take advantage of biophysical properties of CTCs.

Magnetic particles [169] can be manipulated remotely with magnetic forces induced either by a permanent magnet or an electromagnet placed in close proximity. There are several biological related applications for an integrated magnetic Lab-on-Chip (LOC) system which includes cancer cell destruction[170], virus detection[171], immunoassays[172], protein analysis[173] and cell separation[174, 175]. Magnetic beads have become an essential part of these magnetic devices as

they can be easily functionalized by tagging with desired biomolecules such as DNA, antibodies, proteins and cells[48, 176]. After which, they can be manipulated and separated [177, 178] based on the behavior of the magnetic fields applied.

Magnetofluidic devices utilizing both fluid flow and magnetic fields, have detected and isolated magnetically labelled CTC cells within the bloodstream [179-182]. This magnetic manipulation exploits external magnets that sorts and detects magnetically tagged molecules and cells becoming a well-known technique in bio-technology [183, 184].

3.1.3 Microelectromagnetic separation

Most reported magnetic separation schemes utilize bulky permanent magnets, which can generate strong magnetic field. However, the bulky permanent magnets suffer from a number of limitations. Their dimensions are usually much larger than microfluidic channel, increasing the difficulty of building a highly compacted system. In recent years, there has been an increasingly strong interest in coupling microelectromagnets with microfluidics for cell manipulation. Although the field magnitude generated by microelectromagnets is smaller than that of permanent magnets, this shortcoming can be remedied by the unique merits of microelectromagnets. The microelectromagnets hold the ability to control the magnetic field with high flexibility and temporal accuracy. Based on the relationship between magnetic field and electric current, the magnitude and direction of the magnetic field can be tuned by varying electric current, which is easily controlled. In addition, the dimension of microelectromagnets is comparable with the size of microchannel, facilitating the generation of high magnetic field at microscale.

Microelectromagnetic separation works on the principle that a magnetic field can be created by electric current when it passes through a conductive wire. The magnetic field direction can be judged by right-hand rule. The field magnitude is in the proportion to the current and can be

described using well-known Biot-Savart law [185]:

$$\mathbf{B} = \frac{\mu_0 i}{4\pi} \int \frac{d\mathbf{l} \times \mathbf{r}}{r^3} \quad (3-1)$$

where r is the distance from the conductive wire, $\mathbf{r}$ indicates the displacement vector from the wire element to the point at which the field is calculated, μ_0 is the vacuum permeability, i represents the steady current flow in the conductive wire, and $d\mathbf{l}$ is a vector of the differential element of the wire with a direction that follows the current.

Ahn *et al.* presented one of the first examples of microelectromagnets on microfluidic platform [186]. A meander-shape inductor with fully integrated coils was implemented as the core functional component on microfluidic platform. When 500 mA DC current was applied to the inductor, the coils generated electromagnetic field to separate magnetic particles from mainstream flow. The particles first clumped on the electromagnets surface and was then released by turning off the applied current.

Joule heating is a common concern for electromagnets. Song *et al.* presented a microfluidic device implemented with microelectromagnets for cell separation. A cooling channel was fabricated in the device to dissipate the Joule heat from the electromagnets. Creatively, the dissipated heat was used to maintain the biocompatible temperature of approximately 37°C in the device. Immunomagnetic beads and human Jurkat cells were introduced from two separated inlets and mixed under electromagnetic field, resulting in the immunomagnetically labelled cells were separated in the vertical direction [187].

3.1.4 Motivation

Magnetophoresis is the marriage of magnetism and microfluidics [188], which is the concept used in several instances, such as the implementation of permanent magnets in magnetic separation systems [189-191]. The applications of magnetophoresis for the separation of magnetic

particles have been also reported extensively [176, 192-196]. Some researchers utilize ferromagnetic structures with various geometries [197-199] to generate a magnetic field to direct a stream of magnetic particles towards a desired outlet. These methods are widely investigated, however, an alternate method would be to use current conducting electro magnets in the form of micro-coils [200] and micro-strips [201-204] to generate a magnetic field. Few have used such an approach to fabricate an integrated magnetofluidic device with a tunable current generated field to isolate magnetic particles. The tunable nature of the field allows for magnetic particle to flow freely without causing sedimentation in the microchannel obstructing the flow of the fluid, which happens when permanent magnets are used. Isolation of particles using an integrated tunable magnetic field is scarcely explored. Although a number of microelectromagnets have been integrated with microfluidics for cell manipulation, the separation of cancer cells in continuous flow has been rarely reported.

In this study, an integrated portable magneto-fluidic microchip that allows for portability and low cost of fabrication was designed to isolate HeLa cells that are tagged with magnetic microparticles. Under the influence of an applied magnetic field generated by the current carrying microstripline, the HeLa cells are expected to be driven to a desired outlet. The movement of the cells will be controlled by the flow dynamics of the microchannel as well as the strength of the applied magnetic field.

3.2 Design principle

The microchannel design consists of 3 inlets, 2 outlets and a straight channel with 500 μm width, 8 mm length and 50 μm height. The 3-inlets system is used to introduce cell sample and sheath flow for sample focusing. The cells are introduced from the center inlet and the sheath flow are

introduced from the other side inlets. Magnetic beads with diameter of 8 μm are used to label the cells through antigen-antibody interaction. The microstripline is embedded in the silicon substrate and used to generate electromagnetic field when electric current pass through. As the sample and sheath flows are pumped into the channel, the cells will be focused at the center of the channel. Once the focused cell streaming flow is subjected to electromagnetic field, the magnetically labelled cells will be deflected by the magnetic force toward the microstripline and finally flow out from the upper outlet, thus achieving a continuous cell isolation.

Figure 3.2. Schematic of the of the device design consisting a microchannel and a microstripline as two major functional components. The width of the microstripline is 100 μm . The two parallel wires configuration can enhance the electromagnetic field.

3.3 Numerical study

In the theory of fluids at the microfluidic level, it is known that the mass transport within

the fluid is dominated by the viscous effects, while the inertial effects are negligible in comparison due to the fundamental property in the nature of fluid flow, the Reynold's number, R_e . In a microfluidic device, the $R_e \ll 1$, which linearizes the Navier-Stokes Equation to the Stokes Drag Equation given by:

$$F_{drag} = \frac{1}{8} C_D \pi A \rho_{fluid} v^2, \quad (3-2)$$

where C_D is given as the dimensionless drag coefficient, A is the cross sectional area of the object, ρ_{fluid} is the density of the fluid, v is the speed of the object relative to the fluid.

In magnetic sorting theory, the magnetic force is a volume based force and at the microscale, it is not suitable for particle isolation using a permanent magnet as it will cause particles to be trapped and cannot be sophisticatedly removed and controlled accurately. As such, electrically controlled magnetic fields are preferred as it allows the field to be tuned accordingly. The deflection of the magnetic particles v_{defl} (m/s) can be described as the vector sum of the magnetically induced flow velocity of the particle v_{mag} , and the velocity due to the hydrodynamic flow v_{hyd} [205]:

$$v_{defl} = v_{mag} + v_{hyd}, \quad (3-3)$$

The magnetically induced flow, v_{mag} , is the ratio of the magnetic force, $\mathbf{F}_{mag}$ (N), exerted on the particle by the magnetic field to the viscous drag force:

$$v_{mag} = \frac{F_{mag}}{F_{drag}} = \frac{F_{mag}}{6\pi\eta r}, \quad (3-4)$$

The magnetophoretic force on a superparamagnetic bead can be expressed as and utilizing

the vector calculus identity $\nabla(\mathbf{B} \cdot \mathbf{B})\mathbf{B} = 2\mathbf{B} \times (\nabla \times \mathbf{B}) + 2(\mathbf{B} \cdot \nabla)\mathbf{B} = 2(\mathbf{B} \cdot \nabla)\mathbf{B}$,

$$F_{mag} = \frac{V_{bead} \Delta\chi}{\mu_0} (\mathbf{B} \cdot \nabla)\mathbf{B} = \frac{V_{bead} \Delta\chi}{2\mu_0} \nabla B^2, \quad (3-4)$$

where $\Delta\chi$ is the difference between the magnetic susceptibilities of the bead and the fluid, and V_{bead} is the volume of a particle.

Magnetostatic and flow dynamics simulations are performed using COMSOL Multiphysics program. The magnetic simulation results are shown in Figure 3.3. Through the employment of a current through the double microstripline, magnetic flux is generated around it. The average magnetic flux generated by the microstripline when 0.9A current is passing through can be determined by $B_{total} = \sqrt{B_x^2 + B_y^2 + B_z^2}$ and $B_{total} = 347 \text{ G}$, which according to the particle flow simulations will be sufficient to deflect the magnetic particles. The value of the B_{total} for 0.9A are compared with the simulation where a permanent magnet was used to deflect the bead where $B_{total} \sim 320 \text{ G}$. The magnetic simulations are consistent with Bio-Savart's Law ($B \propto I$) as there is a proportionate relation between the magnitude of the magnetic flux and current. The magnetic particles are then attracted to the current carrying microstripline given by the magnetophoretic force equation governed by $F_{mag} = \frac{V_{bead} \Delta\chi}{2\mu_0} \nabla B^2$. The average magnetophoretic force from a 0.9A field generating microstripline was determined to be $(7.5 \pm 0.2) \text{ pN}$ using the mentioned magnetophoretic force equation inputted into MATLAB.

Figure 3.3. Magnetic flux density distribution in the (a) x , (b) y and (c) z components for the current values $0.4\text{A} \leq I \leq 1.0\text{A}$ along a reference line of measurement at the centre of the microstripline. (d) Magnetic field gradient distribution of the y -component in the double microstripline structure with current at 0.4A. (e) Surface temperature distribution along the microstripline for current values ranging $0.4\text{A} \leq I \leq 0.9\text{A}$. (f) Temperature change across the microstripline after a period of 5 minutes for current values ranging $0.4\text{A} \leq I \leq 0.9\text{A}$.

Thermal characterization of the device was done by simulation of joule heating in the microstripline. The average surface temperature was determined after 5 minutes of passing current through the double microstripline. This is done in order to estimate the likely real time temperature increase on the surface of the magneto-fluidic chip. Simulations were performed for different current values from 0.4A to 0.9A with intervals of 0.1A with the initial temperature conditions set as 25°C and heat transfer coefficient of copper with water interface as 13.1W/m²K and the surface emissivity of copper is 0.87. Simulation results have affirmed that joule heating will not be an issue, and this is confirmed experimentally. External cooling was not required. However, in order to ensure complete viability of cells, external cooling was implemented for the experiment conducted with cells. External cooling was done by incorporating a Peltier dielectric chip below the magnetofluidic chip to cool the chip as shown by the experimental setup schematic in Figure 3.8(a). In order to determine the effect of heating on the HeLa cells, cell viability for current values of 0.4A, 0.6A and 0.9A were recorded and the cell viability for 0.9A was determined to be 87.2%. When cooling was implemented, the cell viability was at 100% for all current values within the range 0.4A to 0.9A.

In the fluid dynamics simulation, the laminar flow profile from three inputs is simulated. The flow rates of the three inlets are varied so as to focus the middle streamline close to the centre of the microchannel and this is governed by the Navier-Stokes Equation and the continuity equation. Hydrodynamic focusing occurs when flow streams with different flow rates come into contact with each other [206-208]. Upon the application of a magnetic field the profile of the magnetic particle stream will deviate to the outlet. In the beginning, to simplify the mathematical modeling, a permanent magnet with a magnetization of 320 G was applied [209] and the particles were successfully diverted. The entire magnetofluidic device was simulated and significant deflection

of the magnetic beads occurs at around 0.6A with complete deflection of the magnetic beads occurring at input current of 0.9A. From the simulations and theoretical understanding, the particles are influenced by two forces, the drag force from the fluid and magnetic attraction force from the magnet. The gravitational effect is neglected because it is only acting on z direction. The simulation of particle separation is shown in Figure 3.8(b).

3.4 Experimental procedures

3.4.1 Device fabrication

The device fabrication process includes microchannel fabrication and microstripline fabrication. Figure 3.4 summarizes the fabrication processes for the microchannel and microstripline. The microfluidic channel and microstripline layouts were designed using AutoCAD software and printed on transparent plastic film as photomask (CAD/Art Services, Inc. Bandon, OR). The microchannel was fabricated using standard soft-photolithography technique. A silicon wafer was spin-coated with a 50 μm -thick layer of SU-8 3035 photoresist (MicroChem Corporation, Newton, MA). A mask aligner (Karl Suss MA6, Suss Microtec Inc.) was used to transfer the patterns from photomask to the photoresist. After exposure, the photoresist was immersed into SU8 developer for development to obtain a negative master. The polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, USA) prepolymer and curing agent were mixed at the ratio of 10:1 and poured on the master wafer. After curing at 80 °C for 2 h, the solidified PDMS can be peeled off from the master.

The microstripline fabrication process is shown in Figure 3.5. A thermally grown silicon dioxide (300nm) silicon wafer is used as the substrate for the microstripline fabrication. Positive resist AZ9260 imaging resist (AZ electronic Materials) and LOR-3B (MicroChem

Corporation, Newton, MA) was spun coated onto the wafer subsequently. In order to ensure clean lift-off, ion-milling was done to create a trench to enhance adhesion. A double squared meandering patterned structure is preferred over the single meandering structure as it has higher efficiency in magnetic actuation determined through simulation.

A metallic multilayer comprising of Cr (3nm)/NiFe/SiO₂/Cu/Cr structure is deposited on the resist-patterned wafer using electron-beam evaporation techniques. The Cr layer serves as a seed layer to enhance the bonding of the following conduction layer. The SiO₂ layer between NiFe and Cu was used as a passivation layer which decreases the total resistance of the structure by separating the permalloy and the conducting Cu layer, other alternatives such as photoresist could be used as the dielectric passivation [200], but in this case, SiO₂ was preferred. The Cu layer serves as the main conduction layer for the generation of the magnetic field when electric current flows through it. The ferromagnetic permalloy (NiFe) was used to concentrate the path of the flux lines by creating a new low reluctance path. The final Cr layer serves as a protection capping layer to prevent the process of oxidation to the conducting Cu layer.

After the microstripline structural deposition, a SiO₂ insulating or passivation layer is deposited using the electron beam evaporation technique. This allows the isolation of the metallic microstripline structure from the liquid flow in the microchannel. SiO₂ was used to effectively insulate the microstripline from the liquid. The maximum thickness of the passivation layer was confined to below 300 nm in order to maintain an effective magnetic field experienced by the magnetic particles. The optimized thickness of the passivation layer was determined to be 100 nm so that passivation and good bonding of the PDMS microfluidic channel could be effectively achieved.

Figure 3.4. Procedures of magnetofluidic chip fabrication, including SU-8 mold fabrication, microstripline fabrication and the bonding process.

Upon successful development of the essential fabrication techniques and experimental optimization of the microfluidic system and microstriplines, they were integrated into one compact system.

Figure 3.6 illustrates the microstripline pattern fabricated using AZ 9260 and LOR 3B. LOR 3B is favourable for subsequent lift off of metal films. During the lift off process, the photoresist under the metal film is removed by solvent, leaving only the metal films that was deposited on the silicon substrate.

Figure 3.5. Schematic illustrating the detailed steps of the microstripline fabrication.

Figure 3.6. The microscopic image of microstripline. The width of a wire is $100\mu\text{m}$ and the space between two wires is $100 \mu\text{m}$.

Figure 3.7. The microscopic image of microfluidic channel and microstripline. The microstripline is positioned beneath the microchannel. There is a little offset between the channel centreline and the microstripline to induce the cell's deflection bias the upper outlet.

3.4.2 Cell culture

Adherent HeLa cells were cultured at 37 °C with 5% CO₂ supply in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. To subculture the cells, PBS was used to wash cells and 0.05% trypsin-EDTA solution was used to detach the cells from the bottom of the T25 flask. The detached cells were transferred to 15 ml conical tube and underwent centrifugation. After removing the supernatant, the cells were resuspended in complete medium and reseeded on a new T25 flask.

3.4.3 Measurement setup

The cell separation is observed under stereomicroscope (SZX-16, Olympus, Japan) with a fluorescence light source (KL2500, Schott, Germany). The cell movement in microchannel is recorded by a camera (HDR-XR 500E, Sony, Japan), which is mounted on the microscope via a

C-mount adaptor (DCR TRV 950, LM Scope, Austria). Three syringe pumps (LSP02-1B, China) are used to introduce sample and sheath flow into the microchannel. A Peltier cooling chip is placed under the microfluidic device to dissipate Joule heat (Figure 3.8 (a)).

Figure 3.8. (a) Schematic of Experimental setup of the magnetofluidic device on top of a dielectric Peltier cooling chip fitted with cooling fins for cooling down of the chip. (b) Simulations of magnetic particle flow in a magnetofluidic microchip for current values ranging $0.4A \leq I \leq 0.9A$, partial deflection occurs at 0.6A and complete deflection occurs at 0.9A. The flow is governed by the Navier-Stokes equation and the beads flow is forced to the central line using the hydrodynamic focusing approach.

3.5 Results and discussions

Glycerol mixture (Glycerol: water = 1:1) was first used to simulate the viscosity of blood. Before the application of the magnetic field, the flow rate at the inputs are adjusted so as to confine the particle flow at a position just exactly above the center line of the sorting chamber. Hydrodynamic focusing happens as predicted by simulation results. Hydrodynamic focusing is to ensure that without the application of a field, the particle stream will always flow into outlet 2. In addition, the particle stream is close to the center of the microchannel, the magnetic field strength needed to coerce the particles to flow into

outlet 1 is minimized. Under the function of magnetic field, the magnetic particles will change their path of flow and switch to another streamline closer to the field-generating double microstripline. Thus, by gradually changing the flow path of the magnetic particles the device is able to direct the magnetic particles to outlet 1. Figure 3.9 shows the images of the result of sorting of the magnetic beads going through Outlet 1. This result is repeated with increasing current values from 0.5A to 1.0A. The minimum current of 0.5A would have been sufficient to generate a magnetic field to deflect the microbeads.

Figure 3.9. Sorting of magnetic particles from glycerol mixture. (a) When no current was applied, the magnetic particles flow to Outlet 2 and (b) upon the activation of a minimum current of 0.5A, deflection starts to show. (c) Sorting efficiency relationship with controlled current. At 1.0A, 91% of the particles were deflected.

The isolation efficiency is calculated by $\frac{O_d}{O_d + O_N} \times 100\%$, where O_d is the number of deflected particles and O_N is the number of non-deflected particles. This was then calculated by post-processing the images. As control, at zero current, the stream was adjusted to just flow close to Outlet 2, and when a current is applied, the beads will then be deflected to Outlet 1 due to the presence of the magnetic field. By increasing the current, the magnetic field generated by the microstripline increased. As such, the isolation efficiency increases. At 1.0A current, the magnetic field generated is sufficient to cause an isolation efficiency of 91%. Upon proving the concept of deflection using the magnetofluidic device, this similar principle is brought to the separation of magnetic particle tagged HeLa cells.

3.5.1 Magnetic Particle Tagging of HeLa Cells

Mouse monoclonal Ep-CAM (C-1) antibodies (sc-25308) was chosen for the detection and isolation of cells in this study. The monoclonal antibody recognizes the Ep-CAM antigen that is expressed on the HeLa cells. A large volume of magnetic particle will lead to a larger magnetic force, which can enhance separation efficiency. Hence, we used fluorescent magnetic beads with 8 μ m diameter (COMPEL™, Bangs Laboratories, Inc.) for cell tagging. The beads are superparamagnetic and functionalized by carboxyl group. The magnetic beads are comprised of Fe₃O₄ crystal core coated with polymer. The beads were washed with 1ml of 2-(N-Morpholino) ethane sulfonic acid (MES) (0.1 M, pH 5) buffer for about 3 times. The magnetic particles are isolated by a magnet and washed with PBS. The washed particles are activated by suspending them in MES buffer containing 10mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and rolled for 30minutes at room temperature. The monoclonal antibodies are then added to the activated magnetic particles and incubated on a rocker overnight at room

temperature. Finally, the mouse monoclonal Ep-CAM (C-1) antibodies conjugated magnetic beads was re-suspended in 100 μL PBS. 1×10^6 HeLa cells were re-suspended in 0.9 ml of completed DMEM culture medium. 100 μL of anti-Ep-CAM antibody conjugated magnetic beads in DMEM culture medium was added to the 0.9 ml cell suspension and incubated at 37°C for 60 min. Binding of the anti-Ep-CAM antibody conjugated COMPEL™ 8 μm magnetic beads to HeLa cells was verified using a fluorescence microscope. Figure 3.10 shows the efficient tagging of the HeLa cells with the magnetic beads.

Figure 3.10. High binding efficiency of the Ep-CAM antibodies conjugated with 8 μm magnetic beads to HeLa cells was observed on (a) bright field (b) fluorescence and (c) confocal microscope. Multiple magnetic beads could be found attached to the cell.

3.5.2 Sorting of Magnetically Tagged HeLa Cells

The mixture was then pumped into the sorting chamber with a controlled flow rate $1 \mu\text{L}/\text{min}$ that met the hydrodynamic focusing requirement similar to the magnetic bead only experiment. Video footages were taken at the center of the dialyzer where magnetic field was applied. The strength of magnetic field was adjusted by fine tuning to the optimum field with current provided to the microstripline at 1.0A. As control, zero current was applied. Sorting was done in a process similar

to the magnetic bead only experiment. The cells were then counted in Outlet 1 and Outlet 2 at the end of the experiment. The experiment was repeated and the average isolation efficiency for sorting the Ep-CAM (C-1) antibodies conjugated magnetic beads tagged HeLa cells were sorted into Outlet 1 is 79%. The counting of the tagged cells was repeated and consistent results within $\pm 1\%$ deviation was seen. Figure 3.11 illustrates the optical microscope image of the isolated HeLa cells in the sorted outlet.

The magnetic elements used in magnets-based microfluidic cell sorters can be either permanent magnets or electromagnets. Compared to permanent magnets, microelectromagnets are advantageous in controlling the magnetic field by varying the amplitude of the electric current, resulting in precise control of magnetic targets. On the other hand, the continuous-flow separation mode possesses many important merits, such as high throughput, the possibility of continuous monitoring and high potential for system integration [34]. Although there were a number of microfluidic cell manipulation devices that exploited microelectromagnets, only a few of them worked in continue-flow mode[210-212]. As the prominent advantage of microelectromagnets-based cell sorter is that the magnetic field can be tuned to control cell isolation, it is necessary investigate the cell separation performance under various electric current. Song *et al.* presented a microfabricated electromagnet for Lab-on-a-Chip applications. They used the electromagnet to separate 4.5 μm diameter magnetic beads and obtained a highest separation efficiency of 92.4% at 1.5A [210]. Kong *et al.* demonstrated a 95% magnetic particle separation efficiency using microelectromagnets [211]. Chung *et al.* fabricated magnetic-field-gradient microelectromagnet. When applied 1A electric current, they separated 2.8 μm magnetic beads from 6.0 μm polystyrene particles with efficiency of 95% [212]. Distinctive from these reports, we implemented microelectromagnets for live cell separation. Compared with these high separation efficiency, our

relative low separation efficiency can be partly attributed to the large cell size, which lead to high hydrodynamic drag force. To compete with the hydrodynamic drag force, a stronger magnetic force are required [34, 48, 50]. To our knowledge, our study is the first one that demonstrated the variation of live cell sorting efficiency on applied current of microelectromagnet.

Figure 3.11. (a) HeLa cells tagged with anti Ep-CAM antibody-conjugated 8 μm magnetic beads. (b) Optical microscope image of the deflection of the tagged HeLa cells (indicated by the red circles) when 0.9A electric current was applied.

3.6 Summary

In summary, the microfluidics integrated with double meandered microstripline is capable of sorting immunomagnetically labelled cancer cells with isolation efficiency up to 79%. The overall sorting efficiency is governed by the binding affinity and the number of antibody-conjugated magnetic beads to HeLa cells. Considering only the isolation efficiency, many other devices have demonstrated a slightly higher values. For instance, Song *et al.* [210] has presented an electromagnets-based microfluidic device which achieved 92.4% particle sorting efficiency at 1.5A current. Darabi *et al.* [213] employed permanent magnets as magnetic source for CD4⁺ T cells separation from peripheral blood and obtained 90% purity. In future, we can further improve

the performance of our device through efforts on the following aspects. (i) Increase the NiFe permalloy layer and Cu conducting layer to increase the magnetic field. (ii) Utilize a longer microstripline and a longer separation channel to elongate the cell deflection route, allowing more cells deviated by the magnetic field. This study demonstrates the capability of the integrated magnetofluidic device in cell separation. This capability can also applicable to other types of cancer cells, thus serving as a potential enabling technology for CTCs detection and collection in bloodstream.

Chapter 4

Size and Affinity-based Multiplexed Cell Sorter

4.1 Background

4.1.1 Inertial microfluidics

The motion of viscous fluid is governed by Navier-Stokes equation as shown below:

$$\rho (\partial \mathbf{u} / \partial t + \mathbf{u} \cdot \nabla \mathbf{u}) = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{f} \quad (4-1)$$

where μ is the fluid dynamic viscosity, $\mathbf{u}$ is the fluid velocity field, p represents fluid pressure field, and $\mathbf{f}$ is the body force. $\rho (\partial \mathbf{u} / \partial t + \mathbf{u} \cdot \nabla \mathbf{u})$ corresponds to the inertial forces, $-\nabla p$ corresponds to pressures, and $\mu \nabla^2 \mathbf{u}$ corresponds viscous forces.

Traditionally, inertial phenomenon was neglected in microfluidics, which means that the fluid flow in microchannel is regarded as occurs at low Reynolds number, which is dimensionless parameter used to describe the ratio between inertial and viscous forces in a flow.

$$Re = \rho UH / \mu \quad (4-2)$$

where ρ represents the fluid density, μ is the dynamic viscosity of the fluid, H is the microchannel dimension, and U is the average flow velocity.

In microfluidic channels, the liquid flows are usually taken as Stokes flow, where the inertial forces are much smaller than the viscous forces. Hence the Reynolds number is very slow, *i.e.* $Re \rightarrow 0$. This assumption is based on the fact that the dimension of microfluidic channel is in the region of 1~1000 μm . Hence, the Stokes equation is used to describe the fluid flow in microchannel by setting the right hand side of Navier-Stokes equation to zero,

$$0 = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{f} \quad (4-3)$$

However, this assumption can lead to inaccurate results in some cases [91]. Stokes flow implies laminar flow, while the opposite is not necessarily correct. In most cases, the flow in microfluidic channel can be seen as laminar flow without need to consider the fluid turbulence, which was only observed for $Re > \sim 2000$. However, this approximation is incorrect for some extreme cases, where the right hand side of Navier-Stokes equation should not be seen as zero.

Recently, the usually neglected inertial effects have seen rapid increasing use by microfluidic community. Departing from the common belief that the microfluidics works in the Stokes region (*i.e.* $Re \ll 1$), the inertial works in part of the intermediate region between Stokes region and turbulent region. In inertial microfluidics, the inertial forces and viscous forces are both finite and have important influences on the flow profile. The inertial migration and secondary flow are the predominantly studied effects in inertial microfluidics [214].

Segre and Silberberg first experimental observed the inertial migration phenomenon, where in a large circular pipe, the randomly distributed neutrally buoyant particles focused to an annulus position. The distance between the annulus and the pipe center equals to 0.6 times the radius of the pipe [215]. In this experiment, the Reynolds numbers were in the region $1 < Re < 100$, which indicated that the inertial force played an important role for particle focusing. The practical application of this phenomenon emerged only in recent years with the advances of microfabrication technologies, which enable the realization of microchannel with the feature dimension is comparable with the particle size [214].

The inertial phenomenon is believed to be induced by the interplay between two inertial forces: the shear gradient lift force and the wall lift force. The shear gradient lift force pushes particles in the medium away from the channel center, while the wall lift force repels the particles away from the wall. The equilibrium positions of the particles are the results of the balance of these two

opposite effects.

Secondary flow usually occurs in a curved channel due to the mismatch of fluid velocity between fluid elements around the channel center and close to channel wall. The fluid tends to flow outward due to the larger inertial in the center with respect to that close to channel wall. In the enclosed channel, the fluid elements close to the wall are pushed to circulates inward due to the pressure gradient. Therefore, the two counter-rotating vortices are created at the top and bottom of the cross-sectional plane of the channel. Some parameters are of significance to modify the magnitude and qualitative feature the secondary Dean flow, such as channel dimension, radius of curvature and flow velocity. The secondary flow is described by the dimensionless Dean number, which is defined as:

$$De = Re (H/2R)^{0.5} \quad (4-4)$$

where Re is the Reynolds number, H is the channel dimension and R is the radius of curvature.

Spiral and asymmetric curving microchannels are the two mostly adopted structures using secondary flow to enhance inertial focusing. Spiral channels can be used for differential focusing and size-based particle separation.

In order to reduce the number of focused streams in microchannel, D. Di Carlo *et. al.* introduced alternating channels into microfluidics for particle focusing. Through modify Dean number, in their microchannel with square cross section, the original four equilibrium positions were reduced to two and further to one. The particle focusing positions were determined by the interaction between inertial lift force F_L and Dean drag force F_D . When $F_D \gg F_L$, the particles could not be focused. When $F_D \ll F_L$ and the channel was long enough, the inertial lift alone induced particle focusing occurred in the channel. They concluded that the ratio of lift to drag force scale as

$$\frac{F_z}{F_D} \sim \frac{1}{\delta} \left(\frac{a}{D_h} \right)^3 R_c^n \quad (4-5)$$

R_c is channel Reynolds number, D_h is the hydraulic diameter, defined as $= 2wh/(w + h)$, δ is curvature ratio, defined as $\delta = D_h/2r$, r is the radius of curvature of the channel, w and h are the channel width and height, respectively, a is the particle diameter. This relation implies a third-power dependence on the ratio of particle to channel dimension [214]. Based on this theory, asymmetrical curving channel can focus large particles while above a threshold, while the small particles remain unfocused state.

Comparing with asymmetrical curving channel, Dean flow coupled spiral microfluidics is more promising for particle separation based on some intriguing advantages: 1) the particle equilibrium positions can be modified by the Dean vortex, enabling improved particle separation efficiency; 2) the footprint of the microchannel can be reduced by using secondary flow induced particle later migration; 3) size-based particle separation can be achieved due to the their different equilibrium positions in Dean vortex [214].

4.1.2 Inertial effects for cell separation

Traditional blood analysis relies on complete blood count to carry out disease prognosis. The disadvantages of this method are apparent. First, this method requires large blood volume. Second, the whole blood processing inevitably causes cell phenotype variation. Thirdly, during the blood sample transferring from one to another step, contamination risk is always inevitable.

Although a wealth of information exist in monocytes for disease assessment, technical hurdles limit their clinical screening. A major technical one is the lack of suitable cell separation method to isolate monocytes from blood efficiently.

Cell separation from liquid biopsy is important in many biomedical applications ranging from

HIV diagnosis (CD4+ T-lymphocytes) [216] to inflammatory profiling in diabetes mellitus [95]. Traditional blood analysis requires laborious sample preparations (centrifugation, lysis) and cell sorting methods (FACS, MACS) to isolate leukocytes subtypes. These batch-mode processing methods are not only time-consuming and costly, but may also cause undesired cell activation and cell loss during manual handling. It is therefore essential to develop novel, simple and integrated technologies for rapid cell sorting in point-of-care clinical diagnostics.

In the past decade, there has been a significant progress in the development of lab-on-a-chip/microfluidics platforms for cell manipulation. Microfluidics is an enabling technology for cell separation due to its apparent advantages such as reduced sample volume and portability [21, 35, 217]. As mentioned before, microfluidics cell separation approaches can be categorized into active methods and passive methods. Active methods exploit external fields including electric, magnetic, acoustic and optical forces to fractionate target components based on intrinsic or modified cell surface properties. Passive methods are generally simpler in chip design, and utilise microfabricated structures or microscale hydrodynamic flow phenomenon to achieve separation [218].

As an active cell separation approach, magnetophoresis has emerged as an efficient cell separation technology to achieve high purity and yield [48, 50, 205]. Briefly, generation of magnetic fields can be achieved by passive magnetic elements or by active electromagnets in the vicinity of the microchannel [219]. In the former case, a permanent magnet is often used to provide external field source. This eliminates the requirement for power consumption, but compromises setup portability with the use of bulky permanent magnets. Zhou *et al.* introduced a method to fabricate on-chip microscale permanent magnetic microstructures for yeast separation at sample flow rates of $\sim 1 \mu\text{L}/\text{min}$ [220]. In the latter case, complicated procedures and expensive equipment

are required to fabricate the electromagnets. Although magnetophoretic microfluidics can precisely control the cells of interest, the flow rates in these devices are usually very low ($\sim 1\text{-}10\ \mu\text{L}/\text{min}$) [21] due to the long residence time required to subject the cells to magnetic field [218].

In 2013, Hou *et al.* first introduced Dean Flow Fractionation (DFF) as a novel microfluidics cell sorting technology for size-based separation of diseased cells from whole blood at high volume throughput ($\sim 1 - 10\ \text{mL}/\text{hr}$) [94, 221, 222]. DFF is a passive cell separation technique based on the principles of secondary Dean vortices, and particle lateral migration by inertial forces (F_L) at high flow rates (Reynolds number, $Re \sim 50\text{-}100$) [92]. In DFF system, fluid flowing through a curvilinear (spiral) channel experiences centrifugal acceleration directed radially outwards, leading to the formation of two counter-rotating vortices known as Dean vortices, in the top and bottom halves of the channel [223]. The presence of these inherent transverse Dean flows imposes lateral Dean drag force (F_D) which offers superior separation resolution [222, 224, 225] as both forces (F_L and F_D) scale non-linearly with particle size, and their superposition (F_L/F_D) determines the equilibrium position within the channel cross section. By focusing different cell types at distinct channel positions based on size, we successfully applied DFF technology for separation of circulating tumour cells (CTCs) [94], leukocytes [222] and microorganisms [221] from whole blood for point-of-care testing.

Passive separation technologies have the ability to separate cells at high volume throughput with superior size resolution [94], and integration of both active and passive separation techniques is an attractive method as a new class of particle sorting technology. However, a major technical limitation lies with the flow rate mismatch between the passive and active stages. Toner *et al.* recently reported a hybrid microfluidic cell sorter combining active and passive separation methods. This device consists of three modules: (i) size-based cell separation module using deterministic

lateral displacement (DLD) technique, (ii) inertial focusing module, and (iii) affinity-based cell separation module using magnetic force [226, 227]. However, these three modules were fabricated in 2 separate chips of different materials and connected by an extra tubing, which increased the complexity of the sorting system. Additionally, the magnetophoresis module must be carefully aligned with the bulky off-chip permanent magnets, which further complicated the setup. By combining inertial and magnetic forces in a straight channel and addition of a downstream expansion hydrodynamic separator, Kumar *et al.* developed a multiplexed inertio-magnetic fractionation technique, for duplex to fourplex fraction of magnetic and non-magnetic nanoparticles. However, the device is still limited by the use of a bulky external permanent magnet to provide magnetic field [228].

4.1.3 Motivation

Efficient blood sample preparation remains a crucial and unsolved problem in clinical settings. To address the aforementioned issues, we develop a novel multiplexed cell sorter, which enables separation of cells based on their size and immune affinity subsequently. We combined spiral inertial microfluidics (DFF) with micro-magnets array for high-throughput size and affinity-based cell separation in an integrated device. Noteworthy, in contrast to current inertial microfluidic devices, the multiplexed device enables cells of similar sizes to be further separated based on their surface marker expression. Generally, passive separation methods are featured with high-throughput, while active separation methods possess the advantage of high selectivity. The marriage of passive and active separation techniques has been proved to be promising. Recently, spiral microfluidics that exploiting inertial force has demonstrated great merits for cell separation. On the other hand, microfluidic immunomagnetic cell separation method has reached maturity. In the contrary to other prevalent active methods, such as acoustic, optical, and electric methods,

which rely on sophisticated fabrication and/or external equipment, magnetic force-based cell separation can be achieved using simple permanent magnets. Our goal in this study is to present an integrated, non-expensive and easy use cell sorter, enabling multiplexed cell sorting with high throughput and high selectivity. Due to the large channel dimension and high flow rate required for Dean Flow and inertial focusing operation, the cell sorter can process very high haematocrit blood samples (~20%) at high throughput (1ml/min). The shortcoming of using normal permanent magnets is that the size of a normal magnet is much larger than the dimension of cells, resulting low magnetic force experienced by immunomagnetically tagged cells. To generate high magnetic force within the microchannel region where cells or particles pass through, we fabricated on-chip micro-sized magnets array using a novelty method.

4.2 Design principle

The purpose of device design is to obtain size-based cell separation in spiral microchannel and introduce specific sized cells into the magnetic module for affinity-based separation. The target of this study is to achieve separation >70% and sample processing throughput >1ml/min. To incorporate inertial force based separation module and magnetic force based separation module on one single microfluidic chip, there are some design specification should be fulfilled. (i) The integrated magnets should impart sufficient force on the cells to compete with the high hydrodynamic force due to high flow rates. Therefore, a permanent magnet with jagged structure was fabricated to generated local magnetic field. (ii) To focus the cells along the microchannel centerline, a serpentine microchannel was applied to connect the spiral channel and the magnetic separation channel.

The developed two-inlet, three-outlet cell sorter was fabricated in polydimethylsiloxane (PDMS)

and consisted of three functional stages, namely 1) size-based DFF separation stage, 2) cell alignment stage and 3) affinity-based separation stage (Figure 4.1 A). The channel height was fixed at 115 μm so that larger monocytes ($\sim 15 \mu\text{m}$) can undergo inertial focusing in the spiral device. In the first stage, the size-based separation was based on DFF developed by our group previously [94, 222]. The sample and sheath inlets were fixed at the outer and inner wall of the channel, respectively. Prior bifurcation, the channel width gradually expanded from 500 μm to 1000 μm , and split into one waste outlet at outer wall (outlet 1) and one serpentine microchannel (inner wall) for subsequent cell alignment and magnetic separation. In the second stage, the DFF-sorted cells were aligned based on inertial focusing phenomenon in the serpentine microchannel [92, 93]. The curving channel had a width of 160 μm for the small radius of curvature and a width of 305 μm for the large radius of curvature. This stage comprised of 6 repeating units of one small and one large curvature. The detailed geometric parameters are indicated in Figure 4.2. The design rationales for employing this focusing strategy are to 1) ensure that the unlabelled cells exit the device through the waste outlet at the end of third stage (due to the off-center bifurcation at the end of third module), 2) reduce cell-cell interferences, and 3) minimize the magnetic forces required during the magnetic deflection. In the third stage, immunomagnetically labelled cells

Figure 4.1. Integrated size and affinity-based cell sorter. (A) Design layout of the 2-inlet, 3-outlet cell sorter consisting of 1) size-based Dean Flow Fractionation (DFF), 2) serpentine channel for cell alignment using inertial focusing, and 3) magnetic separation channel with micromagnets. (B) Schematic illustration of cell trajectories at different stages of the device. (i) At the first stage, larger monocytes/neutrophils were fractionated by DFF into the top channel while smaller lymphocytes, RBCs and excess magnetic beads are removed as waste. Randomly distributed monocytes (ii) align along the centreline into a single stream at the end of the serpentine channel (iii) due to inertial focusing. This enables efficient deflection and collection of immunomagnetically labelled monocytes into the lower outlet (O2) while unlabeled cells are sorted into outlet 3 (O3).

were deflected by magnetic forces using micro-sized magnets [229]. The magnetic separation stage comprised of a micromagnets array with a 60 μm gap size next to a long straight microchannel of 240 μm width and 4 cm length. The microchannel bifurcated at the end of the channel into 2 asymmetrical outlets with the upper bifurcated channel of 180 μm width (for unlabeled cells) and a smaller channel of 120 μm width (for labelled cells) at the lower end (magnet side).

Figure 4.2. Two units consisting of small and large curvature. For the large curvature, the center of the two curves is offset by 160 μm . The width of small and large turns is 160 μm and 350 μm , respectively.

4.3 Experimental procedures

4.3.1 Microchannel fabrication

The microchannel layouts were designed using AutoCAD software and fabricated in polydimethylsiloxane polymer (PDMS, Sylgard 184, Dow Corning, USA) using standard softlithography technique which consisted of a double molding process. Standard photolithography was implemented in cleanroom to fabricate the first mold, where the microchannel pattern was transferred from a transparency photo mask (10,000 dpi, CAD/Art

Services Inc) to positive photoresist AZ 9260 (MicroChem Corp, USA) on 6 inch silicon wafer. The ultraviolet source was provided by Mask aligner (Karl Suss MA6, Suss Microtec Inc.). Following photolithography, the photoresist mold was sent to etch using deep reactive ion etching (DRIE) technology to etch the microchannel for 68 min. After etching, the photoresist was stripped using acetone and followed by IPA washing.

The second mold was made by casting PDMS onto the silicon wafer. To facilitate the PDMS release, the silicon wafer was silanized with trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma Aldrich, USA) for 1 h beforehand. PDMS prepolymer was initially mixed with curing agent in 10:1 (w/w) ratio, degassed and poured onto the silanized wafer, followed by curing in oven (brand, type) at 75 °C for 2 hrs for solidification. The obtained PDMS mold was used as a negative replica for subsequent PDMS casting which formed the final PDMS microchannels. It should be noted that, the PDMS master template must be silanized for 1 h before casting the final PDMS to facilitate the final PDMS microchannel release from the negative mold. The final PDMS microchannel was peeled off from the master and some holes were punched for fluidic inlets and outlets. Finally, the PDMS device were irreversibly bonded to glass slides using air plasma machine (Harrick Plasma Cleaner, USA).

Figure 4.3. Schematic of the PDMS microchannel fabrication process. A double-casting process assisted by Deep Reactive Ion Etching (DRIE) was adopted for the PDMS microchannel fabrication.

4.3.2 Micromagnets fabrication

The magnetized microstructure filled with Neodymium Iron Boron (NdFeB) magnetic powder functioned as permanent magnets which resulted in strong deflection of the immunomagnetically

labelled cells. Each microscale magnet had a length of 500 μm and was positioned at 60 μm away from the microchannel. To fabricate the micromagnets, NdFeB magnetic powder (MQFP-B-20076, Magnequench) was mixed with Isopropyl Alcohol (IPA) and manually injected into the microstructure using a plastic syringe. The device was then centrifuged at 1000 rpm for 5 min so that the magnetic particles can be tightly packed in the microstructure, facilitating the generation of high magnetic field (Figure 4.4). After being filled with the magnetic powder, the device was permanently magnetized by a magnetizer (Magnetic Instrumentation Inc., Indianapolis, IN) with 2 Tesla magnetic field.

Figure 4.4. Fabrication of micromagnets. Microscopic images at (A) 4 $\times$ and (B) 10 $\times$ magnification illustrating magnetic powder loading process. The magnetic beads were first manually injected into the microstructure and could not fill up the smaller tooth structures. After centrifugation, the microstructures were completely filled by the beads.

4.3.3 Sample preparation

Blood samples were obtained from healthy donors with informed consent. A total of 4 blood samples from healthy donors were used for monocytes separation. All blood specimens were collected in vacutainer tubes (BD, Franklin Lakes, NJ) containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant and diluted 1:2 (v/v) with buffer solution consisting of 0.1% bovine serum albumin (BSA) and 1x phosphate buffered saline (PBS) prior to processing on device. The function of BSA was to prevent non-specific absorption of the blood cells to the microchannel surface. An average of 4 mL blood volume was processed from healthy donors in monocytes separation experiment.

The peripheral blood mononuclear cells (PBMCs) were isolated from whole blood specimens through density gradient centrifugation. Briefly, the fresh blood was diluted with 4× the volume of PBS and then carefully layered over Ficoll-Paque media in a 15 mL conical tube. The conical tube was centrifuged at 300G for 45 min at 23 °C. After density gradient centrifugation, the PBMCs layer was carefully transfer to a new tube and washed with PBS to remove contaminants.

Figure 4.5 Isolation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque.

4.3.4 Immunofluorescence staining and characterization

To differentiate different cell types, immunofluorescence staining was applied to the PBMCs. CD66b, CD14 and CD 45 antibodies are subsequently added into the PBMC solution to respectively stain neutrophils, monocytes and lymphocytes. Next, the PBMCs were incubated at 4 °C for 30 min. To determine the cell recovery rates, fluorescence-activated cell sorting (FACS) was performed using a flow cytometer (BD LSR II, BD Biosciences, USA) on the inlet and three outlets samples.

4.3.5 Device characterization

During experiment, samples were filled in a 3 ml plastic syringe (BD Company) and pumped into the spiral microfluidic channel using a programmable syringe pump (NE-1000, New Era Pump Systems Inc., USA). A separate mechanical pump (PHD 2000, Harvard Apparatus, USA) was used to pump sheath flow (1X PBS, 2 Mm EDTA supplemented with 0.1 % BSA) which filled in 60 ml plastic syringe into the spiral channel through the sheath inlet. The flow rate ratio between the sample and sheath flow was set at 1:10 to constraint the sample stream at the outer wall. An inverted phase contrast microscope (Olympus IX71) interfaced with a high speed camera (Phantom V9, Vision Research Inc., USA) was used for image capture. The high speed camera was controlled by Phantom software and the captured high speed video was analyzed using open source ImageJ software. Fluorescence images were captured using a 12-bit EMCCD camera (iXon+885, Andor Technology, USA) and Metamorph software (Molecular Devices, USA). A flow cytometer (BD Accuri C6, BD Biosciences, USA) was used to determine the separation efficiency.

For device operation, cell sample containing PBMCs labelled with functionalized paramagnetic beads was pumped into the outer inlet while sheath fluid (1× PBS) was pumped through the inner

inlet at a higher flow rate. The flow rate ratio between the sample and sheath flow was set at 1:10 to constraint the sample stream at the outer wall. At the first stage, the larger monocytes focused near the inner wall due to inertial focusing while smaller platelets, lymphocytes, red blood cells, and free magnetic beads ($\sim 1.5 - 3 \mu\text{m}$) migrated laterally towards the outer wall into the waste outlet (O1) due to dominant Dean forces (Figure 4.1 B) [94]. The immunomagnetically labelled monocytes were then inertially focused (2nd stage) and subjected to magnetic separation (3rd stage). Magnetically-labelled monocytes were deflected towards the magnets and sorted into the smaller outlet (O2), while unlabelled monocytes are sorted into another waste outlet (O3).

4.4 Results and discussion

To achieve blood cell separation with high throughput and high purity, the chip design should fulfil the following target specifications. (i) The device should be able to process very high haematocrit blood sample ($\sim 20\%$) at high throughput (1ml/min). (ii) The magnets should provide sufficient magnetic field to displace the cells. Hence, the magnets should be placed near the long channel (distance $< 60 \mu\text{m}$). (iii) A long channel ($> 2\text{cm}$) was required to allow the magnets to displace the cells within certain time. (iv) The asymmetric curving geometries as shown in Figure 3.2 should be able to focus leukocytes in the center of the long channel.

In the second stage, a serpentine microchannel was utilized to connect the prior spiral channel and subsequent long channel. The purpose of using the serpentine design was to focus cells coming from the first stage based on inertial focusing phenomenon. Di Carlo *et al.* [92] have found that asymmetric curving geometries was useful for reducing the inertial focusing equilibrium positions in rectangular channels to a single position. They presented a curving channel for rapid (1ml/min) separation of blood cells [93]. Based on their successful design, we modified our serpentine channel for our particular situation.

Figure 4.6. Device characterization using monocytes. Sorted monocytes were randomly distributed at (A) stage 1 bifurcation of DFF, and (B) the start part of serpentine channel. (C) Monocytes focused inertially into a single stream at the end of serpentine channel.

To investigate the function of cell alignment stage, cell distribution was recorded by a high speed camera at three different locations of the microchannel as shown in Figure 4.6: (A) stage 1 bifurcation, and the (B) start and (C) end position of the serpentine channel. DFF-sorted monocytes entering the serpentine channel were randomly distributed. At the end of the serpentine channel, the cells were inertially focused into a single stream at the centre of the channel.

Figure 4.7. Phase contrast images indicating monocytes immunomagnetically labelled with 1.5 μm magnetic beads.

For characterization of affinity-based separation, streptavidin-coated paramagnetic particles (1.5 μm diameter) were first functionalized with biotin anti-phycoerythrin (PE) antibody. The magnetic particles were then incubated with monocytes, which were pre-stained with PE anti-human CD14 antibody. After 30 min incubation at 4°C, the monocytes were efficiently bound with magnetic beads (Figure 4.7). Average stack images at different positions of the magnetic separation microchannel (3rd stage) clearly demonstrated deflection of labelled monocytes into outlet 2 (Figure 4.8 C), which was confirmed with bright field images (40 $\times$ magnification) of bead-bound monocytes near O2.

Figure 4.8. Average composite images indicating monocyte flow positions (stage 3) at (A) the start of the micromagnets array, (B) the end of the micromagnets array, and (C) the bifurcation prior outlet 2 and outlet 3. (D) Magnified bright field image at the bifurcation at stage 3. Red arrows indicate the monocytes labelled with magnetic beads.

A

B

Figure 4.9. Immune cell separation using DFF. High recovery of monocytes in outlet 3 (A), and lymphocytes in outlet 1 (B) were achieved for all flow rates tested.

To characterize the efficacy of size-based DFF separation stage, various sheath flow rates (ranging from 1200 to 1600 $\mu\text{L}/\text{min}$) were applied, and PBMCs containing lymphocytes (8~12 μm diameter) and monocytes (12~18 μm diameter) were introduced into the outer inlet with corresponding flow rates (ranging from 120 to 160 $\mu\text{L}/\text{min}$). As shown in Figure 4.9, unlabelled monocytes were efficiently sorted (>90%) into outlet 3 with increasing flow rates, while smaller lymphocytes separated into outlet 1 (waste outlet).

There are only a few reports to date has demonstrated the monocyte sorting using microfluidic devices. Yamada *et al.* presented a lattice-shaped microfluidic pattern as a virtual sieve for size-dependent continuous particle sorting. In this device, monocytes were separated from other blood cells with purity of $78\pm 14\%$. The highest particle suspension flow rate was $400\mu\text{l}/\text{min}$ [230]. Ramachandraiah *et al.* developed an inertial microfluidic device to separate nucleated cells from whole blood. The operational flow rate can reach $1\text{ml}/\text{min}$ and they separated monocytes with purity of 43% [231]. Darabi *et al.* reported a magnetophoretic-based microfluidic chip for monocyte separation. The separation results indicated that a monocyte purity around 90% and a recovery rate of 87% was obtained at a flow rate of $50\text{ml}/\text{h}$ [232]. Compared to these devices, our microfluidic chip is advantageous in higher sample processing throughput and remarkable separation efficiency.

Taken together, our results clearly illustrate high-throughput size and affinity-based cell sorting using the developed device. First, effective size-based monocyte separation from blood or PBMCs was achieved using DFF. Integration of the serpentine structure for cell focusing significantly improved subsequent magnetic separation of immunomagnetically labelled monocytes.

4.5 Summary

In this chapter, we report a size and affinity-based super-high throughput cell separation approach to isolate monocytes from whole blood. The microfluidic device consists of three major functional sections: the spiral channel for size-based cell separation using Dean vortex flows, termed Dean Flow Fraction, a serpentine shape microchannel for cell alignment and a long straight channel for secondary affinity-based cell separation using micro-sized magnets. This microfluidic cell separation platform couples both advantages of size-based separation and affinity-based advantages. It can process 1mL of whole blood within 10 min with >90% cell separation rate. In this work, we combined DFF, inertial focusing in serpentine structure, and magnetophoresis in a single device to achieve continuous size and affinity-based cell separation. To our knowledge, this is the first biochip integrating spiral microfluidics size-based cell sorter with on-chip magnetic separation. The developed platform operates at high-throughput ($\sim 100\text{-}150\ \mu\text{l}/\text{min}$) and facilitates continuous collection of cells for downstream analysis. As proof-of-concept, the device is developed for immune cells (monocytes) isolation, and can be readily applied to purify circulating tumour cells (CTCs) and circulating progenitor cells from whole blood.

Chapter 5

An Enclosed Paper Microfluidic Chip as A Sample Pre-concentrator Based on Ion Concentration Polarization

5.1 Background

To get over the outstanding problem of existing paper-based microfluidic sample concentrator, we proposed an enclosed paper-based concentrator which was incorporated with the following design specification. (i) To simplify the fabrication process and ensure high reproducibility, craft cutting was exploited for the paper channel preparation. (ii) The channel width was optimized and a nozzle-like design was adopted to enhance concentration performance. (iii) To reduce energy cost and guarantee operation safety, the driven voltage must not exceed 50 V. (iv) To be comparable with existing paper-based ICP device, the target concentration factor should reach 100-fold. (v) To surpass the current enclosed paper-based microfluidic devices, the paper channel should possess sufficient mechanical strength, simple sealing strategy and high capability of mitigating sample evaporation. Hence, we developed a Parafilm sealing method for the paper-based concentrator fabrication.

5.2 Experimental procedures

5.2.1 Design principle

For paper-based ICP, the ion transportation is not only governed by conductance of the channel, but also determined by the channel geometry. Therefore, the device design had to be optimized. In

order to achieve better pre-concentration performance, some factors must be taken into consideration. The channel width is the first one that can influence the ICP effect. Obviously, a narrow channel is benefit for concentrating the sample due to the miniaturized dimension. However, it is very challenging to cut a narrow paper channel using craft cutter. Particularly, a narrow channel cut on a bare paper is easily broken, leading to difficult handling of the paper device. On the Parafilm-paper composite, a 600 μm wide narrow channel can be cut with high reproducibility. Further decrease of the channel width will dramatically increase the cutting failure. Second, the nozzle-like part, which can produce a squeeze effect due to channel geometry variation, plays a critical role for sample concentration. Therefore, to choose an appropriate angle for the nozzle-like part is important. In evaluating the angle effect, various nozzle shape with different angle were tested. The nozzle-like part with large angle ($>90^\circ$) showed the weakest concentration enhancement. The concentration enhancement showed in the nozzle-like part with small angle ($<90^\circ$) was much better, but the process is very slow. Hence, 45° was adopted as the nozzle angle.

Sartorius[®] quantitative grade 292 filter paper with average thickness of 180 μm was used in the fabrication of laminar composite with Parafilm. Negatively charged fluorescein isothiocyanate (FITC) (Sigma-Aldrich) solution with concentration of 5 μM was used as fluorescent tracer during the ICP process. Nafion perfluorinated ion exchange resin (Sigma-Aldrich) was directly patterned on paper channel to form a permselective membrane.

An inverted fluorescence microscope (Nikon Eclipse Ti-S) equipped with a CCD camera (Nikon DS-Ri2) was used to capture all images in the experiment. A customized Python program was developed to quantitatively analyze the captured images. A power supply (PLH250-P, Aim and Thurlby Thandar Instruments, UK) was used to provide the external electrical field to induce ICP on the paper chip. The standard tensile strength test was carried out on different substrates using a

mechanical testing machine (YG026B electronic fabric strength tester).

(a)

(b)

Figure 5.1. (a) Schematic illustration of the device fabrication process. (b) The photograph of an enclosed paper-based pre-concentrator. Two gaps were cut on the two wings, leaving the microchannel as the only path connecting the two reservoirs. Two electrode windows were located in the two reservoirs, respectively.

5.2.2 Device fabrication and operation

The procedures of fabricating the enclosed μ PADs were illustrated in Figure 4.1(a), including five major steps. First, the Parafilm and filter paper were laminated and sandwiched between two glass plates using clamps. Next, the parafilm laminated with filter paper was placed in oven at 85 °C for 5 min. Due to the wax property of paraffin, the Parafilm gradually melted and partially penetrated into the porous filter paper, forming a two-layer composite. The melted Parafilm bonded the filter paper very tightly and formed a Parafilm-paper composite. In the third step, the obtained Parafilm-paper composite was sent to cut using a computer controlled craft cutter (Silhouette America, Inc., Silhouette Cameo[®]) to obtain the designed microchannel. Meanwhile, the covering Parafilm bearing sample loading window and electrode windows was also prepared using the craft cutter. In step 4, the two wings of the device were cut manually to leave the microchannel as the only path connecting two reservoirs. Therefore, each wing of the device worked as the extension of one reservoir to absorb more water, enhancing moisture retention capability of the device. On the paper device, 0.5 μ L Nafion perfluorinated resin was directly pipetted at one end of the microchannel and left at room temperature about 1 h for air-drying. In the last step, the covering Parafilm and Parafilm-paper substrate was aligned and placed in oven for 3 minutes at 85 °C to enable the covering layer to tightly bond with the filter paper from top side. To reduce liquid sample leakage, the dimension of the covering Parafilm was slightly larger than that of the Parafilm-paper substrate. The extra margin of covering Parafilm was folded back to wrap the edges of the reservoirs. Finally, an enclosed paper-based device was fabricated with the paper microchannel was sandwiched by two Parafilm from top and bottom side. The electrode windows on the covering Parafilm can allow the electrodes to directly touch the hydrophilic paper reservoirs.

Due to the small dimension of the window, the amount of evaporation loss from the window was very little.

Figure 5.2. (a) Close view of the μ PAD with two electrodes fixed by a 3D printed holder. (b) Schematic illustration of ICP induced by external electric field. The Nafion permselective film only allow cations to pass through. Ion depletion region forms at the anodic side of Nafion, repelling negatively charged analytes toward the anode.

As shown in Figure 5.2(a), two copper electrodes were mounted on a 3D printed holder and

placed on the paper device to provide electric voltage. To operate the device, the channel and reservoirs were firstly pre-wetted with 10 mM CaCl_2 solution, followed by loading 5 μL sample from the sample loading window. Fluorescence images were captured every 10 s for subsequent analysis. The basic working principle of ICP pre-concentration was demonstrated in Figure 5.2(b). The Nafion membrane is composed of nanopores with average diameter of 4 nm. The surfaces of the pores are negatively charged due to the existence of sulfonic acid groups in Nafion, resulting in only cations can transport through the Nafion membrane. Under external voltage, the cations move toward the cathode and can transport through the Nafion membrane. On the contrary, anions at the right side of Nafion cannot travel through the membrane. To satisfy electrical neutrality, the continuous efflux of cations at the anodic side of membrane results in anions move out of this region. The electrophoretic migration (EP) is dominant in the depletion region and pushes the negatively charged FITC ions toward the anodic side. While the electroosmotic flow (EOF), which is dominant downstream of the depletion region, drives the fluorescent tracers to move toward the cathodic side [149]. The balance of the two opposing effects leads to the tracers focused at the depletion boundary.

5.3 Results and discussions

5.3.1 Parafilm sealing method

Parafilm, a thermoplastic material consisting primarily of polyolefins and paraffin waxes, is widely used in biology and chemistry laboratories, and recently has found its special use to fabricate paper-based microfluidic devices [127, 233]. Distinct from the previous reports where the Parafilm were used to form hydrophobic barriers to define the microchannel, in our μPAD , the Parafilm offers a leakproof surface to enclose the paper microchannel. The cross-sectional views

of Parafilm-paper composites were captured by optical microscope and showed in Figure 5.3. Before heating, the boundary between filter paper and Parafilm can be clearly seen in Figure 5.3(a). Figure 5.3(b) showed a filter paper embedded from bottom side. During heating process (85 °C, 5 minutes), the Parafilm melted and became semi-liquid, which gradually penetrated into the microporous paper under the pressure from clamped glass plates, resulting in that the Parafilm and filter paper bond tightly. The fibres were submerged by semi-liquid paraffin wax, forming a condensed composite that was difficult to be peeled off even when the paper is wet. This is advantageous over many other sealing methods that cannot withstand wet situation. The original thicknesses of Parafilm and filter paper were 130 μm and 180 μm respectively. The Parafilm embedding process led to the total thickness decreased to less than 280 μm , indicating that the Parafilm has been pressed into the paper. Figure 5.3(c) indicated that a filter paper was embedded by two Parafilm layers from top and bottom side. The filter paper remained hydrophilic and worked as an enclosed channel. The Parafilm penetration depth depends on heating time, temperature and applied pressure to press the melted Parafilm into porous paper. Through adjusting the three factors, the hydrophilic channel thickness can be well controlled. Additionally, as a mechanical process, craft cutting can guarantee reproduction accuracy and high consistency from sample to sample. Therefore, the fabrication method is highly reproducible and has the potential for mass production of μPADs .

Figure 5.3. Cross sectional view of the Parafilm and paper. (a) a filter paper and parafilm laminated together before heating; (b) a filter paper penetrated by Parafilm from bottom side; (c) a filter sandwiched by two layers Parafilm after heating.

Electric craft cutting provides an ideal choice for rapid-prototype of paper-based devices. However, some researchers reported that the tearing and rupture occurred very often when craft cutter was used for cutting cellulose filter paper [107, 234, 235]. In addition, to fix the paper before implementing craft cutting, the paper substrates must be stick to adhesive surface of a cutting mat. However, when they are peeled off from the adhesive mat, small structures are easily broken due to their low mechanical strength. In our fabrication process, we compared the cutting performance on the Parafilm-paper composite and bare filter paper. As shown in Figure 5.4(a) and (b), in contrary to using bare paper (Figure 5.4(a)), the use of Parafilm-paper laminar composite (Figure 5.4(b)) allowed the cutting process to be performed smoothly without any tears. This can be

attributed to two factors: first, the surface of Parafilm was much smoother than that of bare filter paper; second, the laminar composite was much more robust than bare filter paper. Additionally, thanks to enhanced mechanical strength, the small structures cut on this composite can be easily peeled off from adhesive mat without broken.

Figure 5.4. Comparison of cutting performances on (a) bare filter paper and (b) Parafilm-paper composite. Tears and ruptures occurs very often when filter paper was used as substrate for craft cutting, this can be attributed to the rougher texture of filter paper comparing with printing paper. For the Parafilm-paper composite, since one side was covered with smooth Parafilm, the cutting can be performed smoothly without tears and ruptures.

5.3.2 Mechanical property test

Low mechanical strength is a major weakness of most existing paper-based microfluidic devices. Particularly, the paper channels cut by knife possess relative low strength due to reduced physical dimension. The problem is even more severe when the paper channel is wetted by aqueous samples. In many circumstances, the paper channel becomes sagging when liquid samples are loaded onto the channel. This is because that the mechanical strength of cellulose fibres dramatically decreases when the fibres are wetted. Therefore, to prevent the wet channel from contacting surrounding

solid surfaces (*e.g.* glass plate on microscope stage), the paper devices with open channels usually must be suspended in air, which may cause the operation to be complicated. As mentioned above, the Parafilm-paper laminar composite showed high mechanical strength, which facilitated the uniform appearance and the large-scale fabrication of μ PADs.

Figure 5.5. Mechanical strength test of different paper substrates. Due to the super-low mechanical strength of wet filter paper, the test was only performed on bare filter paper in dry state. Each force value is averaged over $N = 4$ measurements. The error bars represent one standard deviation.

To demonstrate how well the mechanical strength had been enhanced by Parafilm embedding, a standard tensile stress-strain test was carried out on different paper substrates. Figure 5.5 depicted the forces required to pull different substrates to the points where they broke. The paper substrates with double side embedded by Parafilm and single side embedded by Parafilm were tested in dry state and wet state. The bare filter paper without Parafilm embedding was only tested in dry state, due to that wet filter paper possessed super low mechanical strength that below the minimum range of the strength tester. In dry state, the filter paper had the highest breaking force of 25.8 N. While

in dry state, the double side embedded and single side embedded substrates showed highest breaking forces of 160 N and 134 N, respectively. Even in wet state, these two Parafilm embedded substrates remained high mechanical strength. For the paper sealed from double side, the standard deviation regarding the breaking force is much higher than that of single side embedded substrates. This should be attributed to lamination nonuniformity used for fabricating double side embedded paper. When the double side embedded paper underwent tensile strength test, usually only one side Parafilm broke first. Hence, the test results of the double side embedded are similar as those of single side embedded device. The results proved that the Parafilm embedding technique dramatically enhanced the mechanical property of paper substrates.

5.3.3 Fluid flow measurement in enclosed and open channels

To compare the fluid flow in enclosed channel and open channel, two simple paper devices, consisting of straight channels and circular reservoirs, were fabricated. The width and length of the channel are 2 mm and 40 mm, respectively. For the enclosed device, both the straight channel and reservoir were sealed by Parafilm. A small window was opened at the reservoir for sample loading. To start the measurement, 20 μL red color food dye was dropped onto the reservoirs of both enclosed and open channels and the propagation of red dye in both channels were recorded by mobile phone camera. Figure 5.6 and Figure 5.7 show the difference between fluid velocities in an enclosed channel and an open channel. During the Parafilm sealing process, the penetration of Parafilm into the filter paper resulted in the decrease of paper thickness. The reduced paper thickness influences the wicking property of the paper, leading to the slower flow velocity in the enclosed channel. However, due to the protection of Parafilm, the sample evaporation rate in the enclosed channel was dramatically reduced. This can be evidenced by the longer fluid transport time in the enclosed channel comparing with that in the open channel. As observed during

experiment, the flow front of red dye stopped at 400 s. While, the red dye in enclosed channel continued moving forward until 600 s.

Figure 5.6. The representative time sequential images show the propagation of red dye front in enclosed channel (a) and open channel (b) with time. 20 μL red color food dye was dripped in the circular reservoir at the bottom of a straight channel. Under the function of capillary force, the red dye moved along the paper channel.

Figure 5.7. Flow comparison of red dye in enclosed channel and open channel. The results indicate a slightly slower flow velocity in the case of enclosed channel as compared to open channel within 400 s. The flow front of red dye in open channel stopped at 400 s, indicating the red dye has dried in the open channel due to evaporation. Each distance value is averaged over N=4 measurements, and the error bars represent one standard deviation.

5.3.4 ICP demonstration on enclosed μ PADs

At the beginning of the experiment, a drop of 20 μ M fluorescent tracer in deionized water was dipped onto the sample loading window of the paper channel. The entire channel was pre-wetted in 2~3 minutes due to the capillary effect of the cellulose paper. A voltage was applied across the channel through two copper electrodes connected with power supply to trigger ICP.

Experimental results for ion depletion and concentration were shown in Figure 5.8. Fluorescence images were recorded at different time (150 s, 200 s, 250 s, 300 s, 450 s) with a constant voltage of 50 V. Here, the time when the power supply started to provide voltage was set as start time point. The time-lapse images in Figure 5.8(a) demonstrated the ion depletion at the anodic side of the Nafion membrane developed and expanded with time under a DC voltage. The

generation of ICP was based on the permselectivity (cation-biased conductance) of Nafion membrane. Due to the existence of sulfonic acid groups in Nafion membrane, the surfaces of the nanopores were negatively charged, resulting in the selective migration of cations while the anions were blocked at the boundary of the membrane. The cations continuously migrated to the cathode under the function of external electric field. The fluorescent tracer used here was negatively charged and repelled by the ion depletion region toward anode. Consequently, the ion depletion region was induced at the interface of paper channel and nanoporous membrane. Previous studies demonstrated that the geometry of the paper channel can influence the concentration effect. Usually, a convergent channel can generate high pre-concentration effect than a straight channel [148, 236]. Gong *et al.* [148] used this arrangement to enhance sample pre-concentration and Yang *et al.* [236] systematically investigated the effect for paper-based ICP. In our study, we adopted convergent channel design to improve the device performance. The sample loading zone interfaced with the straight channel with a convergent geometry. The variation of channel dimension caused a nozzle-like squeeze effect. The pre-concentration performance was greatly enhanced by the nozzle at 250 s, resulting in more than 100-fold pre-concentration (Fig. 5.8(b)).

Many existing similar devices using the same driven voltage (50V). For example, Gong *et al.* [148] achieved 40-fold concentration using 50V driven voltage, and Yang *et al.* [236] obtained 20-fold enhancement in their paper-based concentrator given an external potential of 50V. Compared with these devices, the performance of our device with 100-fold concentration factor was more prominent. On the other hand, some researchers reported higher sample intensity enhancement than ours. For instance, Han *et al.* [150] obtained high preconcentration performance up to 1000-fold using 200V driven voltage. The high external voltage may lead to safety concern.

(a)

(b)

Figure 5.8. Formation of depletion region and concentration plug in sample loading region of an enclosed μ PAD. The sample loading region has a nozzle-like shape to produce a better concentration effect. (a) Time sequential images of 5 μ M FITC in the sample loading region under an applied voltage of 50V. The time when the power supply started to provide voltage was set as start time point. (b) The corresponding fluorescence intensity profiles at selected time points are plotted. The maximum concentration of FITC fluorescent dye reaches more than 100-fold at 250s.

Figure 5.9(a) demonstrates the pre-concentration plug moving in the enclosed straight channel. The boundary of depletion region became more and more bright, indicating the continuous accumulation of fluorescence ions. The forming of concentration band at the anodic side of depletion region boundary resulted from the balance of electroosmotic flow (EOF) and

electrophoretic migration (EP). The EP was dominant in the depletion region, forcing the negatively charged fluorescent ions migrate toward the anodic direction. While in the downstream of the depletion region, the EOF was dominant and drove the fluorescent tracers to the boundary of the depletion region. The interaction of the two opposing effects finally resulted in the concentration phenomenon. For observation convenience, we set the time when the concentration plug entered the straight channel as start time point. To quantitatively analyze the concentration effect, the corresponding fluorescence intensity profiles were shown in Figure 5.9(b), where the concentration enhancement reached its maximum at 150 s. The calculated maximum pre-concentrator factor is 60-fold. It can also be observed from the images that the pre-concentration plug gradually dispersed after 450 s, indicating the decreased ICP effect. This decrease can be attributed to the accumulated sample evaporation loss through the sample loading window and electrode windows. The results clearly demonstrated that the sensitivity of the paper-based assay was substantially improved by ICP effect.

Moreover, the enclosed configuration also enhanced the ICP durability. The fluorescence profiles showed that the pre-concentration plug can maintain around its maximum level for more than 300 s. The enhanced durability proved that the enclosed ICP device is suitable for practically point-of-care detection. The images of Figure 5.9(a) indicated that the profiles of the pre-concentration plug were more regular and concentrated than that we previously reported [125]. It has been reported that the fibre orientation plays an essential role on fluid flow in porous paper [237, 238]. However, in our fabrication process, the external pressure, which was applied from top and bottom side to press the melted Parafilm into the porous paper, could not change the fibre orientation in the paper. Therefore, we speculate that the porous paper was tightly compressed during Parafilm embedding process, resulting in regular alignment of the micropores.

In contrast to recently reported paper-based ICP pre-concentrator, most of which were fabricated by wax printing and directly exposed to air, the Parafilm embedded paper device can effectively prevent direct sample contact with support substrate, resulting in reduced sample contamination possibility, as well as dramatically mitigated sample evaporation rate. In addition, the mechanical strength of open-channel ICP pre-concentrator is deteriorated in wet state, leading to numerous operation inconvenience. Contrarily, the high mechanical strength of our proposed μ PAD can increase device operation flexibility.

Maintaining steady flow is of great significance to achieve high pre-concentration factor. Through adding external absorbent pad, Han *et al.* achieved up to 1000-fold pre-concentration result. The external absorbent pad maintained the paper channel in fully wet state. However, the addition of an external pad increased the operational complexity. Moreover, since the absorbent is exposed to atmosphere, the liquid evaporation loss is significant. Comparing to the recently reported ICP paper-based devices, which achieved up to several hundred-fold concentration factor [148, 150, 151], our device has its own advantages.

In short, compared with existing devices, our enclosed sample pre-concentrator demonstrated its advantages in the following aspects. (i) The fluorescence profile showed that the pre-concentration plug can maintain around its maximum level for more than 300s, which was much longer than those demonstrated in other paper-based ICP concentrators. (ii) Most of existing similar paper-based ICP device showed the concentration plug with poor profiles, which may bring extra difficulties for further quantification. For example, Phan *et al.* [125] demonstrated the concentration of fluorescent dyes on paper channel with various irregular shapes. Different from many other paper-based ICP concentrator, the profiles of our concentrator were more regular. (iii) Departing from the method using external absorbent pad [150], we fabricated two reservoirs with

microchannel on single paper substrate. The reservoirs act as absorbent pads to keep the pressure difference, which is the driven source of the lateral flow in the paper channel, remaining constant throughout the period of experiment. The sealing configuration not only increased the device portability but also facilitated operational simplicity.

Figure 5.9. Movement of pre-concentration plug in an enclosed straight channel downstream of the sample loading region. (a) Time sequential images and (b) corresponding fluorescence intensity profiles of the pre-concentration plug are recorded at selected time points. The boundaries of the paper channels are indicated by the dashed lines. The concentration enhancement reached its maximum at 150 s and maintained at its maximum level for more than 300 s.

(a)

(b)

Figure 5.10. ICP performance on the pre-wetted enclosed μ PAD that has been left in atmosphere for 2 hours: (a) time sequential images and (b) corresponding fluorescence intensities of pre-concentration plug at different time points. The time point of applying external voltage was set as $t = 0$.

5.3.5 Mitigated evaporation influence

Traditional μ PADs with open channels are susceptible to evaporation. Liquid sample loss and concentration change caused by evaporation are common problems suffered by open paper fluidic

channels. These issues are even more severe when dealing with precious samples. Specifically, in term of paper-based ICP, voltage induced Joule heat could deteriorate the situation. On paper-based ICP device, the ion migration is primarily affected by the conductance of the channel. Hence, the moisture retention is of significance to keep ICP effect on the paper device. One of the most important advantages of the enclosed μ PAD is that it can dramatically mitigate sample evaporation. To demonstrate the capability of our device of mitigating evaporation, fluorescent tracer pre-concentration was performed on an enclosed paper-based ICP device. In this experiment, the pre-wetted paper device was placed in atmosphere at room temperature for 2 hours, followed by applying 50 V voltage to trigger ICP. The corresponding ICP effect was reflected by the images in Figure 5.10(a). The time sequential images demonstrate that the sample was gradually concentrated and moved away from the Nafion membrane. This implies that an ICP effect was induced at the anodic side of the permselective membrane and can maintain for very long time without outside reagent supply. Even was left in atmosphere for 2 hours, an ICP phenomenon could still be induced on the enclosed device, indicating that the paper channel remained good conductance. This should be attributed to the protection of the Parafilm packing, which greatly reduced the evaporation rate of the liquid reagent in the device. Particularly, the two reservoirs sealed by Parafilm played an essential role in moisture retention. We previously reported 60-fold concentration enhancement on another paper-based ICP pre-concentrator, which was cut by craft cutter and sealed using Scotch[®] film to reduce sample evaporation [125]. However, sample leakage occurred in the device due to the deteriorated film adhesion when the channels were wet. In the contrary, the sample leakage is effectively prevented in the Parafilm enclosed device.

5.4 Summary

In summary, we reported a novel and cost-effective enclosed paper microfluidic platform based on

ICP as sample pre-concentrator. A Parafilm embedding technique was introduced to fabricate enclosed paper devices. This technique exploits easy-to-access materials and does not need expensive equipment. And the fabrication process is simple and easy-to-handle, enabling the possibility of large scale production of μ PADs. The strain-stress testing quantitatively proved that the Parafilm-paper composite held great mechanical strength. On the enclosed μ PAD, more than 100-fold pre-concentration factor has been achieved, demonstrating good concentration performance that was comparable with many reported paper-based ICP devices. Comparing to traditional open channel μ PADs, the Parafilm embedded paper device as ICP platform possesses four major advantages: 1) the Parafilm encased chip shows stronger mechanical strength than a pure paper chip; 2) it shows better concentration profile than on open paper device; 3) it greatly reduces sample contamination risk thanks to the enclosed configuration; 4) it holds the capability of dramatically mitigating nature evaporation during a relatively long period. We believe that our enclosed microfluidic devices are suitable for generic biochemical assays and therefore pave an avenue to overcome some major problems of conventional open-channel devices.

Chapter 6

Direct Spraying Method for Fabrication of Paper-based Microfluidic Sample Preconcentrator

6.1 Background

The paper-based ion concentration polarization (ICP) technology provides a promising solution to improve the detection sensitivity of μ PADs. However, most of the paper-based ICP sample concentrator were based on wax printing. Due to the limitation of wax printing, fabrication of simple ICP sample concentrators has not been accessible to many researchers. As a representative example of flexible energy devices, paper-based micro-sized supercapacitors (mSCs) have spurred great interest very recently. There are many valuable propositions of using paper to build mSCs—cost, flexibility, and compatibility to various fabrication process. Importantly, a great potential application of paper-based mSCs lies in working as energy storage component for μ PADs. In this study, we proposed a simple spraying method for the fabrication of ICP concentrator and mSCs on paper substrates.

6.2 Experimental procedures

Both μ PADs fabrication and paper-based mSCs construction involve patterning desired structures on paper substrates. The ideal methods should minimize the total fabrication cost and time by simplifying manufacturing procedures and materials. The approach we present here starts with direct spraying, which is subsequently followed with another low-cost,

bench-top method of microfabrication. Figure 6.1 illustrates the approach. Firstly, we explored non-expensive materials to create masks and obtained fluidic channels through one-step mask-assisted spraying. Based on this hydrophobic patterning method, we subsequently performed two hybrid techniques to fabricate leak-free paper channel and paper electrodes, respectively (Figure 6.1, Part II and Part III). First, we fabricated a μ PAD to perform fluorescence sensing through direct spraying hydrophobic patterning combining with Parafilm embedding. The Parafilm melted and penetrated into the filter paper and served as an isolation substrate to seal the paper bottom, which could greatly reduce contamination risk and increase operation flexibility. Experimental results have shown that a 220-fold preconcentration factor was achieved in the μ PAD. Second, we implemented vacuum filtration and deposited conductive carbon material onto the hydrophobic patterned paper substrate to fabricate paper electrodes. In contrast to previously reported in-plane paper-based mSCs fabrication methods, the combination of one-step spraying and vacuum filtration provided a simple path to fabricate interdigital electrodes. A paper-based mSC was achieved after packing the interdigital electrodes with gel electrolyte, and good performance of the paper-based mSC was demonstrated through electrochemical characterization.

Figure 6.1. A schematic overview of the process described in this paper. Direct hydrophobic spraying method can create hydrophobic-hydrophilic patterns on paper substrate. These patterns can be further modified for broad applications. After Parafilm embedding, the device can be used as sample preconcentrator based on ICP effect (Part II). In addition, we fabricated electrodes using this spraying method (Part III). The electrodes hold a number of application potentialities, such as microsupercapacitors.

6.2.1 Direct spraying hydrophobic patterning

Whatman® filter paper with thickness of 180 μm was chosen as substrate for μPADs fabrication. A commercially available water repellent product (Neverwet®, NeverWet L.L.C.) was purchased from a local retail store and employed here as hydrophobic coating material. Acrylic plastic plates and common paperboard were used to fabricate spray masks. AutoCAD® software (Autodesk, San Rafael, CA, USA) was used to design mask patterns. Computer numerical control (CNC) milling equipment (Stepcraft® 420, Stepcraft CNC system, Germany) amounted with 500 μm diameter endmill was used to cut designed patterns on acrylic plates. To further reduce the fabrication cost, a craft cutter (Silhouette America, Inc., Silhouette Cameo®) was also used to cut designed masks on paperboard. Both the acrylic and paper masks were adopted in this work to demonstrate their feasibility for direct spray patterning.

Figure 6.2 schematically outlines the hydrophobic patterning process for paper-based microdevices. Firstly, the paper substrate was sandwiched between a mask and a piece of glass that provided a back support (Figure 6.2 (B)). Subsequently, the hydrophobic coating was manually sprayed onto the substrate to form hydrophobic barriers along the boundaries of the mask. After the spraying, the substrate was left in a place with good ventilation for 15 min for drying. Finally, the paper substrate was disassembled from the sandwiched structure and the patterned papers were ready to use. The feasibility of the fabricated fluidic device was demonstrated by the time sequential images of blue dye flowing through the channels (Figure 6.3).

Figure 6.2. (A) Schematic illustration of the mask-assisted hydrophobic spraying process. (i) Paper substrate was adopted without any pretreatment. (ii) Paper substrate (filter paper or membrane filter) was covered by a mask. (iii) Hydrophobic substance was evenly sprayed onto the substrate. (iv) The desired patterns were obtained after disassembling the mask from the paper substrate. (B) The sandwiched structure with the implementation of magnetic force (not to scale).

Figure 6.3. Non-metal masks were applied to fabricate fluidic patterns by using direct spraying method. The masks were made out of inexpensive paperboard. The feasibility of the fabricated fluidic device was demonstrated by the time sequential images of blue dye flowing through the channels.

6.2.2 Fabrication and operation of leak-free paper-based ICP preconcentrator

The filter paper and Parafilm were laminated together and sandwiched by glass slides and clamps. When placed in oven at 95 °C for 5 min, the Parafilm melted gradually and penetrated into the filter paper. The penetration depth was controlled by the heating time. Figure 6.4 (A) depicts a representative cross-sectional view of the filter paper embedded by Parafilm after heating. It can be seen that the Parafilm partially penetrated into the filter paper. Following the same workflow (Figure 6.2 (A) and (B)), the hydrophobic barriers were formed after direct spraying. Nafion perfluorinated ion exchange resin (Sigma-Aldrich) was pipetted on the centre of the hydrophilic channel to form the permselective membrane. The device was placed on hotplate to evaporate the solvents of the fresh resin at 90 °C.

The schematic of ICP preconcentrator is shown (Figure 6.5 (A)). To operate the device, two copper electrodes were placed at the two ends of the channels and a voltage was applied to trigger ion depletion at the anodic side of the Nafion membrane. A DC power supply (PLH250-P, Aim and Thurlby Thandar Instruments, UK) was used to provide 50V voltage for ICP. Negatively charged FITC with concentration of 20 μ M was chosen as fluorescent tracer. NaOH solution with concentration of 100 mM was used to pre-wet the paper channel. The fluorescent tracer movement was observed using an inverted fluorescence microscope (Nikon Eclipse Ti-S) interfaced with a CCD camera (Nikon DS-Ri2). A customized Python program was used to analyse the fluorescence intensity.

Figure 6.4. (A) A cross-sectional micrograph of a Parafilm embedded filter paper. The total thickness of Parafilm and filter paper changed as a function of (B) temperature and (C) heating time. Each thickness value is averaged over $N=5$ measurements.

Figure 6.5. Demonstration of ICP phenomenon in the leak-free μ PAD. (A) Schematic illustration of the paper-based ICP preconcentrator. A hybrid substrate was fabricated through embedding Parafilm into filter paper. The hydrophobic barrier was constructed through direct hydrophobic spraying on the hybrid substrate. (B) Representative images of fluorescent tracer at selected time points. The dashed lines indicate the boundaries of hydrophilic channel. (C) Fluorescence intensity profiles corresponding to above time sequential images.

6.2.3 Fabrication of paper-based mSC

The paper-like membrane filter (Shanghai Xinya Purification Instruments) was used as mSC substrate. The paper-based mSC fabrication procedures were schematically illustrated (Figure 6.6 (i)-(v)). Firstly, an interdigital pattern was formed on a paper substrate using the same hydrophobic coating approach demonstrated in Figure 6.2. Secondly, the paper substrate was placed at the bottom a funnel and multi-wall carbon nanotubes (MWCNT)-dispersed solution with concentration of 2 g/L in water (Nink-1000, NanoLab, Inc., MA) was infused to the funnel. Then the vacuum pump connected with the funnel was turned on to suck the aqueous solution, resulting in the solution flow through the paper substrate. Because the size of MWCNT was larger than that of membrane pore, almost all MWCNT were trapped by the membrane filter and the liquid was drawn through the funnel into the flask below by the vacuum suction force. In addition, due to the water-repellent property of the hydrophobic coating, the solution could only flow through the uncoated parts of the paper substrate. Therefore, MWCNT only filled the hydrophilic parts, remaining the hydrophobic parts clean (Figure 6.6 (ii)). The un-deposited areas worked as spacers to separate adjacent electrodes. The closely spaced electrodes gave rise to short ion and electron diffusion pathways. Figure 6.6 (ii) shows the results before and after filtration of MWCNT-dispersed solution. The deposited patterns immediately shaped after the filtration. The extra margins of the substrate were then cut off by a scissor, to ensure the anode and cathode were separated. Sufficient PVA/H₂SO₄ solution was drop-casted onto the paper substrate and dried to form a solid electrolyte (Figure 6.6 (iv)). Finally, silver paint was applied to the common areas of the electrodes to glue two separate copper tapes to the electrodes. The conductive copper tapes were used as the extension of the electrodes to

connect to an electrochemical workstation for measurements (Figure 6.6 (v)). The use of gel electrolyte simplified the formation of a compact micrometer-sized device without the complication of a liquid electrolyte [239].

Figure 6.6. Schematic fabrication of paper-based mSC based on hydrophobic spraying: (i) Interdigital electrodes pattern was fabricated through spraying hydrophobic coating on membrane filter. (ii) Vacuum filtration technique was utilized to deposit conductive MWCNTs onto the uncoated part of the membrane filter (inset: before vacuum suction, the membrane filter was submerged by carbon solution (left), interdigital electrodes appeared on the membrane filter after vacuum suction (right)). (iii) The cartoon of as-fabricated planar carbon electrodes on paper substrate. (iv) Drop-casting of PVA/H₂SO₄ gel electrolyte onto the planar electrodes on paper substrate. (v) Attachment of copper tape to cathodic and anodic electrodes for electrical connection.

6.3 Results and discussions

6.3.1 Direct spray hydrophobic patterning

Filter paper can absorb a liquid material by capillary action due to the chemical composition and micron-sized structure of cellulose fibres. Micropatterns that are defined through spatially tuning the wettability on filter paper provide an approach to manipulate liquid on paper. Super-hydrophobicity, always referred to as the Lotus effect, has been introduced to industrial and academia applications including microfluidic devices fabrication [240]. The hydrophobic barrier forming is the key procedure for paper-based microfluidic device fabrication. Here we coated paper substrates with commercial hydrophobic material, converting the substrates from hydrophilic to hydrophobic. We achieved spatially selective hydrophobic coating by using masks covered on the substrates. Neverwet[®] is a commercially available water repellent coating that contains organic solvent, including acetone, xylene, and liquefied petroleum gas. Liu *et al.* have proved the biocompatibility of the Neverwet[®] treated surface through a series of testing [133]. The coated surface features micro-/nano-scale hierarchical structure that converts the surface from hydrophilic to hydrophobic. A scanning electronic microscope (SEM) (JSM-6700F, JEOL, Japan) was used to analyse the surface features of the paper substrates. An atomic force microscope (AFM) (Dimension SPM, Bruker) was used to probe the 3D morphology of filter paper substrates with and without hydrophobic coating. Images were collected in tapping mode with a cantilever with nominal spring constant of 40 N/m (Tap 300, Budgetsensors). Hydrophobicity of the treated substrates was characterized by water contact angle measurement. The pristine surface is shown in Figure 6.7 (A). The SEM image and AFM 3D morphology of filter paper surface after hydrophobic spraying coating are shown in

Figure 6.7 (B) and (C). AFM topography shows a roughness $R_q=441$ nm for hydrophobic coating area. These morphology pictures revealed that micro-/nano-scale hierarchical structures have been introduced on the filter paper surface by spraying coating, resulting in the cellulose fibres to be fully covered. The hydrophobic feature of the coated substrates is apparent by the water contact angle measurement. Figure 6.7 (D) shows a drop of water maintained almost spherical shape on the hydrophobic substrate, with a contact angle of larger than 120° , indicating that the spraying treatment had converted the paper surface from hydrophilic to hydrophobic.

Figure 6.7. Surface characterization of paper substrates. SEM images of Whatman[®] grade 1 filter paper (A) before and (B) after hydrophobic coating; (C) AFM 3D morphology of filter paper after hydrophobic coating; (D) water contact angle measurement on filter paper after hydrophobic coating.

In order to obtain regular patterns, it is necessary to prevent the diffusion of the sprayed substance into the underneath of the masks. During our experiment, we found that a soft mask was not able to attach to the paper substrate tightly. Hence, some parts of the substrates underneath the mask boundaries were invaded by the sprayed substance, which would break the uniformity of the desired patterns. Two factors are critical for the control of the hydrophobic patterns. First, the mask should be sufficiently rigid to avoid deformation during spraying. Second, the mask should be tightly stacked onto the paper substrate so as to minimize coating substance invasion. In the work reported by Thara *et al.*, before conducting spray-on coating, iron mask, which was produced by laser cutting, was attached on top of the paper substrate via magnetic force with a magnetic plate [134]. Here, we propose a method to further simplify the process of stabilizing the mask. After sandwiching paper substrate with mask and glass slide, a permanent magnet was placed behind the glass and some small stainless steel screws on top of the mask. The small screws were forced to stack the mask to the filter paper. The side-view of the stacking was shown in Figure 6.2 (B). This process allowed the mask to be non-metal, which is generally easier to fabricate. The minimum channel width is determined by the mask and lateral diffusion effect. In our experiment, we can fabricate channel mask with a minimum width of 500 μm . To ensure the mask structure were free from any obstruction, only small screws were used on the top of the mask.

CNC milling was carried out for the fabrication of the mask, where hard acrylic materials were used to mask the substrates efficiently. We also demonstrated that the similar pattern can be formed by using craft cutter on a discarded paperboard. Then, 6 piece of similar masks were laminated together with glue. The stiffness of the laminated paperboard mask

was comparable to that of an acrylic mask. Therefore, the laminated paper mask can be used in the spraying coating fabrication.

Figure 6.3 shows the representative as-fabricated paper-based microfluidic chips and adopted masks. The hydrophilic channels were stained by blue dye for visualization and captured at different time points. The minimum width of the channels was 1 mm. It can be seen from the image that with the increase of total channel area and complexity, the color dye required more time to fully wet all hydrophilic channels. As shown in the images, the fabricated channels look slightly smaller than the masks, and the edges of the channels look a little rough. In the spraying process, the sprayed aqueous hydrophobic materials randomly fell on the paper substrate, resulting in uneven distribution of the hydrophobic materials on the surface. This unevenness was obvious at the edges of the channel and led to the irregularity of the channel edges. Figure 6.8 shows the microscopic views of the channels fabricated on filter paper substrate. It can be clearly seen that the boundaries between the hydrophobic region and the hydrophilic region are not very uniform, which is a common phenomenon for most paper-based microfluidic devices.

Figure 6.8. The microscopic views of paper-based microfluidic channels observed by optical microscope at 4 $\times$ magnification. (A) The boundary between hydrophobic coating (top) and hydrophilic paper channel soaked with blue food dye (bottom). (B) A hydrophilic channel defined by hydrophobic coating.

In the spraying process, the spray depth and lateral diffusion of the hydrophobic materials are two important factors, which can influence the channel profile. The spraying depth relies on spraying time and the distance between spraying nozzle and the substrate. To ensure that the hydrophobic materials can penetrate through the entire thickness of the paper substrate, the substrates were sprayed for 3 seconds with a distance between spraying nozzle and the substrate of around 15 cm. Lateral diffusion is another factor that should be considered. Due to the diffusion, the hydrophobic materials can propagate to the covered area from the side of the mask within the paper, resulting in around 10 % variation of the dimensions of the fabricated channel from the mask.

6.3.2 Leak-free paper-based ICP preconcentrator for fluorescence sensing

The μ PADs fabricated using wax printing contains channels for the entire thickness of the paper. Consequently, when liquid samples are loaded in the device, the device has to be suspended in air to avoid direct contact with other surfaces, which may induce sample contamination or loss. Parafilm is one of the mostly used laboratory consumables with high biocompatibility and chemical inertness. It can melt at above 60 °C due to paraffin wax property. Direct hydrophobic spraying combining with Parafilm embedding provides an easy-to-use approach to fabricate μ PADs, albeit not yet fully reported so far. The microfluidic devices fabricated by this hybrid technique are expected to have the following advantages: 1) the Parafilm seals the paper bottom, providing a flexible and strong support for the device. And the paper device can be placed on a solid surface, such as glass slide. These increase operation versatility whilst reduce the contamination risk; 2) a shallow channel can be achieved through the Parafilm embedding, enabling rapid heat dissipation

and reduced sample consumption during ICP process.

To determine the heating temperature and time applied for Parafilm embedding, the entire thickness of Parafilm and filter paper was measured under different conditions. The variation of entire thickness of Parafilm-filter paper hybrid structure with temperature and heating time are shown in Figure 6.4 (B) and (C) respectively. Initially, the entire thickness of the hybrid structure was around 310 μm . As shown in Figure 6.4 (B), the thickness did not change when the heating temperature was set at 40 $^{\circ}\text{C}$, indicating the Parafilm did not melt. When the temperature was set above 60 $^{\circ}\text{C}$, during a same period, more Parafilm penetrated into the filter paper with increased temperature, resulting in reduced entire thickness. Figure 6.4 (C) shows that the entire thickness decreased gradually with time, suggesting that more Parafilm melted with time. Moreover, when the Parafilm was embedded at 100 $^{\circ}\text{C}$ for 10 minutes, the entire thickness decreased to the thickness of the original filter paper (180 μm).

To demonstrate the practical feasibility of the Parafilm embedded μPAD , a sample preconcentration and sensing experiment based on ICP effect was performed on this μPAD . The ICP experiment was carried out on the μPAD under the applied voltage of 50 V. The device was first pre-wetted by 100 mM NaOH solution, followed by dropping 20 μL FITC tracer with concentration of 20 mM. Fluorescent images were recorded every 10 s for subsequent analysis. Experiment results for fluorescent tracer depletion and concentration are shown in Figure 6.5 (B) and (C). As demonstrated in Figure 6.5 (B), ion depletion region generated within 10 s after electric field applied and continuously expanded. Due to the presence of negatively charged sulfonic acid groups in Nafion, only cations can transport through the Nafion membrane that contains nanopores. At the anodic side of the

permselective membrane, the ion depletion band formed shortly after applying voltage as a result of initial electroosmotic flow (EOF), which driven the negatively charge fluorescent tracer move toward the cathodic direction. In the depletion region, the electrophoretic migration (EPH) is dominant, pushing the fluorescent tracer toward the anodic side. The balance of the two opposing effects resulted in ions focused at the depletion boundary [148]. With time passed, the depletion band extended and more fluorescent ions concentrated at the depletion boundary, forming a preconcentration plug.

The fluorescence intensity profiles at some selected time points corresponding to Figure 6.5 (B) are plotted in Figure 6.5 (C). To quantify the preconcentration performance, the ratio of maximum to initial fluorescence intensity was used as the preconcentration factor. The measured maximum preconcentration factor reached up to 220-fold at 40 s. During the experiment, we observed that the maximum concentration decreased after 40 s. This can be attributed to the evaporation effect, including natural evaporation and Joule heating induced evaporation [236].

6.3.3 Paper-based microsupercapacitors

Direct spraying method also provides an alternative way to fabricate in-plane mSCs by patterning the carbon nanotubes (CNTs) on the surface of film substrate. In order to exploit the potential of direct spraying hydrophobic patterning method, membrane filter was utilized as mSC substrate and vacuum filtration technique was used to deposit electrodes on the substrate. The filter, which is paper-like membrane, is used for filtering aqueous solutions and can retain particles or microorganisms that are larger than membrane pore size. The paper-like membrane filter was chosen as mSC substrate for two reasons: (1) it possesses high flexibility, solvent compatibility, and hydrophilicity. These properties are

similar to filter paper that are commonly used as μ PADs substrates; (2) its high pore density and narrow pore size distribution ensure a high-level particle retaining. Vacuum filtration is a widely used technique for separating solid product from a mixture of solid and liquid. The objective here was to use this technique to deposit conductive materials onto the membrane filter that bears primary interdigital electrodes pattern.

Figure 6.6 shows three steps to fabricate conductive electrodes on paper: (1) hydrophobic patterning, (2) vacuum filtration and (3) packing. As illustrated above, the mask assisted spraying coating method provided a fast hydrophobic patterning approach. Vacuum filtration technique was adopted to deposit conductive materials onto the membrane filter, on which the interdigital electrodes pattern was formed beforehand. The unique micro-architectures of membrane filter with porous structure allows water pass through while retaining substance with certain dimensions. Carbon materials such as carbon nanotubes (CNTs) and graphene could be absorbed into paper membrane filter, rendering its conductivity and even electrochemical activity. Vacuum filtration process can be completed in several minutes, and a conductive membrane is achieved by simply soaking the solutions containing carbon materials. On the paper-based mSC, the electrodes should be highly conductive to work as current collector. As CNTs have been widely accepted for SCs applications, we chose CNTs as conductive material to construct interdigital electrodes.

In the case of in-plane paper-based mSC, the interdigital electrodes on paper substrates are the central parts of the mSC architecture. Figure 6.9 (A) shows the configuration of the interdigitated electrodes. The length of each electrode was 10 mm and the width was 500 μm . The spacing between two neighboring electrodes was 500 μm . The subsequent electrochemical performance characterization was mainly conducted on the electrodes

basis.

A parametric study was conducted by measuring cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) in a three-electrode configuration (Figure 6.9 (B), (C) and (D)). The cycling performance of this in-plane supercapacitor was tested at different scan rates from 5 to 500 mV/s (Figure 6.9 (B)). In the voltage window ranging from 0 to 1.0 V, the CV curves exhibited nearly rectangular shapes, demonstrating the effective formation of an electric double layer (EDL) at the electrode/electrolyte interface and good charge propagation across the electrodes. Furthermore, as the scan rate increased above 100 mV/s, the voltammograms window tended to tilt toward the vertical axis, thereby becoming a quasi-rectangle. This result indicated the dominance of the double layer formation in the energy storage process at lower scan rates.

The calculated areal capacitance of our supercapacitor was 0.32 mF/cm² at 10 mV/s scan rate, which was in accord with many previous reports of carbon-based all-solid-state supercapacitors. Li *et al.* fabricated an in-plane microsupercapacitor using silver nanoparticles as current collectors and obtained 0.59 mF/cm² areal capacitance at 100 mV/s scan rate [241]. Based on the same printing technique, they constructed all-solid-state graphene-based in-plane microsupercapacitors with interdigitated structure on silicon wafers. The devices showed areal capacitance over 0.14 mF/cm² at 5 mV/s scan rate [242]. Notarianni *et al.* presented a flexible thin film supercapacitor, which achieved areal capacitance of 0.4 mF/cm² at 10 mV/s scan rate. The performance of our supercapacitor is comparable to these studies [243].

Figure 6.9 (C) shows the GCD curves of the devices at different current densities, which were used to further understand the capacitive features of the mSC. The symmetric

triangular-shaped GCD curves with a nearly linear variation of voltage as a function of time during charge and discharge can be observed. Both the symmetry and good linear profile of the charge and discharge curves indicated the good capacitive performance of the device. Correspondingly, the volumetric capacitance was calculated to be 42.5 mF/cm^3 at a current density of 2 mA/cm^3 (Figure 6.9 (D)).

Figure 6.9. Performance of the fabricated micro-supercapacitors. (A) The photograph of as-fabricated mSC. (B) Cyclic voltammetry (CV) curves of mSCs at different scan rates from 5 to 500 mV/s. (C) Galvanostatic charge-discharge (GCD) curves of mSC at various charge/discharge rates. (D) Rate capabilities of mSCs.

As an emerging field, so far the number of reported in-plane paper-based mSCs is very limited. Lee *et al.* reported the first in-plane paper-based mSC fabricated with thermal-evaporated gold interdigital electrodes on paper substrate [244]. Chen *et al.* manually drew

interdigital graphite electrodes on paper, followed by coating solid-state PVA/H₂SO₄ electrolyte onto the paper surface [245]. Ma *et al.* introduced a vacuum filtration method to fabricate mSCs with interdigital electrodes [246]. They used SU-8 to pattern interdigital electrodes through typical photolithography technique, and implemented vacuum filtration to deposit carbon nanotubes solution to fill the SU-8 trenches. Following Au layer deposition, the SU-8 was removed using remover PG. This process required photolithography, and added complexity to the process of fabrication. The ideal paper-based microdevices fabrication method should minimize the total fabrication cost and time by simplifying manufacturing procedures and materials. Compared to the current paper-based mSCs, our fabrication method based on hydrophobic spraying provides a simple and novel alternative approach to simplify the fabrication and reduce total cost.

Fabrication of paper-based mSC suggested feasibility and versatility of this proposed hydrophobic patterning method. Compared with the photolithography and related fabrication methods, our approach simplified fabrication process by utilizing manual hydrophobic patterning and vacuum filtration. This approach provides an alternative route to achieve the same device for researchers with limited lab resources. One of the most promising applications of the as-proposed paper mSC is integration of on-chip battery to build a self-powered microfluidic sensor. This integration shall miniaturize the microfluidic system by replacing the bulky external power supplies. As the membrane filter possesses the similar properties as those of filter paper, a μ PAD can be co-fabricated on the same filter membrane substrate.

6.4 Summary

In this work, we demonstrated a simple spraying method to fabricate paper-based microfluidic devices. The fluidic channels on paper were formed through one-step mask assisted hydrophobic spraying. Compared with traditional μ PADs fabrication methods such as wax-printing and photolithography, this direct spraying approach does not require complex facilities. Based on this hydrophobic patterning technique, we further developed two hybrid methods to fabricate functional microdevices: a leak-free ICP preconcentrator for fluorescence sensing, and a paper-based mSC. The versatility of this patterning approach opens up new routes to prototype highly integrated, paper-based microfluidic sensors.

Chapter 7

Summary and Outlook

7.1 Original contribution of this thesis

The demands for integrative, versatile and inexpensive tools facilitating biomedical research and diagnostic applications keep booming. However, there are still numerous unmet needs in each aspect. Driven by those needs in current biomedical research and applications, this dissertation seeks to contribute to the field by developing (1) microfluidic platforms facilitating cell separation, (2) microfluidic devices enabling bio-sample analysis. The original contribution of this thesis can be summarized as following:

- In Chapter 3, we developed a cell separation platform leveraging microelectromagnets as magnetic field source to isolate cancer cells in microfluidic channel. The magnitude of electromagnetic field can be tuned by changing electric current. On this platform, we demonstrated up to 79% cell separation efficiency using 1A current.
- In Chapter 4, a highly integrated microfluidic platform for cell separation was proposed. To our knowledge, this is the first experimental demonstration, where inertial force induced size-based cell separation and magnetic force induced affinity-based cell separation are integrated on one chip. This device can operate at high-throughput ($\sim 100\text{-}150\mu\text{l}/\text{min}$) and facilitates continuous collection of cells for downstream analysis. This processing throughput lies in the highest level among state-of-the-art microfluidic cell separation devices.
- In terms of most microfluidic size-based separation approaches, such as micro-filter, cell clogging and low throughput are the major disadvantages. The integrated multiplexed cell sorter achieves high throughput without exhibiting a clogging problem. And compared to previous Dean Flow Fractionation technique, our integration of spiral channel with micro-magnets enables additional affinity-based cell separation.

- A creative Parafilm sealing method was proposed in Chapter 5. This is the first demonstration of using low-cost Parafilm to fabricate fully-enclosed paper-based microfluidic device.
- A simple spraying patterning method was proposed in Chapter 6. Compared with traditional μ PADs fabrication methods such as wax-printing and photolithography, this direct spraying approach does not require complex facilities.
- Based on the spraying patterning method, a novel and fast approach was developed to fabricate paper-based micro-supercapacitors.

7.2 Future work

All the technologies demonstrated above deserve further improvement for broad biomedical research and applications. My research will continue to investigate how to improve the performances of these devices and expand their applications. Based on the results presented in this study, future research will be focused on the following aspects:

1. Improve the efficiency of microelectromagnets-based cell separation platform.

The cell separation efficiency can be determined by many factors. The cell sorting efficiency of microelectromagnets-based cell sorter can be further improved by optimizing the geometries of microstriplines and microchannel. In Chapter 3, we stated that a cell's trajectory in laminar flow was governed by the balance of two competing forces: flow drag force and magnetic force. In order to move the cells laterally across the streamline, the magnetic force requires enough time working the immunomagnetically tagged cells. It is believed that extending the working time of external field is helpful to displace the cells toward side wall. Hence, replacing the microchannel with a much longer one could increase the cell lateral migration, resulting an amplified separation effect. In addition, the increased separation efficiency could allow for the increasing of flow rate, leading to enhanced processing throughput.

2. Construct tunable cell sorter.

Immunomagnetic cell tagging is a critical step for magnetic cell separation. The cell binding efficiency is relevant to cell type and the antigens expressed on cells' surfaces. In addition, there are significant variations among the magnetic permeabilities of immunomagnetic beads. Hence, the magnetic properties of immunomagnetically tagged cells vary case by case. These variations give rise to the difficulty in constructing a generic magnetofluidic platform since different cell types may require different magnetic fields. Conventional magnetofluidic devices utilize permanent magnets as magnetic source, which can only provide constant magnetic field. As discussed in Chapter 3, the magnetic field generated by microelectromagnets can be controlled by tuning the electric current. In other word, the magnetic field can be adjusted to displace the cells according to their magnetic properties. This feature is advantageous over permanent magnets-based microfluidic sorter since it can enable the construction of a tunable and generic cell sorter for the processing of various cell types, without re-design devices according to different cases.

3. Integrate the microelectromagnets with spiral microfluidics to achieve highly tunable multiplexed cell sorting.

The spiral microfluidics presented in Chapter 4 holds the advantage of high throughput. As mentioned above, the microelelctromagnets-based device holds the potential for constructing tunable cell sorter. Hence, the marriage of spiral microfluidics and microelectromagnets can produce a versatile cell sorter with high throughput. To achieve this goal, the major obstacle is the mismatch of the flow rates in the spiral device and in the magnetofluidic device. The flow rates in the spiral channel are much higher than that being practicable for magnetophoretic separation. One approach to solve this issue is to siphoning effect, which was proposed by Martel *et al.* for bioparticle concentration [247]. In brief, a series of asymmetrically curved channels connected

with the spiral channel are applied to generate a cell focusing region and a cell free region. The cell free regions are linked to a siphon channel, which is used to repetitively remove the cell free fluid, leading to the continuous reduction of the total fluid volume and hence the flow rate. Then the sample with low flow rate can be introduced into the magnetic separation section for cell isolation.

4. Apply the size and affinity-based cell sorter for circulating tumor cells (CTCs) separation.

Inertial microfluidics has been known for its high throughput characterization. And the affinity-based microfluidic cell separation is featured with high selectivity. The coupling of the two functionalities provides an enabling platform for cell separation, which has been successfully demonstrated in Chapter 4. A versatile microfluidic platform for the separation CTCs from whole blood is highly demanded for cancer prognosis. Based on the results showed in Chapter 4, our proposed size and affinity-based cell sorter can also be applied for CTCs isolation. As concluded by Di Carlo [91], the cell inertial migration is dependent on the microchannel dimensions. Comparing with nucleated blood cells, the average size of CTC is much larger. Hence, to apply the size and affinity-based cell sorter for CTCs isolation, a deeper channel should be used.

References

- [1] C. M. Yousuff, E. T. W. Ho, K. I. Hussain, and N. H. B. Hamid, "Microfluidic Platform for Cell Isolation and Manipulation Based on Cell Properties," *Micromachines*, vol. 8, p. 26, Jan 2017.
- [2] C. L. Zhao, Z. W. Ge, and C. Yang, "Microfluidic Techniques for Analytes Concentration," *Micromachines*, vol. 8, p. 32, Jan 2017.
- [3] G. M. Whitesides, "The origins and the future of microfluidics," *Nature*, vol. 442, pp. 368-373, Jul 27 2006.
- [4] S. C. Terry, J. H. Jerman, and J. B. Angell, "Gas-Chromatographic Air Analyzer Fabricated on a Silicon-Wafer," *Ieee Transactions on Electron Devices*, vol. 26, pp. 1880-1886, 1979.
- [5] E. Bassous, H. H. Taub, and L. Kuhn, "Ink Jet Printing Nozzle Arrays Etched in Silicon," *Applied Physics Letters*, vol. 31, pp. 135-137, 1977.
- [6] D. R. Reyes, D. Iossifidis, P. A. Auroux, and A. Manz, "Micro total analysis systems. 1. Introduction, theory, and technology," *Analytical Chemistry*, vol. 74, pp. 2623-2636, Jun 15 2002.
- [7] A. Manz, N. Graber, and H. M. Widmer, "Miniaturized Total Chemical-Analysis Systems - a Novel Concept for Chemical Sensing," *Sensors and Actuators B-Chemical*, vol. 1, pp. 244-248, Jan 1990.
- [8] E. K. Sackmann, A. L. Fulton, and D. J. Beebe, "The present and future role of microfluidics in biomedical research," *Nature*, vol. 507, pp. 181-189, Mar 13 2014.
- [9] Y. N. Xia and G. M. Whitesides, "Soft lithography," *Angewandte Chemie-International Edition*, vol. 37, pp. 550-575, Mar 16 1998.
- [10] Y. N. Xia and G. M. Whitesides, "Soft lithography," *Annual Review of Materials Science*, vol. 28, pp. 153-184, 1998.
- [11] P. Liu and R. A. Mathies, "Integrated microfluidic systems for high-performance genetic analysis," *Trends in Biotechnology*, vol. 27, pp. 572-581, Oct 2009.
- [12] E. W. Esch, A. Bahinski, and D. Huh, "Organs-on-chips at the frontiers of drug discovery," *Nature Reviews Drug Discovery*, vol. 14, pp. 248-260, Apr 2015.
- [13] S. Le Gac and A. van den Berg, "Single cells as experimentation units in lab-on-a-chip devices," *Trends in Biotechnology*, vol. 28, pp. 55-62, Feb 2010.
- [14] J. C. Jokerst, J. M. Emory, and C. S. Henry, "Advances in microfluidics for environmental analysis," *Analyst*, vol. 137, pp. 24-34, 2012.
- [15] A. Gross, J. Schoendube, S. Zimmermann, M. Steeb, R. Zengerle, and P. Koltay, "Technologies for Single-Cell Isolation," *International Journal of Molecular Sciences*, vol. 16, pp. 16897-16919, Aug 2015.
- [16] H. M. Shapiro, *Practical flow cytometry*, 4th ed. New York: Wiley-Liss, 2003.
- [17] S. Miltenyi, W. Muller, W. Weichel, and A. Radbruch, "High-Gradient Magnetic Cell-Separation with Macs," *Cytometry*, vol. 11, pp. 231-238, 1990.
- [18] M. R. Emmert-Buck, R. F. Bonner, P. D. Smith, R. F. Chuaqui, Z. P. Zhuang, S. R. Goldstein, *et al.*, "Laser capture microdissection," *Science*, vol. 274, pp. 998-1001, Nov 8 1996.
- [19] V. Espina, J. D. Wulfschlegel, V. S. Calvert, A. VanMeter, W. D. Zhou, G. Coukos, *et al.*, "Laser-capture microdissection," *Nature Protocols*, vol. 1, pp. 586-603, 2006.
- [20] J. Frohlich and H. Konig, "New techniques for isolation of single prokaryotic cells," *Fems Microbiology Reviews*, vol. 24, pp. 567-572, Dec 2000.

- [21] N. Pamme, "Continuous flow separations in microfluidic devices," *Lab on a Chip*, vol. 7, pp. 1644-1659, 2007.
- [22] C. X. Liu, T. Stakenborg, S. Peeters, and L. Lagae, "Cell manipulation with magnetic particles toward microfluidic cytometry," *Journal of Applied Physics*, vol. 105, May 2009.
- [23] A. A. S. Bhagat, H. Bow, H. W. Hou, S. J. Tan, J. Han, and C. T. Lim, "Microfluidics for cell separation," *Medical & Biological Engineering & Computing*, vol. 48, pp. 999-1014, Oct 2010.
- [24] D. R. Gossett, W. M. Weaver, A. J. Mach, S. C. Hur, H. T. K. Tse, W. Lee, *et al.*, "Label-free cell separation and sorting in microfluidic systems," *Analytical and Bioanalytical Chemistry*, vol. 397, pp. 3249-3267, Aug 2010.
- [25] Y. Gao, W. J. Li, and D. Pappas, "Recent advances in microfluidic cell separations," *Analyst*, vol. 138, pp. 4714-4721, 2013.
- [26] E. L. Jackson and H. Lu, "Advances in microfluidic cell separation and manipulation," *Current Opinion in Chemical Engineering*, vol. 2, pp. 398-404, Nov 2013.
- [27] L. Yu, S. R. Ng, Y. Xu, H. Dong, Y. J. Wang, and C. M. Li, "Advances of lab-on-a-chip in isolation, detection and post-processing of circulating tumour cells," *Lab on a Chip*, vol. 13, pp. 3163-3182, 2013.
- [28] B. L. Zhu and S. K. Murthy, "Stem cell separation technologies," *Current Opinion in Chemical Engineering*, vol. 2, pp. 3-7, Feb 2013.
- [29] P. Chen, Y. Y. Huang, K. Hoshino, and X. J. Zhang, "Multiscale immunomagnetic enrichment of circulating tumor cells: from tubes to microchips," *Lab on a Chip*, vol. 14, pp. 446-458, 2014.
- [30] Y. C. Chen, P. Li, P. H. Huang, Y. L. Xie, J. D. Mai, L. Wang, *et al.*, "Rare cell isolation and analysis in microfluidics," *Lab on a Chip*, vol. 14, pp. 626-645, 2014.
- [31] L. Hajba and A. Guttman, "Circulating tumor-cell detection and capture using microfluidic devices," *Trac-Trends in Analytical Chemistry*, vol. 59, pp. 9-16, Jul-Aug 2014.
- [32] B. D. Plouffe and S. K. Murthy, "Perspective on Microfluidic Cell Separation: A Solved Problem?," *Analytical Chemistry*, vol. 86, pp. 11481-11488, Dec 2014.
- [33] Z. T. F. Yu, K. M. Aw Yong, and J. Fu, "Microfluidic blood cell sorting: Now and beyond," *Small*, vol. 10, pp. 1687-1703, 2014.
- [34] M. Hejazian, W. H. Li, and N. T. Nguyen, "Lab on a chip for continuous-flow magnetic cell separation," *Lab on a Chip*, vol. 15, pp. 959-970, 2015.
- [35] C. W. Shields, C. D. Reyes, and G. P. Lopez, "Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation," *Lab on a Chip*, vol. 15, pp. 1230-1249, 2015.
- [36] M. E. Warkiani, L. D. Wu, A. K. P. Tay, and J. Han, "Large-Volume Microfluidic Cell Sorting for Biomedical Applications," *Annual Review of Biomedical Engineering*, Vol 17, vol. 17, pp. 1-34, 2015.
- [37] G. H. Lee, S. H. Kim, K. Ahn, S. H. Lee, and J. Y. Park, "Separation and sorting of cells in microsystems using physical principles," *Journal of Micromechanics and Microengineering*, vol. 26, p. 15, Jan 2016.
- [38] V. Murlidhar, L. Rivera-Baez, and S. Nagrath, "Affinity Versus Label-Free Isolation of Circulating Tumor Cells: Who Wins?," *Small*, vol. 12, pp. 4450-4463, Sep 2016.
- [39] Y. H. Wang, Q. Chen, C. Q. Gan, B. Yan, Y. Q. Han, and J. H. Lin, "A Review on Magnetophoretic Immunoseparation," *Journal of Nanoscience and Nanotechnology*, vol. 16, pp. 2152-2163, Mar 2016.

- [40] M. Xavier, R. O. C. Oreffo, and H. Morgan, "Skeletal stem cell isolation: A review on the state-of-the-art microfluidic label-free sorting techniques," *Biotechnology Advances*, vol. 34, pp. 908-923, Sep-Oct 2016.
- [41] R. J. Yang, H. H. Hou, Y. N. Wang, and L. M. Fu, "Micro-magnetofluidics in microfluidic systems: A review," *Sensors and Actuators B-Chemical*, vol. 224, pp. 1-15, Mar 2016.
- [42] M. Antfolk and T. Laurell, "Continuous flow microfluidic separation and processing of rare cells and bioparticles found in blood - A review," *Analytica Chimica Acta*, vol. 965, pp. 9-35, May 2017.
- [43] L. Huang, S. T. Bian, Y. N. Cheng, G. Y. Shi, P. Liu, X. Y. Ye, *et al.*, "Microfluidics cell sample preparation for analysis: Advances in efficient cell enrichment and precise single cell capture," *Biomicrofluidics*, vol. 11, p. 23, Jan 2017.
- [44] B. Cetin and D. Q. Li, "Dielectrophoresis in microfluidics technology," *Electrophoresis*, vol. 32, pp. 2410-2427, Sep 2011.
- [45] T. B. Jones and M. Washizu, "Multipolar dielectrophoretic and electrorotation theory," *Journal of Electrostatics*, vol. 37, pp. 121-134, May 1996.
- [46] X. J. Wang, X. B. Wang, and P. R. C. Gascoyne, "General expressions for dielectrophoretic force and electrorotational torque derived using the Maxwell stress tensor method," *Journal of Electrostatics*, vol. 39, pp. 277-295, Aug 1997.
- [47] F. Guo, X. H. Ji, K. Liu, R. X. He, L. B. Zhao, Z. X. Guo, *et al.*, "Droplet electric separator microfluidic device for cell sorting," *Applied Physics Letters*, vol. 96, May 10 2010.
- [48] N. Pamme, "Magnetism and microfluidics," *Lab on a Chip*, vol. 6, pp. 24-38, 2006.
- [49] Z. M. Wang, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Trapping of Bacteria at Low Magnetic Fields," *Scientific Reports*, vol. 6, p. 10, Jun 2016.
- [50] N. T. Nguyen, "Micro-magnetofluidics: interactions between magnetism and fluid flow on the microscale," *Microfluidics and Nanofluidics*, vol. 12, pp. 1-16, Jan 2012.
- [51] R. E. Rosensweig, *Ferrohydrodynamics*. Cambridge ; New York: Cambridge University Press, 1985.
- [52] J. Liu, Y. F. Yap, and N. T. Nguyen, "Numerical study of the formation process of ferrofluid droplets," *Physics of Fluids*, vol. 23, p. 10, Jul 2011.
- [53] V. B. Varma, A. Ray, Z. M. Wang, Z. P. Wang, and R. V. Ramanujan, "Droplet Merging on a Lab-on-a-Chip Platform by Uniform Magnetic Fields," *Scientific Reports*, vol. 6, p. 12, Nov 2016.
- [54] C. Y. Chen, W. K. Tsai, and J. A. Miranda, "EXPERIMENTAL STUDY OF A HYBRID FERROHYDRODYNAMIC INSTABILITY IN MISCIBLE FERROFLUIDS: DROPLET SIZE EFFECTS," *Magnetohydrodynamics*, vol. 45, pp. 3-14, Jan-Mar 2009.
- [55] C. Y. Chen and Z. Y. Cheng, "An experimental study on Rosensweig instability of a ferrofluid droplet," *Physics of Fluids*, vol. 20, p. 8, May 2008.
- [56] J. Liu, S. H. Tan, Y. F. Yap, M. Y. Ng, and N. T. Nguyen, "Numerical and experimental investigations of the formation process of ferrofluid droplets," *Microfluidics and Nanofluidics*, vol. 11, pp. 177-187, Aug 2011.
- [57] G. P. Zhu, N. T. Nguyen, R. V. Ramanujan, and X. Y. Huang, "Nonlinear Deformation of a Ferrofluid Droplet in a Uniform Magnetic Field," *Langmuir*, vol. 27, pp. 14834-14841, Dec 2011.
- [58] A. Ray, V. B. Varma, P. J. Jayaneel, N. M. Sudharsan, Z. P. Wang, and R. V. Ramanujan, "On demand manipulation of ferrofluid droplets by magnetic fields," *Sensors and Actuators B-Chemical*, vol. 242, pp. 760-768, Apr 2017.

- [59] V. B. Varma, R. G. Wu, Z. P. Wang, and R. V. Ramanujan, "Magnetic Janus particles synthesized using droplet micro-magnetofluidic techniques for protein detection," *Lab on a Chip*, vol. 17, pp. 3514-3525, Oct 2017.
- [60] G. P. Zhu and N. T. Nguyen, "Rapid magnetofluidic mixing in a uniform magnetic field," *Lab on a Chip*, vol. 12, pp. 4772-4780, Nov 2012.
- [61] Z. M. Wang, V. B. Varma, H. M. Xia, Z. P. Wang, and R. V. Ramanujan, "Spreading of a ferrofluid core in three-stream micromixer channels," *Physics of Fluids*, vol. 27, p. 10, May 2015.
- [62] Z. M. Wang, V. B. Varma, Z. P. Wang, and R. V. Ramanujan, "Tuning magnetofluidic spreading in microchannels," *Journal of Micromechanics and Microengineering*, vol. 25, Dec 2015.
- [63] X. H. Wang, Z. M. Wang, V. B. Varma, Z. P. Wang, A. Ray, W. S. Lew, *et al.*, "Instability-Induced Mixing of Ferrofluids in Uniform Magnetic Fields," *Ieee Magnetism Letters*, vol. 7, p. 5, 2016.
- [64] S. Lone and I. W. Cheong, "Fabrication of polymeric Janus particles by droplet microfluidics," *Rsc Advances*, vol. 4, pp. 13322-13333, 2014.
- [65] T. Nisisako, "Recent advances in microfluidic production of Janus droplets and particles," *Current Opinion in Colloid & Interface Science*, vol. 25, pp. 1-12, Oct 2016.
- [66] J. D. Adams, U. Kim, and H. T. Soh, "Multitarget magnetic activated cell sorter," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, pp. 18165-18170, Nov 2008.
- [67] A. Ashkin, "Acceleration and trapping of particles by radiation pressure," *Physical Review Letters*, vol. 24, pp. 156-&, 1970.
- [68] X. L. Wang, S. X. Chen, M. Kong, Z. K. Wang, K. D. Costa, R. A. Li, *et al.*, "Enhanced cell sorting and manipulation with combined optical tweezer and microfluidic chip technologies," *Lab on a Chip*, vol. 11, pp. 3656-3662, 2011.
- [69] M. P. MacDonald, G. C. Spalding, and K. Dholakia, "Microfluidic sorting in an optical lattice," *Nature*, vol. 426, pp. 421-424, Nov 27 2003.
- [70] A. Lenshof, C. Magnusson, and T. Laurell, "Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems," *Lab on a Chip*, vol. 12, pp. 1210-1223, 2012.
- [71] L. Johansson, F. Nikolajeff, S. Johansson, and S. Thorslund, "On-Chip Fluorescence-Activated Cell Sorting by an Integrated Miniaturized Ultrasonic Transducer," *Analytical Chemistry*, vol. 81, pp. 5188-5196, Jul 1 2009.
- [72] X. Y. Ding, Z. L. Peng, S. C. S. Lin, M. Geri, S. X. Li, P. Li, *et al.*, "Cell separation using tilted-angle standing surface acoustic waves," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, pp. 12992-12997, Sep 9 2014.
- [73] M. Yamada and M. Seki, "Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics," *Lab on a Chip*, vol. 5, pp. 1233-1239, 2005.
- [74] M. Yamada, K. Kano, Y. Tsuda, J. Kobayashi, M. Yamato, M. Seki, *et al.*, "Microfluidic devices for size-dependent separation of liver cells," *Biomedical Microdevices*, vol. 9, pp. 637-645, Oct 2007.
- [75] M. Yamada, M. Nakashima, and M. Seki, "Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel," *Analytical Chemistry*, vol. 76, pp. 5465-5471, Sep 15 2004.
- [76] J. Takagi, M. Yamada, M. Yasuda, and M. Seki, "Continuous particle separation in a

- microchannel having asymmetrically arranged multiple branches," *Lab on a Chip*, vol. 5, pp. 778-784, 2005.
- [77] T. Morijiri, S. Sunahiro, M. Senaha, M. Yamada, and M. Seki, "Sedimentation pinched-flow fractionation for size- and density-based particle sorting in microchannels," *Microfluidics and Nanofluidics*, vol. 11, pp. 105-110, Jul 2011.
 - [78] Y. Sai, M. Yamada, M. Yasuda, and M. Seki, "Continuous separation of particles using a microfluidic device equipped with flow rate control valves," *Journal of Chromatography A*, vol. 1127, pp. 214-220, Sep 15 2006.
 - [79] L. R. Huang, E. C. Cox, R. H. Austin, and J. C. Sturm, "Continuous particle separation through deterministic lateral displacement," *Science*, vol. 304, pp. 987-990, May 14 2004.
 - [80] S. H. Au, J. Edd, A. E. Stoddard, K. H. K. Wong, F. Fachin, S. Maheswaran, *et al.*, "Microfluidic Isolation of Circulating Tumor Cell Clusters by Size and Asymmetry," *Scientific Reports*, vol. 7, May 26 2017.
 - [81] S. H. Holm, J. P. Beech, M. P. Barrett, and J. O. Tegenfeldt, "Separation of parasites from human blood using deterministic lateral displacement," *Lab on a Chip*, vol. 11, pp. 1326-1332, 2011.
 - [82] B. H. Wunsch, J. T. Smith, S. M. Gifford, C. Wang, M. Brink, R. L. Bruce, *et al.*, "Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20 nm," *Nature Nanotechnology*, vol. 11, pp. 936-940, Nov 2016.
 - [83] R. Quek, D. V. Le, and K. H. Chiam, "Separation of deformable particles in deterministic lateral displacement devices," *Physical Review E*, vol. 83, May 2 2011.
 - [84] S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, *et al.*, "Isolation of rare circulating tumour cells in cancer patients by microchip technology," *Nature*, vol. 450, pp. 1235-U10, Dec 20 2007.
 - [85] S. L. Stott, C. H. Hsu, D. I. Tsukrov, M. Yu, D. T. Miyamoto, B. A. Waltman, *et al.*, "Isolation of circulating tumor cells using a microvortex-generating herringbone-chip," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, pp. 18392-18397, Oct 26 2010.
 - [86] H. M. Ji, V. Samper, Y. Chen, C. K. Heng, T. M. Lim, and L. Yobas, "Silicon-based microfilters for whole blood cell separation," *Biomedical Microdevices*, vol. 10, pp. 251-257, Apr 2008.
 - [87] S. Zheng, H. Lin, J. Q. Liu, M. Balic, R. Datar, R. J. Cote, *et al.*, "Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells," *Journal of Chromatography A*, vol. 1162, pp. 154-161, Aug 2007.
 - [88] J. P. Brody, T. D. Osborn, F. K. Forster, and P. Yager, "A planar microfabricated fluid," *Sensors and Actuators a-Physical*, vol. 54, pp. 704-708, Jun 1996.
 - [89] H. Mohamed, J. N. Turner, and M. Caggana, "Biochip for separating fetal cells from maternal circulation," *Journal of Chromatography A*, vol. 1162, pp. 187-192, Aug 2007.
 - [90] S. Yang, A. Undar, and J. D. Zahn, "A microfluidic device for continuous, real time blood plasma separation," *Lab on a Chip*, vol. 6, pp. 871-880, 2006.
 - [91] D. Di Carlo, "Inertial microfluidics," *Lab on a Chip*, vol. 9, pp. 3038-3046, 2009.
 - [92] D. Di Carlo, D. Irimia, R. G. Tompkins, and M. Toner, "Continuous inertial focusing, ordering, and separation of particles in microchannels," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, pp. 18892-18897, Nov 27 2007.
 - [93] D. Di Carlo, J. F. Edd, D. Irimia, R. G. Tompkins, and M. Toner, "Equilibrium separation

- and filtration of particles using differential inertial focusing," *Analytical Chemistry*, vol. 80, pp. 2204-2211, Mar 15 2008.
- [94] H. W. Hou, M. E. Warkiani, B. L. Khoo, Z. R. Li, R. A. Soo, D. S. W. Tan, *et al.*, "Isolation and retrieval of circulating tumor cells using centrifugal forces," *Scientific Reports*, vol. 3, Feb 12 2013.
 - [95] H. W. Hou, C. Petchakup, H. M. Tay, Z. Y. Tam, R. Dalan, D. E. K. Chew, *et al.*, "Rapid and label-free microfluidic neutrophil purification and phenotyping in diabetes mellitus," *Scientific Reports*, vol. 6, p. 12, Jul 2016.
 - [96] K. H. Lee, S. B. Kim, K. S. Lee, and H. J. Sung, "Enhancement by optical force of separation in pinched flow fractionation," *Lab on a Chip*, vol. 11, pp. 354-357, 2011.
 - [97] J. D. Adams, P. Thevoz, H. Bruus, and H. T. Soh, "Integrated acoustic and magnetic separation in microfluidic channels," *Applied Physics Letters*, vol. 95, Dec 21 2009.
 - [98] C. W. Shields, J. L. Wang, K. A. Ohiri, E. D. Essoyan, B. B. Yellen, A. J. Armstrong, *et al.*, "Magnetic separation of acoustically focused cancer cells from blood for magnetographic templating and analysis," *Lab on a Chip*, vol. 16, pp. 3833-3844, 2016.
 - [99] E. Ozkumur, A. M. Shah, J. C. Ciciliano, B. L. Emmink, D. T. Miyamoto, E. Brachtel, *et al.*, "Inertial Focusing for Tumor Antigen-Dependent and -Independent Sorting of Rare Circulating Tumor Cells," *Science Translational Medicine*, vol. 5, p. 11, Apr 2013.
 - [100] J. N. Jiang, H. Zhao, W. L. Shu, J. Tian, Y. Q. Huang, Y. X. Song, *et al.*, "An integrated microfluidic device for rapid and high-sensitivity analysis of circulating tumor cells," *Scientific Reports*, vol. 7, p. 11, Feb 2017.
 - [101] M. Mizuno, M. Yamada, R. Mitamura, K. Ike, K. Toyama, and M. Seki, "Magnetophoresis-Integrated Hydrodynamic Filtration System for Size- and Surface Marker-Based Two-Dimensional Cell Sorting," *Analytical Chemistry*, vol. 85, pp. 7666-7673, Aug 20 2013.
 - [102] D. Kirby, J. Siegrist, G. Kijanka, L. Zavattoni, O. Sheils, J. O'Leary, *et al.*, "Centrifugomagnetophoretic particle separation," *Microfluidics and Nanofluidics*, vol. 13, pp. 899-908, Dec 2012.
 - [103] A. Shamloo, A. Selahi, and M. Madadelahi, "Designing and modeling a centrifugal microfluidic device to separate target blood cells," *Journal of Micromechanics and Microengineering*, vol. 26, p. 9, Mar 2016.
 - [104] D. Huh, J. H. Bahng, Y. B. Ling, H. H. Wei, O. D. Kripfgans, J. B. Fowlkes, *et al.*, "Gravity-driven microfluidic particle sorting device with hydrodynamic separation amplification," *Analytical Chemistry*, vol. 79, pp. 1369-1376, Feb 15 2007.
 - [105] C. H. Hsu, D. Di Carlo, C. C. Chen, D. Irimia, and M. Toner, "Microvortex for focusing, guiding and sorting of particles," *Lab on a Chip*, vol. 8, pp. 2128-2134, 2008.
 - [106] X. Li, W. Q. Chen, G. Y. Liu, W. Lu, and J. P. Fu, "Continuous-flow microfluidic blood cell sorting for unprocessed whole blood using surfacemicromachined microfiltration membranes," *Lab on a Chip*, vol. 14, pp. 2565-2575, 2014.
 - [107] E. M. Fenton, M. R. Mascarenas, G. P. Lopez, and S. S. Sibbett, "Multiplex Lateral-Flow Test Strips Fabricated by Two-Dimensional Shaping," *Acs Applied Materials & Interfaces*, vol. 1, pp. 124-129, Jan 2009.
 - [108] A. W. Martinez, S. T. Phillips, M. J. Butte, and G. M. Whitesides, "Patterned paper as a platform for inexpensive, low-volume, portable bioassays," *Angewandte Chemie-International Edition*, vol. 46, pp. 1318-1320, 2007.
 - [109] W. K. T. Coltro, D. P. de Jesus, J. A. F. da Silva, C. L. do Lago, and E. Carrilho, "Toner and paper-based fabrication techniques for microfluidic applications," *Electrophoresis*, vol. 31,

- pp. 2487-2498, Aug 2010.
- [110] D. R. Ballerini, X. Li, and W. Shen, "Patterned paper and alternative materials as substrates for low-cost microfluidic diagnostics," *Microfluidics and Nanofluidics*, vol. 13, pp. 769-787, Nov 2012.
 - [111] X. Li, D. R. Ballerini, and W. Shen, "A perspective on paper-based microfluidics: Current status and future trends," *Biomicrofluidics*, vol. 6, p. 13, Mar 2012.
 - [112] A. K. Yetisen, M. S. Akram, and C. R. Lowe, "Paper-based microfluidic point-of-care diagnostic devices," *Lab on a Chip*, vol. 13, pp. 2210-2251, 2013.
 - [113] J. Hu, S. Q. Wang, L. Wang, F. Li, B. Pingguan-Murphy, T. J. Lu, *et al.*, "Advances in paper-based point-of-care diagnostics," *Biosensors & Bioelectronics*, vol. 54, pp. 585-597, Apr 2014.
 - [114] J. Adkins, K. Boehle, and C. Henry, "Electrochemical paper-based microfluidic devices," *Electrophoresis*, vol. 36, pp. 1811-1824, Aug 2015.
 - [115] D. M. Cate, J. A. Adkins, J. Mettakoonpitak, and C. S. Henry, "Recent Developments in Paper-Based Microfluidic Devices," *Analytical Chemistry*, vol. 87, pp. 19-41, Jan 2015.
 - [116] Y. He, Y. Wu, J. Z. Fu, and W. B. Wu, "Fabrication of paper-based microfluidic analysis devices: a review," *Rsc Advances*, vol. 5, pp. 78109-78127, 2015.
 - [117] X. Jiang and Z. H. Fan, "Fabrication and Operation of Paper-Based Analytical Devices," in *Annual Review of Analytical Chemistry, Vol 9*. vol. 9, P. W. Bohn and J. E. Pemberton, Eds., ed Palo Alto: Annual Reviews, 2016, pp. 203-222.
 - [118] Y. Y. Xia, J. Si, and Z. Y. Li, "Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review," *Biosensors & Bioelectronics*, vol. 77, pp. 774-789, Mar 2016.
 - [119] C. Dixon, J. Lamanna, and A. R. Wheeler, "Printed Microfluidics," *Advanced Functional Materials*, vol. 27, p. 11, Mar 2017.
 - [120] E. W. Washburn, "The dynamics of capillary flow.," *Physical Review*, vol. 17, pp. 273-283, Mar 1921.
 - [121] A. K. Yetisen, M. S. Akram, and C. R. Lowe, "Paper-based microfluidic point-of-care diagnostic devices," *Lab Chip*, vol. 13, pp. 2210-51, Jun 21 2013.
 - [122] X. Li, D. R. Ballerini, and W. Shen, "A perspective on paper-based microfluidics: Current status and future trends," *Biomicrofluidics*, vol. 6, pp. 11301-1130113, Mar 2012.
 - [123] E. Carrilho, A. W. Martinez, and G. M. Whitesides, "Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics," *Analytical Chemistry*, vol. 81, pp. 7091-7095, Aug 15 2009.
 - [124] J. F. Nie, Y. Z. Liang, Y. Zhang, S. W. Le, D. N. Li, and S. B. Zhang, "One-step patterning of hollow microstructures in paper by laser cutting to create microfluidic analytical devices," *Analyst*, vol. 138, pp. 671-676, 2013.
 - [125] D. T. Phan, S. A. M. Shaegh, C. Yang, and N. T. Nguyen, "Sample concentration in a microfluidic paper-based analytical device using ion concentration polarization," *Sensors and Actuators B-Chemical*, vol. 222, pp. 735-740, Jan 2016.
 - [126] J. Olkkonen, K. Lehtinen, and T. Erho, "Flexographically Printed Fluidic Structures in Paper," *Analytical Chemistry*, vol. 82, pp. 10246-10250, Dec 15 2010.
 - [127] L. Yu and Z. Z. Shi, "Microfluidic paper-based analytical devices fabricated by low-cost photolithography and embossing of Parafilm (R)," *Lab on a Chip*, vol. 15, pp. 1642-1645, 2015.
 - [128] Y. Lu, W. W. Shi, L. Jiang, J. H. Qin, and B. C. Lin, "Rapid prototyping of paper-based

- microfluidics with wax for low-cost, portable bioassay," *Electrophoresis*, vol. 30, pp. 1497-1500, May 2009.
- [129] K. Abe, K. Suzuki, and D. Citterio, "Inkjet-printed microfluidic multianalyte chemical sensing paper," *Analytical Chemistry*, vol. 80, pp. 6928-6934, Sep 15 2008.
 - [130] X. Li, J. F. Tian, T. Nguyen, and W. Shen, "Paper-Based Microfluidic Devices by Plasma Treatment," *Analytical Chemistry*, vol. 80, pp. 9131-9134, Dec 1 2008.
 - [131] P. Spicar-Mihalic, B. Toley, J. Houghtaling, T. Liang, P. Yager, and E. Fu, "CO₂ laser cutting and ablative etching for the fabrication of paper-based devices," *Journal of Micromechanics and Microengineering*, vol. 23, Jun 2013.
 - [132] M. J. Hancock, J. K. He, J. F. Mano, and A. Khademhosseini, "Surface-Tension-Driven Gradient Generation in a Fluid Stripe for Bench-Top and Microwell Applications," *Small*, vol. 7, pp. 892-901, Apr 4 2011.
 - [133] C. C. Liu, S. C. Liao, J. Z. Song, M. G. Mauk, X. W. Li, G. X. Wu, *et al.*, "A high-efficiency superhydrophobic plasma separator," *Lab on a Chip*, vol. 16, pp. 553-560, 2016.
 - [134] T. Nurak, N. Praphairaksit, and O. Chailapakul, "Fabrication of paper-based devices by lacquer spraying method for the determination of nickel (II) ion in waste water," *Talanta*, vol. 114, pp. 291-296, Sep 30 2013.
 - [135] B. Jung, R. Bharadwaj, and J. G. Santiago, "Thousandfold signal increase using field-amplified sample stacking for on-chip electrophoresis," *Electrophoresis*, vol. 24, pp. 3476-3483, Oct 2003.
 - [136] J. Lichtenberg, E. Verpoorte, and N. F. de Rooij, "Sample preconcentration by field amplification stacking for microchip-based capillary electrophoresis," *Electrophoresis*, vol. 22, pp. 258-271, Jan 2001.
 - [137] B. Jung, R. Bharadwaj, and J. G. Santiago, "On-chip millionfold sample stacking using transient isotachophoresis," *Analytical Chemistry*, vol. 78, pp. 2319-2327, Apr 1 2006.
 - [138] S. S. Bahga, R. D. Chambers, and J. G. Santiago, "Coupled Isotachophoretic Preconcentration and Electrophoretic Separation Using Bidirectional Isotachophoresis," *Analytical Chemistry*, vol. 83, pp. 6154-6162, Aug 15 2011.
 - [139] T. Matsui, J. Franzke, A. Manz, and D. Janasek, "Temperature gradient focusing in a PDMS/glass hybrid microfluidic chip," *Electrophoresis*, vol. 28, pp. 4606-4611, Dec 2007.
 - [140] D. Ross and L. E. Locascio, "Microfluidic temperature gradient focusing," *Analytical Chemistry*, vol. 74, pp. 2556-2564, Jun 1 2002.
 - [141] H. G. Kunkel and A. Tiselius, "Electrophoresis of proteins on filter paper," *Journal of General Physiology*, vol. 35, pp. 89-118, 1951.
 - [142] L. Ge, S. W. Wang, S. G. Ge, J. H. Yu, M. Yan, N. Q. Li, *et al.*, "Electrophoretic separation in a microfluidic paper-based analytical device with an on-column wireless electrogenerated chemiluminescence detector," *Chemical Communications*, vol. 50, pp. 5699-5702, 2014.
 - [143] S. S. Chen, C. W. Hu, I. F. Yu, Y. C. Liao, and J. T. Yang, "Origami paper-based fluidic batteries for portable electrophoretic devices," *Lab on a Chip*, vol. 14, pp. 2124-2130, 2014.
 - [144] L. Luo, X. Li, and R. M. Crooks, "Low-Voltage Origami-Paper-Based Electrophoretic Device for Rapid Protein Separation," *Analytical Chemistry*, vol. 86, pp. 12390-12397, Dec 16 2014.
 - [145] B. Y. Moghadam, K. T. Connelly, and J. D. Posner, "Isotachophoretic Preconcentration on Paper-Based Microfluidic Devices," *Analytical Chemistry*, vol. 86, pp. 5829-5837, Jun 2014.

- [146] B. Y. Moghadam, K. T. Connelly, and J. D. Posner, "Two Orders of Magnitude Improvement in Detection Limit of Lateral Flow Assays Using Isotachophoresis," *Analytical Chemistry*, vol. 87, pp. 1009-1017, Jan 2015.
- [147] T. Rosenfeld and M. Bercovici, "1000-fold sample focusing on paper-based microfluidic devices," *Lab on a Chip*, vol. 14, pp. 4465-4474, 2014.
- [148] M. M. Gong, P. Zhang, B. D. MacDonald, and D. Sinton, "Nanoporous Membranes Enable Concentration and Transport in Fully Wet Paper-Based Assays," *Analytical Chemistry*, vol. 86, pp. 8090-8097, Aug 19 2014.
- [149] M. M. Gong, R. Nosrati, M. C. S. Gabriel, A. Zini, and D. Sinton, "Direct DNA Analysis with Paper-Based Ion Concentration Polarization," *Journal of the American Chemical Society*, vol. 137, pp. 13913-13919, Nov 4 2015.
- [150] S. I. Han, K. S. Hwang, R. Kwak, and J. H. Lee, "Microfluidic paper-based biomolecule preconcentrator based on ion concentration polarization," *Lab on a Chip*, vol. 16, pp. 2219-2227, 2016.
- [151] S. H. Yeh, K. H. Chou, and R. J. Yang, "Sample pre-concentration with high enrichment factors at a fixed location in paper-based microfluidic devices," *Lab on a Chip*, vol. 16, pp. 925-931, 2016.
- [152] K. M. Schilling, A. L. Lepore, J. A. Kurian, and A. W. Martinez, "Fully Enclosed Microfluidic Paper-Based Analytical Devices," *Analytical Chemistry*, vol. 84, pp. 1579-1585, Feb 7 2012.
- [153] X. Jiang and Z. H. Fan, "Fabrication and Operation of Paper-Based Analytical Devices," *Annual Review of Analytical Chemistry, Vol 9*, vol. 9, pp. 203-222, 2016.
- [154] C. K. Camplisson, K. M. Schilling, W. L. Pedrotti, H. A. Stone, and A. W. Martinez, "Two-ply channels for faster wicking in paper-based microfluidic devices," *Lab on a Chip*, vol. 15, pp. 4461-4466, 2015.
- [155] C. L. Cassano and Z. H. Fan, "Laminated paper-based analytical devices (LPAD): fabrication, characterization, and assays," *Microfluidics and Nanofluidics*, vol. 15, pp. 173-181, Aug 2013.
- [156] C. Renault, J. Koehne, A. J. Ricco, and R. M. Crooks, "Three-Dimensional Wax Patterning of Paper Fluidic Devices," *Langmuir*, vol. 30, pp. 7030-7036, Jun 2014.
- [157] E. T. S. G. da Silva, M. Santhiago, F. R. de Souza, W. K. T. Coltro, and L. T. Kubota, "Triboelectric effect as a new strategy for sealing and controlling the flow in paper-based devices," *Lab on a Chip*, vol. 15, pp. 1651-1655, 2015.
- [158] Y. C. Wang, A. L. Stevens, and J. Y. Han, "Million-fold preconcentration of proteins and peptides by nanofluidic filter," *Analytical Chemistry*, vol. 77, pp. 4293-4299, Jul 15 2005.
- [159] J. H. Lee, Y. A. Song, and J. Y. Han, "Multiplexed proteomic sample preconcentration device using surface-patterned ion-selective membrane," *Lab on a Chip*, vol. 8, pp. 596-601, 2008.
- [160] C. H. Chen, A. Sarkar, Y. A. Song, M. A. Miller, S. J. Kim, L. G. Griffith, *et al.*, "Enhancing Protease Activity Assay in Droplet-Based Microfluidics Using a Biomolecule Concentrator," *Journal of the American Chemical Society*, vol. 133, pp. 10368-10371, Jul 13 2011.
- [161] D. T. Phan, Y. Chun, and N. T. Nguyen, "A continuous-flow droplet-based concentrator using ion concentration polarization," *Rsc Advances*, vol. 5, pp. 44336-44341, 2015.
- [162] D. T. Phan, C. Yang, and N. T. Nguyen, "Fabrication of nanoporous junctions using off-the-shelf Nafion membrane," *Journal of Micromechanics and Microengineering*, vol. 25,

Nov 2015.

- [163] S. J. Kim, Y. A. Song, and J. Han, "Nanofluidic concentration devices for biomolecules utilizing ion concentration polarization: theory, fabrication, and applications," *Chemical Society Reviews*, vol. 39, pp. 912-922, 2010.
- [164] M. Bacac and I. Stamenkovic, "Metastatic cancer cell," *Annu. Rev. pathmechdis. Mech. Dis.*, vol. 3, pp. 221-247, 2008.
- [165] Y. Dong, A. M. Skelley, K. D. Merdek, K. M. Sprott, C. S. Jiang, W. E. Pierceall, *et al.*, "Microfluidics and Circulating Tumor Cells," *Journal of Molecular Diagnostics*, vol. 15, pp. 149-157, Mar 2013.
- [166] A. C. Chiang and J. Massagué, "Molecular basis of metastasis," *New England Journal of Medicine*, vol. 359, pp. 2814-2823, 2008.
- [167] C. Jin, S. M. McFaul, S. P. Duffy, X. Y. Deng, P. Tavassoli, P. C. Black, *et al.*, "Technologies for label-free separation of circulating tumor cells: from historical foundations to recent developments," *Lab on a Chip*, vol. 14, pp. 32-44, 2014.
- [168] H. J. Yoon, T. H. Kim, Z. Zhang, E. Azizi, T. M. Pham, C. Paoletti, *et al.*, "Sensitive capture of circulating tumour cells by functionalized graphene oxide nanosheets (vol 8, pg 735, 2013)," *Nature Nanotechnology*, vol. 8, Nov 2013.
- [169] N.-T. Nguyen, K. M. Ng, and X. Huang, "Manipulation of ferrofluid droplets using planar coils," *Applied Physics Letters*, vol. 89, p. 052509, 2006.
- [170] D.-H. Kim, E. A. Rozhkova, I. V. Ulasov, S. D. Bader, T. Rajh, M. S. Lesniak, *et al.*, "Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction," *Nat Mater*, vol. 9, pp. 165-171, 02//print 2010.
- [171] W.-C. Lee, K.-Y. Lien, G.-B. Lee, and H.-Y. Lei, "An integrated microfluidic system using magnetic beads for virus detection," *Diagnostic Microbiology and Infectious Disease*, vol. 60, pp. 51-58, 1// 2008.
- [172] C. T. Lim and Y. Zhang, "Bead-based microfluidic immunoassays: The next generation," *Biosensors and Bioelectronics*, vol. 22, pp. 1197-1204, 2/15/ 2007.
- [173] J.-W. Choi, K. W. Oh, J. H. Thomas, W. R. Heineman, H. B. Halsall, J. H. Nevin, *et al.*, "An integrated microfluidic biochemical detection system for protein analysis with magnetic bead-based sampling capabilities," *Lab on a Chip*, vol. 2, pp. 27-30, 2002.
- [174] D. W. Inglis, R. Riehn, R. H. Austin, and J. C. Sturm, "Continuous microfluidic immunomagnetic cell separation," *Applied Physics Letters*, vol. 85, pp. 5093-5095, 2004.
- [175] M. Hejajian, W. Li, and N.-T. Nguyen, "Lab on a chip for continuous-flow magnetic cell separation," *Lab on a Chip*, vol. 15, pp. 959-970, 2015.
- [176] M. A. Gijs, F. Lacharme, and U. Lehmann, "Microfluidic applications of magnetic particles for biological analysis and catalysis," *Chemical reviews*, vol. 110, pp. 1518-1563, 2009.
- [177] J.-W. Choi, T. M. Liakopoulos, and C. H. Ahn, "An on-chip magnetic bead separator using spiral electromagnets with semi-encapsulated permalloy," *Biosensors and Bioelectronics*, vol. 16, pp. 409-416, 8// 2001.
- [178] T. Deng, M. Prentiss, and G. M. Whitesides, "Fabrication of magnetic microfiltration systems using soft lithography," *Applied physics letters*, vol. 80, pp. 461-463, 2002.
- [179] E. Dolgin, "New technologies aim to take cancer out of circulation," *Nature medicine*, vol. 17, pp. 266-266, 2011.
- [180] S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, *et al.*, "Isolation of rare circulating tumour cells in cancer patients by microchip technology," *Nature*, vol. 450, pp. 1235-1239, 2007.

- [181] N. Pamme and C. Wilhelm, "Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis," *Lab on a Chip*, vol. 6, pp. 974-980, 2006.
- [182] S. J. Tan, L. Yobas, G. Y. H. Lee, C. N. Ong, and C. T. Lim, "Microdevice for the isolation and enumeration of cancer cells from blood," *Biomedical microdevices*, vol. 11, pp. 883-892, 2009.
- [183] G. M. Whitesides, R. J. Kazlauskas, and L. Josephson, "Magnetic separations in biotechnology," *Trends in Biotechnology*, vol. 1, pp. 144-148, 11// 1983.
- [184] S. Roath, A. R. Smith, and J. H. P. Watson, "High-gradient magnetic separation in blood and bone marrow processing," *Journal of Magnetism and Magnetic Materials*, vol. 85, pp. 285-289, 1990/04/01 1990.
- [185] J. R. Basore and L. A. Baker, "Applications of microelectromagnetic traps," *Analytical and Bioanalytical Chemistry*, vol. 403, pp. 2077-2088, Jun 2012.
- [186] C. H. Ahn, M. G. Allen, W. Trimmer, Y. N. Jun, and S. Erramilli, "A fully integrated micromachined magnetic particle separator," *Journal of Microelectromechanical Systems*, vol. 5, pp. 151-158, Sep 1996.
- [187] S. H. Song, H. L. Lee, Y. H. Min, and H. I. Jung, "Electromagnetic microfluidic cell labeling device using on-chip microelectromagnet and multi-layered channels," *Sensors and Actuators B-Chemical*, vol. 141, pp. 210-216, Aug 18 2009.
- [188] N.-T. Nguyen, "Micro-magnetofluidics: interactions between magnetism and fluid flow on the microscale," *Microfluidics and Nanofluidics*, vol. 12, pp. 1-16, 2012.
- [189] Y. Haik, V. Pai, and C.-J. Chen, "Development of magnetic device for cell separation," *Journal of Magnetism and Magnetic Materials*, vol. 194, pp. 254-261, 4// 1999.
- [190] G. P. Hatch and R. E. Stelter, "Magnetic design considerations for devices and particles used for biological high-gradient magnetic separation (HGMS) systems," *Journal of Magnetism and Magnetic Materials*, vol. 225, pp. 262-276, // 2001.
- [191] M. Zborowski, L. Sun, L. R. Moore, P. Stephen Williams, and J. J. Chalmers, "Continuous cell separation using novel magnetic quadrupole flow sorter," *Journal of Magnetism and Magnetic Materials*, vol. 194, pp. 224-230, 4// 1999.
- [192] D. Melville, F. Paul, and S. Roath, "Fractionation of blood components using high gradient magnetic separation," *IEEE Transactions on Magnetics*, vol. 18, pp. 1680-1685, 1982.
- [193] D. Melville, F. Paul, and S. Roath, "High gradient magnetic separation of red cells from whole blood," *IEEE Transactions on Magnetics*, vol. 11, pp. 1701-1704, 1975.
- [194] S. Miltenyi, W. Müller, W. Weichel, and A. Radbruch, "High gradient magnetic cell separation with MACS," *Cytometry*, vol. 11, pp. 231-238, 1990.
- [195] J. Oberteuffer, "High gradient magnetic separation," *IEEE Transactions on Magnetics*, vol. 9, pp. 303-306, 1973.
- [196] A. J. Richards, O. S. Roath, J. S. Smith, and J. H. P. Watson, "The mechanisms of high gradient magnetic separation of human blood and bone marrow," *IEEE Transactions on Magnetics*, vol. 32, pp. 459-470, 1996.
- [197] B. B. Yellen, R. M. Erb, H. S. Son, J. R. Hewlin, H. Shang, and G. U. Lee, "Traveling wave magnetophoresis for high resolution chip based separations," *Lab on a Chip*, vol. 7, pp. 1681-1688, 2007.
- [198] J. H. Kang and J. K. Park, "Magnetophoretic Continuous Purification of Single-Walled Carbon Nanotubes from Catalytic Impurities in a Microfluidic Device," *Small*, vol. 3, pp. 1784-1791, 2007.
- [199] K. Smistrup, B. G. Kjeldsen, J. L. Reimers, M. Dufva, J. Petersen, and M. F. Hansen, "On-

- chip magnetic bead microarray using hydrodynamic focusing in a passive magnetic separator," *Lab on a Chip*, vol. 5, pp. 1315-1319, 2005.
- [200] S.-H. Song, H.-L. Lee, Y. H. Min, and H.-I. Jung, "Electromagnetic microfluidic cell labeling device using on-chip microelectromagnet and multi-layered channels," *Sensors and Actuators B: Chemical*, vol. 141, pp. 210-216, 8/18/ 2009.
 - [201] C. Derec, C. Wilhelm, J. Servais, and J.-C. Bacri, "Local control of magnetic objects in microfluidic channels," *Microfluidics and Nanofluidics*, vol. 8, pp. 123-130, 2009.
 - [202] J. Joung, J. Shen, and P. Grodzinski, "Micropumps based on alternating high-gradient magnetic fields," *IEEE transactions on magnetics*, vol. 36, pp. 2012-2014, 2000.
 - [203] T. F. Kong, H. S. Sugiarto, H. F. Liew, X. Wang, W. S. Lew, N.-T. Nguyen, *et al.*, "An efficient microfluidic sorter: implementation of double meandering micro striplines for magnetic particles switching," *Microfluidics and nanofluidics*, vol. 10, pp. 1069-1078, 2011.
 - [204] Z. Jiang, J. Llandro, T. Mitrelias, and J. Bland, "An integrated microfluidic cell for detection, manipulation, and sorting of single micron-sized magnetic beads," *Journal of applied physics*, vol. 99, p. 08S105, 2006.
 - [205] N. Pamme and A. Manz, "On-chip free-flow magnetophoresis: Continuous flow separation of magnetic particles and agglomerates," *Analytical Chemistry*, vol. 76, pp. 7250-7256, Dec 2004.
 - [206] C.-C. Chang, Z.-X. Huang, and R.-J. Yang, "Three-dimensional hydrodynamic focusing in two-layer polydimethylsiloxane (PDMS) microchannels," *Journal of Micromechanics and Microengineering*, vol. 17, p. 1479, 2007.
 - [207] A. Jahn, W. N. Vreeland, M. Gaitan, and L. E. Locascio, "Controlled vesicle self-assembly in microfluidic channels with hydrodynamic focusing," *Journal of the American Chemical Society*, vol. 126, pp. 2674-2675, 2004.
 - [208] C. Simonnet and A. Groisman, "Two-dimensional hydrodynamic focusing in a simple microfluidic device," *Applied Physics Letters*, vol. 87, p. 114104, 2005.
 - [209] O. Osman, S. Toru, F. Dumas-Bouchiat, N. Dempsey, N. Haddour, L.-F. Zanini, *et al.*, "Microfluidic immunomagnetic cell separation using integrated permanent micromagnets," *Biomicrofluidics*, vol. 7, p. 054115, 2013.
 - [210] S. H. Song, B. S. Kwak, J. S. Park, W. Kim, and H. I. Jung, "Novel application of Joule heating to maintain biocompatible temperatures in a fully integrated electromagnetic cell sorting system," *Sensors and Actuators a-Physical*, vol. 151, pp. 64-70, Apr 2009.
 - [211] T. F. Kong, H. Shin, H. S. Sugiarto, H. F. Liew, X. H. Wang, W. S. Lew, *et al.*, "An efficient microfluidic sorter: implementation of double meandering micro striplines for magnetic particles switching," *Microfluidics and Nanofluidics*, vol. 10, pp. 1069-1078, May 2011.
 - [212] Y. C. Chung, C. M. Wu, and S. H. Lin, "Particles Sorting in Micro Channel Using Designed Micro Electromagnets of Magnetic Field Gradient," *Journal of Magnetism and Magnetic Materials*, vol. 407, pp. 209-217, Jun 2016.
 - [213] J. Darabi and C. Guo, "On-chip magnetophoretic isolation of CD4+T cells from blood," *Biomicrofluidics*, vol. 7, p. 14, Sep 2013.
 - [214] J. Zhang, S. Yan, D. Yuan, G. Alici, N. T. Nguyen, M. E. Warkiani, *et al.*, "Fundamentals and applications of inertial microfluidics: a review," *Lab on a Chip*, vol. 16, pp. 10-34, 2016.
 - [215] G. Segre and A. Silberberg, "Radial Particle Displacements in Poiseuille Flow of Suspensions," *Nature*, vol. 189, pp. 209-&, 1961.

- [216] X. H. Cheng, D. Irimia, M. Dixon, K. Sekine, U. Demirci, L. Zamir, *et al.*, "A microfluidic device for practical label-free CD4+T cell counting of HIV-infected subjects," *Lab on a Chip*, vol. 7, pp. 170-178, 2007.
- [217] J. Autebert, B. Coudert, F. C. Bidard, J. Y. Pierga, S. Descroix, L. Malaquin, *et al.*, "Microfluidic: An innovative tool for efficient cell sorting," *Methods*, vol. 57, pp. 297-307, Jul 2012.
- [218] S. Yan, J. Zhang, D. Yuan, and W. H. Li, "Hybrid microfluidics combined with active and passive approaches for continuous cell separation," *Electrophoresis*, vol. 38, pp. 238-249, Jan 2017.
- [219] S. A. Khashan and E. P. Furlani, "Effects of particle-fluid coupling on particle transport and capture in a magnetophoretic microsystem," *Microfluidics and Nanofluidics*, vol. 12, pp. 565-580, Jan 2012.
- [220] R. Zhou, Q. B. Yang, F. Bai, J. A. Werner, H. L. Shi, Y. F. Ma, *et al.*, "Fabrication and integration of microscale permanent magnets for particle separation in microfluidics," *Microfluidics and Nanofluidics*, vol. 20, p. 12, Jul 2016.
- [221] H. W. Hou, R. P. Bhattacharyya, D. T. Hung, and J. Han, "Direct detection and drug-resistance profiling of bacteremias using inertial microfluidics," *Lab on a Chip*, vol. 15, pp. 2297-2307, 2015.
- [222] L. D. Wu, G. F. Guan, H. W. Hou, A. A. S. Bhagat, and J. Han, "Separation of Leukocytes from Blood Using Spiral Channel with Trapezoid Cross-Section," *Analytical Chemistry*, vol. 84, pp. 9324-9331, Nov 6 2012.
- [223] W. R. Dean, "The stream-line motion of fluid in a curved pipe. (Second paper.)," *Philosophical Magazine*, vol. 5, pp. 673-695, Apr 1928.
- [224] S. S. Kuntaegowdanahalli, A. A. S. Bhagat, G. Kumar, and I. Papautsky, "Inertial microfluidics for continuous particle separation in spiral microchannels," *Lab on a Chip*, vol. 9, pp. 2973-2980, 2009.
- [225] A. A. S. Bhagat, S. S. Kuntaegowdanahalli, and I. Papautsky, "Continuous particle separation in spiral microchannels using dean flows and differential migration," *Lab on a Chip*, vol. 8, pp. 1906-1914, 2008.
- [226] E. Ozkumur, A. M. Shah, J. C. Ciciliano, B. L. Emmink, D. T. Miyamoto, E. Brachtel, *et al.*, "Inertial Focusing for Tumor Antigen-Dependent and -Independent Sorting of Rare Circulating Tumor Cells," *Science Translational Medicine*, vol. 5, Apr 3 2013.
- [227] N. M. Karabacak, P. S. Spuhler, F. Fachin, E. J. Lim, V. Pai, E. Ozkumur, *et al.*, "Microfluidic, marker-free isolation of circulating tumor cells from blood samples," *Nature Protocols*, vol. 9, pp. 694-710, Mar 2014.
- [228] V. Kumar and P. Rezai, "Multiplex Inertio-Magnetic Fractionation (MIMF) of magnetic and non-magnetic microparticles in a microfluidic device," *Microfluidics and Nanofluidics*, vol. 21, p. 14, May 2017.
- [229] E. P. Furlani, "Analysis of particle transport in a magnetophoretic microsystem," *Journal of Applied Physics*, vol. 99, p. 11, Jan 2006.
- [230] M. Yamada, W. Seko, T. Yanai, K. Ninomiya, and M. Seki, "Slanted, asymmetric microfluidic lattices as size-selective sieves for continuous particle/cell sorting," *Lab on a Chip*, vol. 17, pp. 304-314, Jan 2017.
- [231] H. Ramachandraiah, H. A. Svahn, and A. Russom, "Inertial microfluidics combined with selective cell lysis for high throughput separation of nucleated cells from whole blood," *Rsc Advances*, vol. 7, pp. 29505-29514, 2017.

- [232] J. Darabi and C. Guo, "Continuous isolation of monocytes using a magnetophoretic-based microfluidic Chip," *Biomedical Microdevices*, vol. 18, p. 10, Oct 2016.
- [233] E. M. Dunfield, Y. Wu, M. T. Koesdjojo, T. P. Remcho, and V. T. Remcho, "Simple and rapid fabrication of paper microfluidic devices utilizing Parafilm®," *Lab Chip - Chips & Tips*, 2012.
- [234] X. E. Fang, S. S. Wei, and J. L. Kong, "Paper-based microfluidics with high resolution, cut on a glass fiber membrane for bioassays," *Lab on a Chip*, vol. 14, pp. 911-915, 2014.
- [235] R. A. G. de Oliveira, F. Camargo, N. C. Pesquero, and R. C. Faria, "A simple method to produce 2D and 3D microfluidic paper-based analytical devices for clinical analysis," *Analytica Chimica Acta*, vol. 957, pp. 40-46, Mar 8 2017.
- [236] R. J. Yang, H. H. Pu, and H. L. Wang, "Ion concentration polarization on paper-based microfluidic devices and its application to preconcentrate dilute sample solutions," *Biomicrofluidics*, vol. 9, Jan 2015.
- [237] K. Tenda, R. Ota, K. Yamada, T. G. Henares, K. Suzuki, and D. Citterio, "High-Resolution Microfluidic Paper-Based Analytical Devices for Sub-Microliter Sample Analysis," *Micromachines*, vol. 7, May 2016.
- [238] Y. Xu and T. Enomae, "Paper substrate modification for rapid capillary flow in microfluidic paper-based analytical devices," *Rsc Advances*, vol. 4, pp. 12867-12872, 2014.
- [239] S. K. Kim, H. J. Koo, A. Lee, and P. V. Braun, "Selective Wetting-Induced Micro-Electrode Patterning for Flexible Micro-Supercapacitors," *Advanced Materials*, vol. 26, pp. 5108-5112, Aug 13 2014.
- [240] S. Y. Xing, J. Jiang, and T. R. Pan, "Interfacial microfluidic transport on micropatterned superhydrophobic textile," *Lab on a Chip*, vol. 13, pp. 1937-1947, 2013.
- [241] J. T. Li, F. Ye, S. Vaziri, M. Muhammed, M. C. Lemme, and M. Ostling, "Efficient Inkjet Printing of Graphene," *Advanced Materials*, vol. 25, pp. 3985-3992, Aug 7 2013.
- [242] J. T. Li, V. Mishukova, and M. Ostling, "All-solid-state micro-supercapacitors based on inkjet printed graphene electrodes," *Applied Physics Letters*, vol. 109, Sep 19 2016.
- [243] M. Notarianni, J. Z. Liu, F. Mirri, M. Pasquali, and N. Motta, "Graphene-based supercapacitor with carbon nanotube film as highly efficient current collector," *Nanotechnology*, vol. 25, Oct 31 2014.
- [244] X. Wang, A. Sumboja, W. L. Foo, C. Y. Yan, K. Tsukagoshi, and P. S. Lee, "Rational design of a high performance all solid state flexible micro-supercapacitor on paper," *Rsc Advances*, vol. 3, pp. 15827-15833, 2013.
- [245] B. Yao, L. Y. Yuan, X. Xiao, J. Zhang, Y. Y. Qi, J. Zhou, *et al.*, "Paper-based solid-state supercapacitors with pencil-drawing graphite/polyaniline networks hybrid electrodes," *Nano Energy*, vol. 2, pp. 1071-1078, Nov 2013.
- [246] C. W. Ma, P. C. Huang, and Y. J. Yang, "A Paper-Like Micro-Supercapacitor with Patterned Buckypaper Electrodes Using a Novel Vacuum Filtration Technique," *2015 28th IEEE International Conference on Micro Electro Mechanical Systems (Mems 2015)*, pp. 1067-1070, 2015.
- [247] J. M. Martel, K. C. Smith, M. Dlamini, K. Pletcher, J. Yang, M. Karabacak, *et al.*, "Continuous Flow Microfluidic Bioparticle Concentrator," *Scientific Reports*, vol. 5, p. 12, Jun 2015.

Appendix A: COMSOL Simulation of Magnetic Flux Density Distribution

In this appendix, parts of the numerical analysis results presented in Chapter 3 are detailed. The magnetic fields generated by single and double striplines are compared.

When a magnetic spherical particle is released in the magnetofluidic channel, it is subjected to two dominant forces, i.e. magnetic force and hydrodynamic drag force are the dominant forces. The magnetic force exerted on the particle can be expressed as

$$F_m = \frac{V\Delta\chi}{\mu_0} (\vec{B} \cdot \nabla) \vec{B}, \quad (\text{A-1})$$

where μ_0 is the magnetic constant, V is the volume of the particle, $\Delta\chi$ indicates the difference in volume susceptibility between magnetic particle and the surrounding liquid medium.

The hydrodynamic drag force experienced by the particle in uniform flow is given by the Stokes' Law:

$$F_d = 6\pi\eta R \cdot v, \quad (\text{A-2})$$

where η is the solution viscosity, R is the radius of a sphere, v is the velocity of the sphere moving through the medium.

To estimate the hydrodynamic drag force, the following parameters are used: $\eta=3.5\times10^{-3}\text{Pa}\cdot\text{s}$, $R=1\times10^{-6}\text{m}$, $v=1\times10^{-4}\text{m/s}$. The calculated hydrodynamic drag force was $F_d=6.58\times10^{-12}\text{N}$. Hence, to compete with the hydrodynamic drag force, the minimum magnetic force required to deflect the particle should be in the order of pico-newton.

Single stripline

In the simulation, the magnetic fields generated by the single stripline and the double stripline are investigated under the following conditions: 1. the current-carrying wire is the only source of the magnetic field; 2. the current flow in the wire is steady; 3. the magnetic field is not permeating the air box surface; 4. the boundary condition of the stripline is continuity; 5. the boundary conditions of the encapsulating box are assumed to be magnetic insulation and electric insulation.

Figure A.1 shows the single stripline electromagnets used in COMSOL simulation. The material of the stripline is copper (conductivity= 5.998×10^7 S/m). The current density applied in the stripline is 1.5×10^7 A/m². The outermost cubic box showed in this figure is used as a boundary to limit the computation area. The arrow plots in Figure A.2 demonstrate the directions of local magnetic fields. The multislice view of magnetic flux density distribution in Figure A.3 clearly shows that the maximum magnetic field appears at the corners of stripline due to the superposition of magnetic fields generated by the connected current-carrying wires. This implies that the particle deflection in microfluidic channel is mainly determined by the stripline corners. Therefore, positioning the stripline edges close to the center of microfluidic channel will enhance the deflection effect. Figure A.4, A.5 and A.6 demonstrates the X, Y and Z component of magnetic flux density distribution, respectively. As shown in Figure A.4, due to the current flow direction change, the directions of magnetic fields generated by current-carrying wires alternates between neighboring wires. The intensity of magnetic flux density along x axis vary from -4.75×10^{-3} T to 4.69×10^{-3} T. As the magnetic force exerted on a magnetic particle is mainly determined by magnetic field strength and magnetic field gradient, the variation of magnetic field direction does not affect the magnetic particle's trajectory in the microfluidic channel. Figure A.5 indicates that the Y component of the magnetic flux density appear at the short edges of the stripline and have similar intensity with the

X component. In fact, the Z component of magnetic flux density showed in Figure A.6 can not contribute to the bias deflection of the magnetic particle in microfluidic channel. Therefore, the particle deflection is mainly determined by the X and Y components of the magnetic flux density. From Figure A.4 and A.5, we can also conclude that the increase of the number of stripline corners will generate more magnetic flux density peaks, enabling enhanced particle deflection effect.

In summary, finite element method (FEM) analysis of the current-carrying single stripline provides information for the estimates of the magnetic field strength in the proximity of microfluidic channel. In conjunction with experimental observation of a magnetic particle's position, these simulations provide an overall model to predict the flow path of the particle in the microfluidic channel.

Figure A.1. Single stripline used in the COMSOL simulation.

Figure A.2. Simulated distribution of magnetic fields in the single stripline. The magnetic field generation is governed by the Biot-Savart Law.

Figure A.3. Simulated distribution of magnetic flux density in single stripline. The unit of the flux density is tesla (T). The current density in the stripline is 1.5×10^7 A/m². The intensity of the magnetic flux density is presented with different colors. The maximum magnetic flux intensity appear at the corners of the stripline due to the superposition of neighboring magnetic fields. The magnetic field generated at the corners are the major contributors to the particle deflection.

Figure A.4. Simulated distribution of X component of magnetic flux density in single stripline. The blue color represents the maximum magnetic fields along the negative x axis. While the orange color represents the maximum magnetic fields along the positive x axis. The variation of magnetic field direction does not affect the particle deflection.

Figure A.5. Simulated distribution of Y component of magnetic flux density in single stripline. The blue color indicates that the maximum magnetic fields appear at the short edges of the stripline. The values of magnetic flux density vary between $-4.67 \times 10^{-3} \text{ T}$ and $4.68 \times 10^{-3} \text{ T}$. The X component and Y component of magnetic flux density have similar intensity.

Figure A.6. Simulated distribution of Z component of magnetic flux density in single stripline. Different from the X and Y components, the maximum values of Z component of the magnetic flux density mainly appear in the gaps of stripline. As the direction of the Z component is perpendicular to the microfluidic chip substrate, this component dose not contribute to the bias deflection of the magnetic particles in the microfluidic channel.

Figure A.7. Double stripline used in the COMSOL simulation.

Figure A.8. Simulated arrow plots show the magnetic field distributions of the double stripline. The arrows indicate the direction of the magnetic field of the stripline.

Figure A.9. Simulated distribution of magnetic flux density in the double stripline. The unit of the flux density is tesla (T). The intensity of the magnetic flux density is presented with different colors. Comparing with single stripline, more magnetic flux density peaks appear in the double stripline, resulting in enhanced magnetic field strength and field gradient. Therefore, the double stripline has great potential to increase particle deflection efficiency.

Figure A.10. Simulated distribution of X component of magnetic flux density in double stripline. The blue color and orange color represents the direction of magnetic field in negative and positive x axis, respectively. The intensity of the X component of magnetic flux density range from $-5.6 \times 10^{-3} \text{ T}$ to $5.63 \times 10^{-3} \text{ T}$, indicating enhanced magnetic flux density comparing with those generated by single stripline.

Figure A.11. Simulated distribution of Y component of magnetic flux density in the double stripline. The values of magnetic flux density vary between $-5 \times 10^{-3} \text{ T}$ to $4.8 \times 10^{-3} \text{ T}$. Similar as in the single stripline, the maximum magnetic fields appear at the short edges of the double stripline, which are indicated by blue color. Comparing with single stripline, the overall areas of blue color are much larger, indicating more magnetic field peaks in the double stripline.

Figure A.12. Simulated distribution of Z component of magnetic flux density in the double stripline. The maximum values of Z component of the magnetic flux density mainly appear in the gaps of the stripline. Same as the analysis above, the Z component of the magnetic flux density can be ignored as they do not contribute to the particle deflection.

Figure A.13. The measurement positions in the vicinity of the double stripline. The red line is parallel with the double stripline along the X direction. The yellow line indicates the distance away from the double stripline. The distance of the red line away from the stripline is 150 μm . The yellow line indicates the distance away from the stripline from 0 to 500 μm .

Figure A.14. Line graph of the simulated magnetic flux density distribution (x component) along the red line. The values of magnetic flux density vary from -1.5 to 1.4mT from 0 to 8mm along the x-axis. The distribution of the flux density looks like a periodic wave, corresponding to the shape of the double stripline. The magnetic flux density peaks correspond to the corners of the stripline.

Figure A.15. Line graph of the simulated magnetic flux density distribution (y component) along the red line. The maximum absolute values of magnetic flux density can reach 1.4 mT. The minimum values appear near the gaps between neighboring edges. The magnitude of magnetic flux density is proportional to the electric current. With 1.2A current, the stripline can generate the strongest magnetic field.

Figure A.16. Line graph of the simulated magnetic flux density distribution (z component) along the red line. Comparing with the plots of x and y components, the z component plot shows more clear peaks corresponding to the corners of the stripline.

Figure A.17. Line graph of magnetic flux density distribution along the yellow line. With the increase of the distance away from the stripline, the magnetic flux densities drop rapidly. The slope of the magnetic flux density plot is the magnetic field gradient. Within 200 μ m distance, the stripline shows high magnetic flux density and high magnetic field gradient. Therefore, a magnetic particle will experience strong magnetic force in this range.

Figure A.18. Magnetic force distribution of single stripline (YZ plane). The unit of the force is newton (N). Corresponding to the distribution of magnetic flux density, the peaks of the magnetic force distribution appear near the corners of the stripline. The average force exerted on a magnetic particle by the single stripline is around 4 pico-newton.

Figure A.19. Magnetic force distribution of double stripline (YZ plane). The unit of the force is newton (N). Due to the relative larger size of each unit of the double stripline, the number of force peaks is less than that of single stripline. However, the average magnitude of double stripline is much larger than those of single stripline. This can be attributed to the enhanced magnetic flux density and magnetic field gradient in the double stripline.

Figure A.20. The oblique view of magnetic force distribution of double stripline. The unit of the force is newton (N). The maximum magnetic force experience by a magnetic particle appear near the two arms of the stripline due to the larger electric current density in the arms. As seen from the plot, some force peaks appear above the zero plane, while some appear below the zero plane. This is determined by the direction of magnetic fields in different parts of the stripline.

Double stripline

Figure A.7 represents the double stripline used for COMSOL simulation. The material of the double stripline is same as single stripline. The current density applied in double stripline is $1.5 \times 10^7 \text{ A/m}^2$. Figure A.8 indicates the local magnetic fields directions of current-carrying wires. Figure A.9 demonstrates the magnetic flux density distribution. Within the range of double stripline, it is obvious that magnetic flux density has the highest value at the corners while the lowest values in the gaps between neighboring wires. Comparing with Figure A.3, the magnetic field magnitudes of double stripline are larger than those generated by single stripline. Figure A.10, A.11 and A.12 demonstrates the X, Y and Z component of magnetic flux density distribution, respectively. Due to the superposition of magnetic fields, double stripline can generate stronger magnetic field than single stripline with same electric current density. From Figure A.10, it can be seen that the directions of magnetic fields change alternatively. This is due to the variation of current flow in the stripline. Figure A.11 clearly indicates that double stripline can generate more magnetic field peaks than single stripline. The magnetic peaks are the major contributors of magnetic force. As seen from the Figure A.12, the Z component mainly appears near the gaps of the double stripline. The contribution to the particle deflection of the Z component of magnetic flux density can be ignored.

To study the change of magnetic field, different components of magnetic flux density were measured along the red line in Figure A.13. The measurement range along X-axis is set from 0 to 8 mm. The results were plotted in Figure A.14, A.15 and A.16. For X and Y component, the absolute values of magnetic flux density vary between 0 and 1.4 mT. For Z component, the absolute values of magnetic flux density change between 0 and 1.0 mT, indicating relatively weak of magnetic field in Z component. Corresponding to Figure A.10, the variation of the signs of

magnetic flux density values in Figure A.14 indicates the magnetic field direction change at different positions along the red line. From the plots, we can see that the values of magnetic flux density at 1.2A are larger than those generated at lower electric currents. Hence, we can obtain high magnetic field through increasing the current. However, due to the Joule heating effect, overload current may damage the stripline and live cells. To investigate the decay of the magnetic field, the variation of magnetic flux density with respect to the increase of the distance away from the stripline have been measured. The line plot in Figure A.17 indicates that within 200 μm range, the strength of magnetic field decreases rapidly. This plot provides helpful information to position the magnetic particles or cells near the stripline to ensure that they can experience high magnetic force.

To estimate the magnitude of the magnetic forces, the magnetic force experienced by a magnetic particle (Fe_2O_3 magnetic particle with 8 μm diameter) was calculated using Matlab. Based on equation A-1 and the magnetic flux density values extracted from COMSOL simulations, we can obtain the magnetic force distribution as shown in Figure A.18, A.19, and A.20. Comparing with magnetic force in single stripline device (Figure A.17), the magnetic force experienced by a magnetic particle in double stripline device (Figure A.18) was obviously enhanced. In addition, the force plots indicate the magnetic force is in the order of piconewton, which agrees well with the force estimation at the beginning of this Appendix.

In summary, our approach, based on the use of FEM analysis and Matlab calculation, enables to clearly estimate the range of magnetic force required to deflect particles in microfluidic channel. This approach is very useful to assist micro-electromagnets design and fabrication, especially when different geometric parameters of stripline have to be tested. Moreover, this simulation and calculation does not take into account the NiFe magnetic field concentrator layer of the stripline,

but the general principle proposed here can be extended to study more complicated electromagnetic systems.

Appendix B: Comparative Review of the Fabricated Devices

In this appendix, we compare our devices with those reported in literature with regard to the aspect of performance or fabrication. The relevant paragraphs in the thesis are restated here with four tables to give a clear comparison.

Table B-1. Comparison of various electromagnetic devices for cell separation

<i>No.</i>	<i>Device features</i>	<i>Separation efficiency</i>	<i>Reference</i>
1	Copper microcoil conductor, electroplated nickel plate	92.4%	[210]
2	Copper conduction layer, Square meandering micro stripline	95%	[211]
3	Electroplated copper layer	95%	[212]
4	Magnetofluidic device described in Chapter 3	91%	This thesis

Both permanent magnets and electromagnets can be used as magnetic field sources for immunomagnetic cell separation. Compared to permanent magnets, electromagnets have some unique merits. For example, the magnetic field can be tuned by varying the amplitude of the electric current, enabling precise manipulation of cells. However, the number of reports on electromagnets for live cell separation in continuous manner is very limited. Song et al. developed a microfluidic device integrated with micro-electromagnets for magnetic particles separation. In their device, a copper microcoil fabricated by electroplating was used as a conductor. In addition, a 25 μm thick nickel layer was electroplated outside the microcoil to enhance the magnetic field.

This device was used to separate 4.5 μm magnetic beads and achieved maximum separation efficiency of 92.4% [210]. Kong et al. fabricated a copper microelectromagnets on silicon substrates and aligned it with microfluidic channel. They achieved 95% magnetic particle separation efficiency [211]. Chung et al. fabricated a microelectromagnets by electroplating 5 μm copper on glass substrate. They used this microelectromagnets to separate 2.8 μm magnetic beads from polystyrene particles with separation efficiency of 95% [212]. However, these devices did not prove their feasibilities by separating live cells. Compared with the devices mentioned above, the efficiency of our device ($>90\%$) is satisfying. To our knowledge, our device is the first one used to investigate the dependence of live cell sorting efficiency on electric current of microelectromagnets.

Table B-2. Comparison of various devices for monocytes separation

<i>No.</i>	<i>Flow rate</i>	<i>Device performance</i>	<i>Reference</i>
1	400 $\mu\text{l}/\text{min}$	Separation purity: 78 $\pm$ 14%	[230]
2	1ml/min	Separation purity: 43%	[231]
3	50ml/h	Separation purity: 90% Recovery rate: 87%	[232]
4	1300 $\mu\text{l}/\text{min}$	Separation efficiency: $>90\%$	This thesis

Although obtaining human monocytes with high purity is important for biomedical research, only a few microfluidic devices have been reported for this purpose so far. Using lattice-shaped pattern as virtual sieve, Yamada et al. presented a microfluidic chip for size-based continuous particle sorting. This device can separate monocytes from other blood cells with purity of 78 $\pm$ 14% and can run at highest flow rate of 400 $\mu\text{l}/\text{min}$ [230]. An inertial microfluidic chip was developed

by Ramachandraiah et al. for separating nucleated cells from whole blood. Under maximum flow rate of 1ml/min, they separated monocytes from blood with purity of 43% [231]. To obtain monocytes from blood, Darabi et al. developed a magnetophoretic-based device. At flow rate of 50ml/h, they can isolate monocytes with ~90% purity and 87% recovery rate [232]. Compared to these devices, our integrated device showed higher processing throughput (>1300 μ l/min) with high separation efficiency (>90%) for monocytes separati

Table B-3. Comparison of various paper-based concentrators

<i>No.</i>	<i>Device feature</i>	<i>Device performance</i>	<i>Reference</i>
1	Fabricated by craft cutter, enclosed by plastic film	60-fold concentration enhancement within 200s	[125]
2	External stamp-like device	44-fold concentration enhancement within 155s	[148]
3	Three layers wax printed device	1000-fold concentration enhancement using 200V voltage	[150]
4	Wax printed device, clamped by magnets	20-fold concentration enhancement using 50V voltage	[236]
5	Sprayed device,	100-fold concentration enhancement using 50V voltage	This thesis

Very recently, some groups have reported the application of paper-based microfluidic devices for sample pre-concentration. Phan et al. presented a paper channel for fluorescent dyes concentration. Their device was fabricated by craft cutter and enclosed by plastic film. Using this

device, they achieved 60-fold concentration enhancement within 200s. However, the profile of concentration plug is irregular, leading to difficulty of further quantification [125]. Gong et al. developed a stamp-like sample concentrator using paper as substrate and achieved 40-fold concentration at 50V driving voltage [148]. Using same voltage, Yang et al. achieved 20-fold sample signal enhancement on a wax printed paper-based concentrator [236]. To main longer concentration time, Han et al. fabricated a three-layer paper device integrated with absorbent pad. The applied 200V voltage across the paper channel and achieved >1000-fold sample concentration factor. However, the high external voltage may lead to safety concern. On the contrary, our device can obtain >200-fold concentration factor at 50V voltage. In addition, our enclosed paper device can maintain around its maximum concentration level for more than 300s, which was much longer than other devices. Moreover, our sealing configuration not only increase the portability of the device, but also can facilitate operational simplicity.

Table B-4. Comparison of various in-plane paper-based microsupercapacitors

<i>N</i> <i>o.</i>	<i>Fabrication method</i>	<i>Fabrication shortcoming</i>	<i>Reference</i>
1	Thermal-evaporated gold interdigital electrodes on photo paper	Complex and expensive	[244]
2	Manually drew interdigital graphite electrodes on paper	Low productivity	[245]
3	Vacuum filtration, Au layer deposition	Complex and expensive	[246]
4	Sprayed device, Carbon nanotubes electrodes	Simple, low-cost	This thesis

Although promising, in-plane paper-based micro-supercapacitors (mSCs) have been rarely reported so far. In 2003, the first in-plane paper-based micro-supercapacitor was reported by Lee's group. The gold interdigital electrodes of the device were fabricated on photo paper through thermal evaporation [244]. Ma et al. first reported a vacuum filtration method for the fabrication of interdigital electrodes. The electrodes were patterned using photolithography, followed by deposition of carbon nanotubes solutions through vacuum filtration. At last, Au layer was deposited on the top of the carbon electrodes [246]. However, due to the involvement of expensive materials and equipment, these two fabrication methods are not available for many researchers. To reduce fabrication cost, Chen et al. developed a hand-painting method. They manually drew interdigital graphite electrodes on paper substrate [245]. However, low productivity of hand-painting limits its wider application. Compared to the current paper-based micro-supercapacitors,

our fabrication method based on hydrophobic spraying provides a low-cost and simple approach for the massive production of micro-supercapacitors.