On-chip flow cytometry for cell analysis

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On-Chip Flow Cytometry

For Cell Analysis

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SCHOOL OF CHEMICAL & BIOMEDICAL ENGINEERING

NANYANG TECHNOLOGICAL UNIVERSITY

2014

On-Chip Flow Cytometry For Cell Analysis

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School of Chemical & Biomedical Engineering

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In fulfillment of the requirement for the degree of Doctor of Philosophy

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This work is dedicated with affection to my girlfriend

Lei Wen, whose care, courage, and patience

have been an inspiration to me,

and to my dear parents and sister,

whose love, support and encouragement

have put me where I am now.

Abstract

On-chip flow cytometry provides a powerful tool to characterize the cell samples for point of care diagnosis since they provide an effective solution for low-cost and rapid evaluation of disease prognosis. The on-chip flow cytometry can implement some basic functions, such as loading, focusing, detection and sorting toward even single-cell on-chip analysis. Therefore the overall objective of my dissertation research is to develop the microfluidic cytometry for manipulation and detection of cells and other biological particles.

The dissertation first reviewed the background of the on-chip flow cytometry including cell manipulation technique, cell detection techniques and cell sorting techniques. The acoustic manipulation and electrical, optical and image detection of cells are detailed discussed in the following chapters. In the chapter 3, we have conducted a study of standing surface acoustic wave (SSAW) induced acoustophoresis to manipulate the bioparticles in a microchannel. The SSAW generated by two parallel interdigital transducers (IDTs) induce the acoustic radiation force to propel particles toward the pressure node which is tunable. Therefore, the particles can assemble at the desired pressure node. The theoretical analysis also gives the approximated time consuming for particle assembling and the **experimental** result validates the theoretical value. Due to the advantages of easy-fabrication, lower energy consuming, and easy integration, this method can significantly benefit the development of future on-chip flow cytometry. In chapter 4, we have presented a DC-based microfluidic sensor for the detection and enumeration of circulating tumor cells (CTCs). The working principle of this cytometer is based upon the detection mechanism of bias-potential modulated pulse originating from the biological particle's physical blockage of the micropore. 7 μm , 10 μm and 16 μm polystyrene particles were used to test and calibrate the proposed chip, respectively. Furthermore, Hela cells (a type of CTC) blood cells including red blood cells (RBCs) and white blood cells (WBCs) are used to assess the performance of this microcytometer for counting and detecting the tumor cells. The proposed microfluidic sensor is able to provide a promising platform to address the current unmet need for the point-of-care clinical diagnostics. In chapter 5, we have presented an AC-based Microfluidic

impedance sensor. Conventional microfluidic impedance sensor usually requires the patterned gold electrodes directly in contact with the carrying buffer to measure the electrical current change due to the blockage of cells. However, patterning metal electrode probes on the silicon or glass substrate is a non-trivial task, which increases the fabrication cost of the impedance sensor. In this **chapter**, we demonstrate an alternating current (AC) impedance based microfluidic cytometer built on a printed circuit board (PCB) coated with polydimethylsiloxane (PDMS) thin film. In addition, circulating tumor cells (*Hela cells*) are used to successfully demonstrate the feasibility of the microfluidic AC impedance sensor in tumor cell detection. The electrodes pre-deposited PCB costs less than US\$2.00 and is widely available in the market. This device has a good potential for point-of-care diagnosis in resource-poor settings. In chapter 6, we have also developed a planar optofluidic chip for circulating tumor cell apoptosis by exciting dual fluorescence through the integrated fibers. In contrast to conventional cell apoptosis study that requires commercial flow cytometer with a very high price tag and bulky optics, our design was realized via a simple single-layer soft lithography fabrication process. This study demonstrated the capability of the proposed optofluidic chip for cell apoptosis assay. In chapter 7, we demonstrate a portable resistive pulse activated lens-free cell imaging system. The system consists of the microfluidic channel and CMOS imaging sensor. Both the particle modulated pulse profile and images can be concurrently recorded in the system which is very useful for the correlation analysis (peak amplitude & pixel size) of biological cells.

The advantages of this on-chip flow cytometry include simple fabrication process, compact components, reliable performance, and low manufacturing cost is capable of making it a promising platform for future mass-producible, inexpensive, and disposable on-chip investigation of biological samples

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Vita

Guo Jinhong was born on January 1, 1988, in Chengdu, Sichuan, People's Republic of China. He obtained a Bachelor of Engineering degree in July 2010 after four years undergraduate study at University of Electronic Science and Technology of China, Chengdu, Sichuan, China. In August 2011, he joined Nanyang Technological University as a postgraduate research student for a PhD degree in Chemical and Biomedical Engineering.

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Chapter 1

Introduction

1.1 Background

Flow cytometry is a highly efficient method to measure physical and chemical characteristics of cells or other biological particles while they pass, in single file, through the interrogation area in a fluid stream ([Shapiro, 1995](#)). The modern flow cytometry can do rapid screening for hundreds of thousands of cells within minutes with single-cell resolution, and generate a snapshot of the statistical distribution of the cell subpopulations based on multiple phenotypical or biochemical gating criteria. Using some flow cytometers, cells can be sorted on the fly subsequent to the detection according to their particular characteristics for further analysis. Since flow cytometry was first developed in 1950s as amperometric electric signal based Coulter counter and in 1960s as fluorescence-based commercial device, it has revolutionized the hematologic assay and become the gold standard for clinical diagnosis of the blood-borne infectious diseases, such as AIDS ([Marrone, 2009](#)). Precise and fast enumeration of the peripheral blood CD4⁺ T lymphocytes by flow cytometry analysis provides a key parameter for determining disease progression and monitoring efficacy of the treatment. This versatile technology has been later applied to analyze biological particles at even smaller scales, such as bacteria ([Wang et al, 2010](#)) and DNA fragments ([Ferris et al, 2004](#)).

However, the typical commercial bench-top flow cytometer system is highly expensive and the availability is only limited to some major research centers or referenced laboratories. Although the flow cytometric data analysis and explanation require especial training and skills, the stunning cost, which usually ranges from \$75,000 to \$125,000 plus 10% annually for maintenance, is the major obstacle preventing this enabling technology from being widely accessible to home and personal users or in resource-poor settings. As suggested by Shapiro ([Shapiro and Perlmutter, 2006](#)), a “personal cytometer” should cost less than \$5,000 or even lower to be technically and economically feasible for wide range of applications in both developing and developed countries.

Since late 1990s, microfluidics based lab-on-a-chip technology has been rapidly and widely implemented to develop miniature biomedical microdevices. This emerging technology has a profound impact on the healthcare industry (Toner and Irimia, 2005; Dittrich and Manz, 2006; El-Ali and Sorger, 2006; Dittrich et al, 2006) and nurtured many new industrial players that are dedicated to research and marketing of novel lab-on-a-chip products, such as Caliper, AVIVA BioLifeSciences, Fluidigm, Eksigent, Applied Biosystems, RainDance Technologies etc. (Stephan, 2004). Since a fluidic system always constitutes the core of the modern flow cytometry, it is natural that the microfluidics based on-chip flow cytometry has become one of the hottest areas and attracted numerous investigators and companies (Sims et al, 2007). Although the cost, size, and complexity of the present bench-top commercial systems have been greatly reduced over the past fifty years, the on-chip flow cytometry are promising to acquire a decent share of the competitive market and represent a future technology to change people's daily life. The great potential of this technology lies in its several important characteristics: size, automation, and cost.

Size: Miniaturization is the most obvious benefit using on-chip flow analysis. The overall system can be significantly reduced in size and weight, however with complete integration of handling, processing, and analyzing components. With a size of only a fraction of a desk top computer, the future on-chip flow cytometry system can be highly portable for onsite diagnosis. The disposable micro-chips for sample collection and analysis may be in the same size as a credit card or even smaller for easy carrying. The fluidic system comprises channels ranges from tens of micrometers to submicron scale. Large amount of sheath fluids as used in the current standard flow cytometers are no longer necessary.

Automation: Micro and nanotechnologies have enabled on-chip flow cytometry to integrate automated functions, such as mixing, reaction, focusing, optical or electric detection, and on-line sorting. The future technology advances and innovation will further push the integration to a higher level with sample preparation, parallel processing, and multi-parameter analysis. The user interface can be much simplified and targeted for particular biomedical assays. The analytical results can be easily printed and even transferred wirelessly to the patient's doctor through WiFi or cellular networks to realize

practical telediagnosis. All the effort is to make the on-chip flow cytometer a self-contained, fully automatic system which can be operated by lay persons.

Cost: Reduction of size also implies reduced manufacturing cost since they can be built up with much less materials. The disposable micro-chips are mostly made of inexpensive and bio-degradable polymeric materials, such as PDMS (Stevens, 1998), rather than glass and thus will have much less environmental impact. Mass production through standardized manufacturing protocols and commonality of technological platforms can further drive down the system cost and make the chips available right off the shelf in any pharmacy drug store. The sampling amount can be significantly smaller, with tens of microliters down to nanoliters for a complete analysis, compared with milliliters for traditional diagnosis. And consumption of the expensive reagents, such as fluorescent biochemical markers, can be minimized. Therefore the average cost per assay will be much more affordable. Furthermore, because of the high-level automation, there is no need to hire or train a professional operator or bear the extra maintenance cost.

The above visionary observations are based on the recent rapid development and wide applications of the microfluidics based on-chip flow cytometry and the micro/nanotechnology in general. This paper aims to provide an overview of the technology advancement made by numerous scientists from various disciplines, and their promising steps towards the future on-chip flow cytometry. This review will begin from the basic principle of the standard flow cytometry. Since the fabrication process of microfluidic flow cytometers has been discussed in details in another review article ([Huh et al, 2005](#)), we will only focus on the technology advancement, which will be highlighted according to the three key functionalities of a flow cytometer: focusing, detection, and sorting. In each section, the working principle, the state-of-the-art methodologies, and important applications developed in the recent decades will be highlighted. The challenges and perspective on some future directions of the on-chip flow cytometry will be summarized in the end. It should be noted that there are many other innovative methods for on-chip cytometry using trapping arrays ([Voldman, 2002](#); [Kovac et al, 2007](#); [Wlodkowic et al, 2009](#)), biochemical affinity chromatography ([Cheng et al, 2007](#); [Cheng et al, 2007](#)), or opto-electronic sensor arrays ([Seo et al, 2009](#)). All these methods are performed in a non-flow environment for detection of massive cell

population and require image post-processing to identify and count individual cells. The discussion in this review will only be limited to the dynamic on-chip cytometry “in the stream” or “on the fly” with single cell resolution.

1.2 Basics of flow cytometry

The anatomy of a typical flow cytometer comprises three sections according to its major functionalities: particle focusing, detection or interrogation, and sorting ([Fig.1](#)). Focusing is the most crucial step of particle handling for preparation of detection in single profile. To ensure every particle passes the interrogation area one at each time, the original dispersed particle suspension needs to be focused into a thin stream with cross-sectional diameter close to that of a single particle. The most popular and widely applied method is to use sheath fluid in the periphery of sample input. By adjusting the relative hydrodynamic pressure between the sheath and the sample flow, the particles can be confined in an extremely thin stream in which a train of particles can be detected individually ([Fig.1-1a](#)). Usually there are several preset sheath flow rates that correspond to different transit times for a single particle to pass the interrogation area and are well tuned for the sensors to get better signal. Users can choose among different options according to the sample volume, density of the particle suspension, or signal strength of the fluorescent emission.

Detection component is usually the most complex sub-system in a flow cytometer and is probably where the researchers and developers spend most of their effort in. Although there are many methods to detect a single particle with size from a cell to a macromolecule, as will be discussed in detail in the following sections, detection using laser optics is still so far the dominating one. There is no doubt that, compared with other sensing methods, optical detection offers many more degrees of freedom in terms of multi-parameter characterization. Light scatters provide information of the cell size, shape, granularity, membrane roughness. In addition, with thousands of fluorescent markers available in the present market, people can obtain various biochemical information of the cell membrane and intracellular structures from their fluorescent emission. The typical optical detection requires light illuminations, beam path manipulation, and optical sensing ([Fig.1-1b](#)). One or more highly collimated laser sources

with different wavelengths are used to excite the particular fluorophores on the cells. Before the lasers reach the cell, a set of focal optics will focus all the laser beams into a tiny spot in the flow chamber where the particles will pass right through the center of the spot. This configuration maximizes the excitation energy and hence the fluorescent emission intensity on each cell. And this is also why stable particle focusing is a key for high fidelity detection. The incident fluorescent emission and scattered light are collected, filtered, and diverted to different photodetectors (photodiodes or photomultipliers), which convert the analog signals into digital signals. Signals from every single particle will be recorded and processed by a computer system and the statistical information of all the subpopulations in the sample can be shown in a real-time fashion according to their particular phenotypes.

Figure 1 the schematic of Cell sorting and detection system

Although sorting is not a standard function for most of the current commercial flow cytometers, it represents one of the most important applications. Since the on-chip flow cytometry is advantageous in integrating various functional components, sorting cell while keeping the cell viability for subsequent analysis is naturally the most sought after key function for on-chip integration. Various state-of-the-art techniques for on-chip particle sorting will be summarized in this review. In the most popular sorting technique used by the conventional flow cytometry, the focused stream of particles, after they pass the interrogation area, is broken into tiny droplets through a vibrating nozzle. Every single particle is encapsulated in a droplet that is selectively charged according to their

optical signals in the detection system. Then the charged droplets pass a strong electrostatic field, and the particular charge intensities and polarities cause the droplets to be deflected into different collectors ([Fig.1-1c](#)). This typical method is also known as fluorescence activated cell sorting (FACS), which can efficiently sort up to tens of thousands of cells per second.

Chapter 2

Literature Review

The flow cytometry system mainly consists of three parts, which are focusing part, detection part, sorting part respectively. The following literature review gives a brief introduction to each part.

2.1 Particle Focusing

As discussed previously, focusing of the particles randomly dispersed in the original suspension is a prerequisite for subsequent analysis in single file. Most of the conventional flow cytometers are using hydrodynamic focusing with sheath flow. The fluidic design for sheath flow focusing is relatively simple and straightforward without any complex components involved. Hydraulic pressure or peristaltic pumps are mature products that are widely available and can be easily integrated into the system. And significantly high throughput can be achieved under high flow rate without sacrificing the focusing effect. These advantages make this method the mainstream to focus particles in the modern flow cytometry and popular for even the latest developers for new generation of flow cytometers. However, hydrodynamic focusing has its inherent drawbacks. The most obvious one is its requirement of large amount of sheath fluids. Most flow cytometers consume from one to several liters of sheath fluids every day for regular uses. There are tanks or bottles of various sizes built in or attached on side of these machines, some of which need to be regularly refilled for fresh sheath fluids and others be emptied with waste liquids. Extensive use of tubing and hoses adds up the system complexity and maintenance cost. There is no doubt that sheath fluid is one of the major reasons to make the conventional flow cytometers bulky, heavy, and hence lack of portability. Requirement for frequent maintenance also limits their ability for full automation. Recently Life Technology developed and marketed the first commercial focusing flow cytometer, Attune, based on an innovation licensed from Los Alamos National Laboratory.

Micro and Nanotechnology provide many more options in terms of focusing biological particles in micron or submicron size. In addition to sheath flow focusing

with various microfluidics design, other methods by applying a body force directly on individual particles offer more flexibility for design, integration, and miniaturization of innovative on-chip flow cytometry. As will be explained later, body force such as electrokinetic force and acoustic radiation force can be favorably scaled down in micro or nanosystems, which is a powerful tool for manipulation of biological particles of different sizes. In this review, the microfluidic particle focusing techniques are categorized into sheath flow focusing and sheathless focusing.

2.1.1 Sheath flow focusing

Different from the glass tubes and cuvettes used for fluidics design in the conventional flow cytometry, microfabricated channels with polymeric materials are commonly used to develop on-chip flow cytometer. Using the soft lithography rapid prototyping (Duffy et al, 1998), developers can easily fabricate fluid conduits of any dimension, shape, and configuration (Li, 2008). There are two typical configurations of microfluidic hydrodynamic focusing (Fig.2). One is using co-axial channels (Lee et al, 2001; Lee et al, 2005) as in Fig.2a; the other is using cross-junction structure and its variations (Yang et al, 2006; Bermiñan et al, 2006; Bang et al, 2006; Rodriguez-Trujillo et al, 2007; Sakamoto et al, 2007; Tung et al, 2007) as in Fig.2b. In these designs, the core sample flow can be pinched into an extremely thin stream by the sheath flow introduced from both sides laterally. Width of the core can be easily decreased by increasing the relative flow rate of the sheath flow.

Figure 2-1. 2D sheath flow focusing in microchannels. (a) co-axial channel configuration. Reproduced with permission from Ref. (Lee et al, 2001). Copyright (2001), ASME; (b) cross-junction channel configuration. Reproduced with permission from Ref. (Rodriguez-Trujillo et al, 2007). Copyright (2007), Springer.

The major difference between the conventional off-chip and on-chip focusing fluidics lies in their different ways of generating sheath flow and the distinct cross-sectional profiles of the sample core. In a conventional flow cytometer the co-axial tubing generates a cylindrical core surrounded by an annular sheath (Fig. 3a); whereas the on-chip hydrodynamic focusing generates core flow with rectangular cross-sectional shape by sheath from lateral direction due to its planar structure (Fig. 3b). By adjusting the differential hydrodynamic pressures or flow rates of the co-axial tubing, one can easily change the diameter of the cylindrical core flow d to match the size of the target particles. However, there might be a problem with on-chip focusing because one cannot change the height H but the width of the core flow w for a fixed planar channel. Fortunately microchannels can be easily fabricated down to a few micrometers in height, so that particles such as blood cells can pass through in a single file with regard to the channel height dimension. But if one wants to focus submicron particles, such as bacteria, DNA, and other macromolecules, or if a microchannel of high aspect ratio (W/H) has to be used, further decreasing the height might cause the polymeric channel to collapse. In addition,

some biological particles, such as proteins, need to be totally shielded by the sheath fluid to prevent potential surface interaction, such as sticking or adsorbing. In order to address these problems, many researchers later developed innovative methods for three-dimensional (3D) hydrodynamic focusing at both lateral and vertical directions.

Figure 2-2. Difference between off-chip and on-chip hydrodynamic focusing. (a) off-chip focusing with co-axial cylindrical tubing; (b) on-chip 2D (lateral) focusing inside a microchannel; (c) on-chip 3D (lateral and vertical) focusing inside a microchannel. Partly reproduced with permission from Ref. (Chang et al, 2007). Copyright (2007), IOP Publishing.

In the literature, some researchers (Ateya et al, 2008; Simonnet et al, 2005; Simonnet et al, 2006) refer to the lateral focusing (Fig.2 and Fig.3b) as one-dimensional (1D) and the combination of the lateral and vertical focusing as two-dimensional (2D); while others (Chung et al, 2003; Nieuwenhuis et al 2003; Chang et al, 2007; Sundararajan

et al, 2004) refer the former as 2D and the latter as 3D. In order to unify the terminology, we prefer to follow most of the literature and name the lateral focusing in the horizontal plane as 2D and combinational focusing in both horizontal and vertical planes as 3D.

The earlier effort to confine the sample core in the vertical direction was made by (Chung et al, 2003), who realized a focused sample flow at the bottom center of the channel by fabricating a lower sample input channel at the cross-junction. Sheath flow from the higher sheath input channel squeeze the sample core both from the top and the lateral directions. Similarly but also effectively, (Wolff et al, 2003) input the sample core using a chimney structure arising from the bottom of the channel creating a coaxial flow profile for the core and sheath. (Nieuwenhuis et al, 2003) proposed a design that can generate independent sheath flows both from the top and the bottom in addition to the regular lateral sheath, which was later refined by (Simonnet et al. 2005) and (Chang et al, 2006). In this design (Fig.3c), the sample input is from the bottom, *B*; while the sheath is introduced from four ports, which located at upstream, *A*, downstream bottom, *C*, and downstream lateral sides, *D*, respectively. *A* and *C* provide focusing at vertical direction, and *D* provide focusing at lateral direction. Precise diameter and position of the sample stream can be easily controlled by adjusting the relative flow rates of the four sheath ports (Nieuwenhuis et al. 2003). (Sundararajan et al. 2004) fabricated a 3D microfluidic assembly using multi-layer micro-molding procedures, which introduced independent lateral and vertical sheath flows at the same location and was able to tailor dimension and shape of the focused sample stream. However, these methods require 3D or multi-layer microfluidic structures and pose more challenges for the fabrication. To make full use of the single-layer planar structure of a typical microfluidic channel that can be fabricated using simple soft lithography, (Mao et al. 2007, 2009) recently demonstrated a 3D hydrodynamic focusing device combining lateral sheath flow with microfluidic drifting. The feature structure of this innovative design is a 90 degree curved channel right after the sample input from the inner side (Fig.4). When sheath and sample flow are running side by side in this curve, the centrifugal effect generates a pair of vortices in the sheath, which pinch the sample stream in the vertical direction. It looks like the sample stream becomes thinner and is drifting to the outer side the curve. At the downstream end of this

curved channel, two sheath streams are introduced in the horizontal direction to accomplish the overall sample stream focusing in 3D.

Figure 2-3. 3D hydrodynamic focusing using microfluidic drifting and lateral sheath flow with a curved channel. Reproduced with permission from Ref. (Mao et al, 2007). Copyright (2007), the Royal Society of Chemistry.

Rather than relying on the additional sheath flow in the vertical direction, some researchers use certain passive channel structures to achieve vertical focusing and combine traditional lateral sheath for 3D focusing. (Yang et al, 2005) fabricated an inner nozzle structure with a slope from the channel bottom. This converging geometry force the sample stream to be focused as it moves up along the slope. Similar passive focusing in vertical direction has also been applied by (Scott et al, 2008) using stepped top channel wall, and by (Fu et al, 2008; Tsai et al, 2008) using a micro-bump at the bottom of the channel. A unique design utilizing the hydrodynamic interaction of the sheath flow and passive channel structures was demonstrated by Ligler and colleagues (Howell et al, 2008; Kim et al, 2009; Golden et al, 2009). They built up chevron-shaped groove features on the top and bottom walls of the channel, which direct the sheath flow from the lateral sides to the top and bottom, thus completely shielding the sample stream in the channel center (Fig.5).

Figure 2-4. 3D focusing using interaction between lateral sheath and chevron grooves. Reproduced with permission from Ref. (Golden et al, 2009). Copyright (2009), the Royal Society of Chemistry.

All above investigations are based on the dominating technique of pressure-driven hydrodynamic sheath flow focusing. There are also other methods to generate sheath flow on a micro-chip, such as aerodynamic focusing using sheath gas (Huh et al. 2002) and electrokinetic focusing using electroosmotic sheath flow (Jacobson et al, 1997; Schrum et al. 1999; Fu et al, 2004; Yang et al. 2005; Xuan et al. 2005). (Huh et al. 2002) developed a micro flow cytometer using the ambient air as sheath to focus the sample liquid into a thin stream for analysis. This air sheath focusing method completely eliminates the use of sheath liquid required in the conventional flow cytometry and makes their system extremely light and portable. However, the aerodynamic focusing has yet been widely implemented because of some inherent limitations. As pointed out by (Huh et al. 2002), effective aerodynamic focusing depends on the hydrophobicity of the channel wall. The sample solutions are limited to those free of any surface-wetting components, such as amphiphiles and surfactants. In addition, when the sample flow rate falls below certain critical value, the focused liquid stream will become unstable and break up into droplets due to surface tension.

Electroosmotic flow (EOF) arises from the electrokinetic interaction between the liquid solution and the channel wall. When an aqueous medium is in contact with a solid surface, there is electric charge separation at the interface due to ionization, ion adsorption and dissolution (Probstein et al, 1994). A diffuse layer in the electrolyte solution is created close to the interface with non-zero net charge. Upon application of a tangential electric field, the Coulombic body force causes migration of the mobile ions in the diffuse layer and the momentum is transported to the bulk liquid by viscosity, resulting in an electroosmotic flow. EOF provides an option for liquid and particle transport inside a microsystem. Ramsey and colleagues (Jacobson et al, 1997; Schrum et al, 1999) are among the earliest scientists to use electroosmotic flow sheath focusing for on-chip flow cytometry applications. And this approach has been later followed by Lee and colleagues (Fu et al, 2004; Yang et al, 2005) and (Xuan et al, 2005). The pressure building capability makes EOF a good substitute for the conventional hydrodynamic pressure pumping system. Using the classic cross-junction configuration, width and the flow rate of the focused sample stream can be easily controlled by adjusting the voltage level and ratio between the sample input channel and the sheath input channels. Although the EOF focusing has advantages of easy control and uniform flow profile, there are some limitations related to high-voltage electric field. Firstly, the flow rate of EOF is proportional to the electric field strength. In order to improve the flow rate and throughput, significantly high voltage need to be used. This may induce issues in safety and energy efficiency. Secondly, strong electric field in the cell suspension will impose extra high trans-membrane potential that may crash the cell or change its physiological properties (Voldman et al, 2006). Thirdly, an obvious effect of applying electric field over electrolyte solution is the temperature elevation due to Joule heating. Temperature change of 4 C above physiological temperature will cause rapid death of mammalian cells. Even minor temperature increase may also have negative physiological effects (Voldman et al, 2006). All above reasons limit wide application of EOF as a focusing or driving mechanism in practical flow cytometry.

2.1.2 Sheathless focusing

As mentioned earlier, the body force can be implemented for confining suspended particles into a tiny stream at desired locations. The body force acts solely on the particles without disturbing the suspending fluid flow, and it can easily realize particle focusing in 3D. Although there are many forms of body forces that can be used for focusing microparticles (Xuan et al, 2010), the most important and popular two are electric force (Voldman et al, 2006; Kang et al, 2009) and acoustic force (Wiklund et al, 2006; Laurell et al, 2007). Detailed formulation of the electric and acoustic body forces can be found in several comprehensive review papers (Voldman et al, 2006; Kang et al, 2009; Wiklund et al, 2006; Laurell et al, 2007), here we only highlight the general expression of three important body forces in Table 1. Voldman (Voldman et al, 2006) presented a simple scaling analysis to explain why two electric body forces, namely electrophoretic and dielectrophoretic forces, can be favorably scaled down to microscale. Simply speaking, keeping the overall voltage (or electric energy) constant, decreasing the characteristic length of the system can generate stronger electrophoretic and dielectrophoretic forces. Similarly, the acoustic radiation force scales as $F \sim \varepsilon/\lambda$, where ε is the acoustic energy. If the wavelength λ of the applied acoustic wave (reverse of system resonance frequency) or the characteristic length of the microstructure to generate the wave decreases, keeping the acoustic energy constant, the acoustic radiation force becomes stronger. In addition, miniaturization of the system can significantly increase the surface-to-volume ratio and hence the heat dissipation, which can relieve the negative effects of temperature rise due to Joule heating or acoustic heating.

Table 2-1. Important body forces used for particle focusing

Body Forces	Expression	Scaling
Electrophoretic (Kang et al, 2009)	$F = Eq$ Eq. (1)	V/L
Dielectrophoretic (Kang et al, 2009)	$F = \frac{3}{2}\varepsilon_m K_{CM} V_p \nabla E ^2$ Eq. (2)	V^2/L^2
Acoustic Radiation (Laurell et al, 2007)	$F = \frac{2\pi}{\lambda} \phi \varepsilon V_p \sin(\frac{4\pi x}{\lambda})$ Eq. (3)	$\frac{1}{L}$

Electrophoretic force was used by (McClain et al, 2001) to focus and transport *E. coli* bacteria in microfluidic device for flow analysis. Differential electric potentials were applied to fluid reservoirs connected to four branches of a cross-junction to establish electric field within the channels. Although the electrophoretic focusing can effectively confine the bacteria for single-cell scatter and fluorescence detection, their device suffered from low throughput under plain electrophoretic migration of the bacterial particles, which is much slower than electroosmotic flow or pressure driven flow.

As shown in Table 1, dielectrophoretic (DEP) force scales more strongly down to microscale than electrophoretic force (Voldman et al, 2006). For the same energy input, decreasing characteristic length can generate much stronger DEP force than electrophoretic force. Furthermore, DEP force is proportional to the spatial gradient of the electric field strength $\nabla |E|^2$, which implies that it is a short-range force and is confined where there is maximum non-uniformity of electric field. Rather than establishing a global electric field in the whole microfluidic channel, people can use localized electric field to generate strong DEP force, minimizing negative effects due to Joule heating or electric cell damage. These unique characteristics make DEP a much better option than electrophoresis and have been used by numerous investigators for particle manipulation in microsystems (Kang et al, 2009). (Yu et al, 2005) designed and fabricated a microfluidic channel of elliptic cross-section with series of ring electrodes patterned on the circumference of the channel wall (Fig.2-6a). Alternating current (AC) electric fields were created in the channel, and the original dispersed cell suspensions were pushed away from the regions with high electric field gradient (edges of the electrodes) and focused into the center line of the channel by negative DEP force. Microbeads and human leukemia HL60 cells were be successfully focused by applying AC voltage at amplitude up to 15 Vp-p and a frequency below 100 kHz. Also using negative DEP, (Holmes et al, 2005; Holmes et al, 2006; Holmes et al, 2007) came up with a simpler design with two pairs of planar electrodes patterned on the top and bottom of a rectangular channel to achieve particle focusing in 3D (Fig. 2-6b). Rather than using physical metal electrodes inside the channel, (Lin et al, 2008) developed a particle focusing scheme using image DEP, which was firstly developed by (Chiou et al, 2005).

This method creates virtual electrodes by projecting optical image onto a photoconductive layer (Fig.2-6c), where the electric impedance of the illuminated area becomes significantly smaller than other ‘dark’ area and acts as electrode. The digital micro-mirror projector is controlled by a computer. Image DEP greatly reduces the fabrication complexity and provides a simple way to dynamically re-configure the virtual electrodes for better focusing.

Figure 2-5. Negative DEP particle focusing in (a) an elliptic cross-sectional microfluidic channel with ring-electrode array patterned on its circumference; Reproduced with permission from Ref. (Yu et al, 2005). Copyright (2005), IEEE. (b) a rectangular channel with planar electrodes patterned on top and bottom of the channel. Reproduced with permission from Ref. (Holmes et al, 2006). Copyright (2006), Elsevier. (c) a channel coupled with optically induced virtual electrodes. Reproduced with permission from Ref. (Lin et al, 2008). Copyright (2008), Elsevier.

The above DEP focusing methods rely on either physical or virtual electrodes to provide AC electric field in a microchannel in order to induce DEP force. There are alternative methods using DC voltage to achieve similar effects without any form of electrodes inside a channel. As discussed by (Kang et al, 2009), due to conservation of the electric current density in a microchannel made by insulating material and subjected to a global DC voltage, the electric field becomes non-uniform at certain heterogeneous structures, such as constrictions created by turns, hurdles, and blocks. As a result, strong DEP force can be generated at these constrictions where the electric field strength has drastic change. (Zhu et al, 2009; Church et al, 2009) fabricated a serpentine microfluidic channel with large number of turns in the flow path, where the particles experience negative DEP force in the lateral direction every time they pass each corner. The overall effect is that after certain number of turns, dependent on the DC voltage level applied to the channel, the particles are all focused in a stream along the channel centerline (Fig.2-7a). The same group of researchers also tested channel constriction with a symmetric pair of hurdles opposite to each other (Zhu et al, 2009). With DC-biased AC electric field, the DEP focusing can be significantly enhanced by increasing the AC to DC voltage amplitude ratio (Fig.2-7b).

Figure 2-6. Insulator-based dielectrophoretic focusing using turns and constrictions inside a microchannel: (a) dielectrophoretic focusing of 5 μm particles in a serpentine microchannel under different DC voltage strengths. Reproduced with permission from Ref. (Zhu et al, 2009). Copyright (2009), Springer. (b) dielectrophoretic focusing of 10

um particles by a channel constriction at various AC to DC voltage ratios. Reproduced with permission from Ref. (Zhu et al, 2009). Copyright (2009), Wiley-VCH Verlag GmbH & Co. KGaA

Acoustic radiation force is another important body force for effective manipulation of microparticles. From Eq. (3) in Table 1, the acoustic radiation force on micron sized particles is inversely proportional to the sound wavelength. Therefore ultrasonic standing waves have been widely used for non-intrusive manipulation of suspended microparticles for bioanalysis (Wiklund et al 2006; Laurell et al, 2007). Most of the early investigators used bulky piezoelectric transducers (PZT) attached or bonded with the large a chamber holding suspended particles. However these devices usually suffer from inefficient energy coupling between the PZT and the chamber. Because the chamber size is large, much acoustic energy is lost before it reaches the center of the chamber due to attenuation as waves are propagating in the suspending liquid. Furthermore, some secondary effects in the macroscopic fluid domain, such as acoustic streaming, may occur and will make stable particle manipulation difficult. The effort to integrate acoustic particle manipulation onto a microchip started in the most recent decade. (Laurell et al, 2004; Petersson et al, 2004) fabricated a microfluidic channel with highly straight and vertical side walls by anisotropic etching on a silicon wafer. A flat piezoelectric ceramic was bonded on the bottom side of the silicon substrate. They applied and tuned the acoustic signal to match the resonance of the channel dimension, creating a planar acoustic standing wave between the side walls that has a single pressure node in the channel center (Fig. 2-8a). The suspended particles are subject to acoustic radiation force and pushed toward the nodes (pressure minimum) or the antinodes (pressure maximum) of the standing waves depending on their acoustic contrast with regard to the suspending medium. This method was used successfully to focus blood cells, lipid particles (Petersson et al, 2004), and yeast cells (Jung et al, 2008). Rather than using bulk wave and acoustic coupling through lays of different materials, (Shi et al, 2008) developed a novel particle focusing technique using a microfluidic channel directly bonded with a surface acoustic wave (SAW) device (Fig.2-8b). This device is fabricated by depositing a pair of metal interdigital transducers (IDTs) on a lithium niobate (LiNbO_3) piezoelectric wafer. The microchannel is positioned in the center between two IDTs.

When an AC voltage is supplied to both IDTs, two series of planar acoustic waves are created propagating in opposite directions along the LiNbO₃ substrate. The waves will penetrate the bonding interface between the channel wall and the substrate causing leaking waves in the fluid medium. The overall effect is a standing wave and corresponding pressure nodes/antinodes are generated in the channel. By matching the channel width with half wavelength, particle suspension can be focused into a thin stream in the center of the channel. Compared with the bulk wave devices, the SAW devices can be more easily integrated on a chip using standard photolithography. The flexible design of IDTs can offer strong ability to localize the acoustic energy in a specific region for single particle manipulation. In addition, the SAW vibration is limited to tens of nanometers in depth on the substrate, and hence is much more energy efficient than the lossy bulk wave coupling through layers of different materials.

Figure 2-7. Acoustic focusing of particles in microfluidic channel using (a) bulk acoustic waves (Laurell et al, 2004; Petersson et al, 2004); and (b) surface acoustic waves (Shi et al, 2008). Reproduced with permission from Ref. (Laurell et al, 2004; Petersson et al, 2004; Shi et al, 2008). Copyright (2004, 2008), the Royal Society of Chemistry.

There is another advantage of acoustic particle focusing worth to note. In addition to the primary focusing effect, the acoustic force can induce a secondary orientation effects on non-spherical particles, such as oblate spheroidal, prolate spheroidal, disk-shaped, and needle-shaped particles. Upon excitation of acoustic wave, all the particles will rotate and align themselves so that their major axis is orthogonal to the direction of wave propagation ([Fig.2-9](#)). This unique feature can be very beneficial for analysis of biological particles with irregular shapes, such as erythrocytes, some strains of bacteria and algae cells. The preferential orientation of the biological particles causes a substantial planar region facing the detector and hence can significantly improve the optical signal collection and measurement ([Kaduchak et al, 2009](#); [Auer et al, 2010](#)).

Figure 2-8 Acoustic alignment of carbon rods suspended in water. Particle sizes are 8 μm in width and have variable lengths. (a) when acoustic field is off, particles has random orientation; (b) when acoustic field is on, particles are focused in the center and aligned with major axis orthogonal to the wave propagation.

Before closing this section, we should note that there are pros and cons of using body forces for sheathless particle focusing. Although they can relieve us from heavy dependence of sheath fluid, people should be aware of some negative consequences of using body force, especially electric force, when handling biological cells. As discussed

earlier, the body force works by acting directly on the cells. Application of external electric field can induce irreversible physiological change in the cells and cause cell death or lysis because of excessive membrane stress, Joule heating, and thermo-electrically generated free radicals (Voldman 2006; Kang et al, 2009). Compared with electric field, high-frequency acoustic field is much more amicable to the biological cells. However cavitation of suspending liquid and acoustic heating under high energy input could also be devastating to the biological cells.

2.2 Detection Method

2.2.1 Electrical Detection Method

Detection part plays the most important role in micro total analytical system (μ -TAS). The conventional detection methods are based mainly on electrical and optical methods. In the electrical detection region, impedance spectroscopy is frequently chosen as the cell analyzer. Impedance microfluidic cytometry is a non-invasive, label-free technology that can rapidly characterize the dielectric properties of particles and cells. The working principle is based on the fact that, when a particle carried by the fluid translocates a micro/nano scale aperture, the electrical impedance of the aperture will significantly increase due to the blockage by the particle, resulting in the significant change of the electrical current (a pulse) across the aperture. Every single pulse corresponds to an event of a single particle; while the higher pulse amplitude implies larger particle size. By characterizing the amplitude of the electrical pulses, statistical information of the size and the numbers of the microparticles can be obtained. As showed in Fig. 2-10, a pair of parallel electrodes are employed to perform a voltage excitation and impedance measurement. One cell translocation leads to one corresponding peak.

Figure 2-9 the schematic of the Coulter Counter.

Flow cytometry has established for decades as a powerful technology for multi-parametric analysis of biological cells both in fundamental research and clinical practice, especially for hematological disorders, such as blood cancer and AIDS (acquired immunodeficiency syndrome). In order to develop miniature flow cytometer for future healthcare, compact lab-on-chip systems have been introduced recently. Compared to the conventional cytometer, on-chip diagnostic devices offer many advantages, such as the capability of analyzing extremely small sample volumes with low density of bioparticles of interest. In the last decade, many different versions of microcytometers based upon electrical impedance detection have been introduced. However most of them suffered from lower sensitivity and screening throughput, which significantly limited their functionality for practical research or clinical applications. The optical detection-based microfluidic chips enable precise sample delivery and, at the same time, maintains the sensitivity, specificity, reliability, and versatility of the well-established optical detection modality.

2.2.2 Optical detection method:

A novel on chip flow cytometry is reported for fluorescence detection, enumeration, and the sizing of microparticles in a PDMS chip with the external fiber excitation. As illustrated in Fig. 2-11, the detection system consists of a pair of external optical fibers and other optical components for particle counting, sizing and fluorescence analysis. By characterizing the back scattered light signal, the total number and the size of particles

can be obtained from the detection window. Different size particles corresponds to different fluorescence scattered light.

Figure 2-10 the schematic of the external light excitation flow cytometry. Reproduced with permission from Ref. (Chen et al, 2009). Copyright (2008), Springer.

In addition, these microfluidic technology also make it possible to integrate sophisticated sample handling and detection components on a single chip, which enables the development of compact and standalone platform for real point-of-care applications. Miniaturization of flow cytometer was first attempted by Kamensky in the mid-1960s. Nowadays, with the advanced development of silicon and polymer waveguide, it allows the easy integration the fiber or wave guide with the microchip. The paper reports that the SU-8 based polymer wave guide is integrated to determine the microdroplet features in microfluidic chip. By monitoring the optofuidic signal scattered at the fluid-PDMS interface, the droplet size and content can be detected. Fig. 2-12 shows the polymer wave guide and the air lens integrated with chip. This make it promising for biochemical and biosensing applications.

Figure 2-11. the schematic of the droplet characterization chip, top view of the structure of the device, microscope image of the device with inserted multimode optical fiber. Reproduced with permission from Ref. (Shen et al, 2012). Copyright (2012), the Royal Society of Chemistry.

Ring resonator sensor plays the significant role in optical detection. The working principle of micro-ring is that the refractive index change can lead to an obvious transmission property difference. For achieving the multiplexing detection, the ring resonator sensor array is reported. As illustrated in Fig. 2-13, the high sensitivity of the slot-waveguide resonators yields a volume detection limit of 5×10^{-6} refractive index units (RIUs) and surface mass density detection limit of 0.9 pg mm^{-2} which parameters are the best for the currently published integrated planar ring resonators.

Figure 2-12. A top view of the layout of optical chip: light is injected at the surface grating coupler (C) and split by the multi-mode interference splitter (B) to the six transducer channels M1-M6 and the two reference channels REF1 and REF2. Insets are an optical micrograph of the splitter (B); electron micrographs of the grating coupler(C); and a slot wave guide ring resonator(A); with an enlargement of the coupling region. Reproduced with permission from Ref. (Carlborg et al, 2010). Copyright (2010), the Royal Society of Chemistry.

On chip cell analysis enable to determine the refractive index of living cell in the real time and provide low cost, small size, easy operation and high accuracy. By integrating the cavity laser, polymer fiber, micro air lens and some microfluidic chip on a glass substrate, the on-chip flow cytometry potentially has a series application in point of care disease diagnosis, biomedical and biochemical analysis. On the other sides, the flow cytometry also has its pitfall of optical detection: limits of detection degrade rapidly with reducing analysis volumes; it does not scale favorably upon miniaturization than electric or electrochemical methods; difficult alignment procedures; fluorescent markers; light source. Currently, the cost of integration of laser source on chip is much higher, however, optical detection shows its indispensable role in the bio-detection.

2.3 Particle Sorting and Manipulation Method

2.3.1 Acoustic manipulation

The particle sorting and trapping is important part of the integrated analysis system. Particles with different property need to be collected. Currently the main particle trapping and sorting method are focusing on surface standing acoustic wave (SSAW) method, Dielectrophoresis method (DEP) and the optofluidic trapping method.

Acoustic method employs the acoustic radiation force to align the particles flowing in the channel. By the modulation of the operating frequency, different size particles are collected at different position. The standing surface acoustic wave (SSAW) is more preferable rather than the bulk acoustic wave (BAW). The previous one is easy to integrate with the chip. Using the SSAW-based method, particles in a continuous are separated based on their volume, density and compressibility through acoustophoresis. As Fig. 2-14 shows, the larger acoustic force exerted on particle of larger volume, reassembling them at the wave pressure node. This separation method is simple and versatile regardless of charge/polarization or optical properties about the particles with high separation efficiency and lower power consumption.

Figure 2-13 the schematic of the separation mechanism showing particles beginning to translate from the sidewall to the center of the channel due to axial acoustic force exerting on the particles when they enter the working region of the SSAW. The different acoustic force cause differing displacements, repositioning larger particles closer to the

channel center and smaller particles farther from the center. The optical image of the device used in the test. Reproduced with permission from Ref. (Shi et al, 2009). Copyright (2009), the Royal Society of Chemistry

2.3.2 DEP based particle sorting

Dielectrophoresis refers to the motion of a polarized cell in a dielectric medium under an applied nonuniform electric field. By the approximation of dipole moment method, the time-average DEP force exerting on a spherical homogeneous cell of radius a in dielectric medium with permittivity ϵ_m is (Nuttawut et al, 2011):

$$\langle \vec{F}_{DEP} \rangle = 2\pi\epsilon_m a^3 \text{Re}[K(\omega)] \nabla |\vec{E}_{rms}|^2 \quad (2.1)$$

The paper presents a PDMS based microfluidic device using the AC dielectrophoresis to separate the live cells from interfering particles of similar size by their polarizabilities.

Figure 2-14 (a) the PDMS-based microfluidic device with sidewall AgPDMS electrodes. (b) Schematic of the continuous separation process for a mixture of two different particles/Cells of similar size, achieved by hydrodynamic focusing of samples from

branch A toward the electrodes and DEP force induced by the AgPDMS electrodes. Reproduced with permission from Ref. (Nuttawut et al, 2011). Copyright (2011), the American Chemical Society.

2.3.3 Optofluidic sorting

Optical manipulation of particles is the procedure to control the particle motion using optically induced electromagnetic field which is generated by the radiation of the light from optical source operating different light spectrum. The force exerting on particles depends primarily on their size. Particles with size smaller than the wavelength of light are propelled toward to the region with higher electromagnetic field distribution. The paper presented a system sorting a broad range of different biological specimens by an automated optimization of the optical tweezer path and velocity profile with manipulation of small mitochondrial, yeast cell, rod-shaped bacteria and large protoplasts. The system achieves a high separation efficiency and the complex classification can be operated by encoding the algorithms to control.

Figure 2-15 (a) cells within the microchannel can be localized and classified with bright field and fluorescence illumination through a high NA objective. Once a cell is classified, it is moved by optical tweezer to a streamline of laminar flow which leads to the outlet for sorted cells. (b,c) photographs of microfluidic chamber inserted into its chip holder and connected to tubing. (d) Fluid flow diagram with 3 inlets and 2 outlets. Reproduced with permission from Ref. (Landenberger et al, 2012). Copyright (2012), the Royal Society of Chemistry

Chapter 3

Microparticle Focusing By Standing Surface Acoustic Wave

On-chip flow cytometry provides a powerful tool to characterize the cell samples for point of care diagnosis. The focusing of sample at the center of microchannel is crucial to ensure the accuracy of the system. In this [chapter](#), we present a study of standing surface acoustic wave (SSAW) induced acoustophoresis to assemble the particle samples in a microchannel. The SSAW generated by two parallel interdigital transducers (IDTs) induce the acoustic radiation force to propel particles toward the pressure node which is tunable. Therefore, the particles can assemble at the desired pressure node. The theoretical analysis gives the approximated time consuming for particle assembling and the experiment result validates the theoretical value. Due to the advantages of easy-fabrication, lower energy consuming, and easy integration, this method can significantly benefit the development of future on-chip flow cytometry.

3.1 Introduction

Detection and characterization of biological particles, such as cells and biomolecules, is a fundamental technique in biology and medical biotechnology. Since the bio-particles usually exist in natural fluidic environment, microfluidics-based lab-on-a-chip devices provide excellent platforms for various biomedical manipulations and assays. The amazingly fast development of lab-on-a-chip technology in recent decades has had profound impact on the food and healthcare industries ([Toner and Irimia, 2005](#); [El-Ali et al, 2006](#); [Chin et al, 2007](#)) and bio-defense against bioterrorism and bio-warfare ([Lim et al, 2005](#)). Previous techniques are mainly focused on colloidal particle manipulation using biochemical ([Liu et al, 2004](#)), electrokinetic ([Guo et al, 2010](#)), optical ([Wang et al, 2011](#)), and magnetic ([Loureiro et al, 2011](#)) methods that have been reported in numerous studies.

Most recently, another promising method combining ultrasonic and micro/nano-technologies has shown its potential as a powerful tool for manipulation of colloidal microparticles ([Wiklund et al, 2006](#); [Kuznetsova et al, 2007](#); [Hill et al, 2007](#)). Using various types of ultrasonic transducers, the acoustic energy can be transmitted into colloidal particle suspensions in a confined geometry. The acoustic radiation force due to

the standing wave in the carrier medium drives the particles to the local pressure nodes. This unique phenomenon can be applied for particle concentration, positioning, and fractionation. Compared with other popular techniques, acoustic method is fluorescent or magnetic label-free; it avoids implementation of electric field and hence reduces undesirable electrochemical reaction and joule heating; it does not affect the bioelectricity and causes less stress on the biological cell membrane, and therefore has higher biocompatibility for biomedical applications. A typical and interesting application is to use acoustic focusing of biological cells into a stream of particles for sample preparation and flow cytometer system ([Goddard and Kaduchak, 2005](#)).

Most previous devices that apply acoustic manipulation to microparticles create bulk standing waves through piezoelectric transducers. Recently surface acoustic wave (SAW) devices have become more popular because of their design flexibility, ability for further downscaling and on-chip integration by interdigitated transducers (IDT) ([Yeo and Friend, 2009](#)). Subtle positioning in 1-D or 2-D arrays with finer resolution down to the size of a single cell can be achieved by controlling the excitation frequency and layout of the IDTs ([Wood et al, 2008](#); [Wood et al, 2009](#); [Shi et al, 2008](#); [Shi et al, 2009](#)). A sinusoidal pressure wave in the suspending medium is generated from the fluid-piezoelectric substrate interaction. One of the most important issues to be addressed when interfacing the SAW substrate and the microfluidic chip, such as those made of soft polymeric materials by rapid prototyping, is how to transmit the acoustic energy in the desired location inside the channel in an efficient way. Therefore, in this chapter, we propose a novel ultrasonic waveguide structure for the purpose of convenient and highly efficient interfacing for a SAW-microfluidic system. As another major objective, the dynamics of the microparticle aligning under acoustic radiation force inside a microchannel is investigated using the proposed device.

Fig.3-1 Fishbone design of hollow PDMS-LiNbO₃ chambers as SAW wave guides.

3.2 Mathematical Analysis

It is known that when a particle is suspended in a carrier liquid on top of a piezoelectric substrate, the particle is subject to pressure fluctuation on the particle surface due to longitudinal leakage waves transmitted from the piezoelectric solid surface to the liquid medium (James and Leslie, 2011). The characteristic pressure fluctuation in a standing acoustic field leads to an acoustic radiation force exerting on the particle, which consists of two major components due to both rigid and compressible mechanical properties of the particle. Assuming the wavelength of the acoustic wave is λ , the wave number is $k = 2\pi / \lambda$, and the particle radius is a , we can introduce a characteristic parameter to indicate the ratio between the wavelength and the particle size, ka . For a small rigid sphere ($ka \ll 1$) that is subject to acoustic standing wave propagating in x direction, Nyborg (Nyborg, 1954) developed a simple theoretical model that represents the rigid component of the acoustic radiation force.

$$F_{rigid} = V_0 \left[\frac{3\delta - 3}{2\delta + 1} \left(\frac{\partial \bar{T}}{\partial x} \right) - \frac{\partial \bar{U}}{\partial x} \right] \quad (3.1)$$

where V_0 is the volume of particle, $\delta = \rho_p / \rho_f$ is the density ratio between spherical particle and the suspending fluids. $\bar{T}$ is the time-averaged kinetic energy density; and $\bar{U}$ is the time-averaged potential energy density. The transient forms of the kinetic energy and potential energy density are given by

$$T = \frac{1}{2} \rho_f u^2(x, t) ; \quad U = \frac{1}{2} \frac{p^2(x, t)}{\rho_f c_f^2} \quad (3.2)$$

where u is the fluid material particle velocity, p is acoustic pressure. For a planar standing wave, the acoustic pressure and its gradient along x-axis are described as follows:

$$p_A(x, t) = P_A \sin(kx) \cos \omega t \quad (3.3)$$

$$\nabla p_A(x, t) = P_A k \cos(kx) \cos \omega t \quad (3.4)$$

$$u(x, t) = \frac{P_A}{\rho_f c_f} \cos(kx) \cos(\omega t) \quad (3.5)$$

Assuming p_A is the amplitude at location z , for the sake of simplicity, ρ and c represent the fluidic density and the acoustic speed in fluids, while ρ^* and c^* are the density and the sound velocity in the particle material, respectively.

$$\bar{T} = \frac{1}{T_0} \int_{-\frac{1}{2T_0}}^{\frac{1}{2T_0}} T dt = \frac{P_A^2}{4\rho c^2} \frac{(1 + \cos 2kx)}{2} \quad (3.5a)$$

$$\bar{U} = \frac{1}{T_0} \int_{-\frac{1}{2T_0}}^{\frac{1}{2T_0}} U dt = \frac{P_A^2 k}{4\rho c^2} \frac{(1 - \cos 2kx)}{2} \quad (3.5b)$$

$$\frac{\partial \bar{T}}{\partial x} = -\frac{P_A^2 k}{4\rho c^2} \sin 2kx \quad (3.5c)$$

$$\frac{\partial \bar{U}}{\partial x} = \frac{P_A^2 k}{4\rho c^2} \sin 2kx \quad (3.5d)$$

Substituting Eq. (3.5) in Eq. (3.1), the acoustic radiation force on a rigid sphere is obtained as

$$F_{rigid} = -\frac{5\delta - 2}{2\delta + 1} \frac{V_0 P_A k \sin 2kx}{4\rho c^2} \quad (3.6)$$

For a compressible small spherical particles, if $ka \ll 1$, the time-averaged acoustic radiation force exerted on it is obtained by Eller (Eller, 1968):

$$F_{compressible} = -\left\langle V_{p(t)} \cdot \frac{\partial p_A(x, t)}{\partial x} \right\rangle \quad (3.7)$$

where V is the instantaneous particle volume and p_A is the instantaneous acoustic pressure along x-axis. For compressible small sphere, V can be written as:

$$V(t) = V_0(1 - P_A \frac{\sin(kx)}{\rho^*(c^*)^2} \cos \omega t) \quad (3.8)$$

Substituting Eqs. (3.4) and (3.8) into Eq.(3.7), we obtain instantaneous fore

$$F_{inst} = -kP_A \cos(kx) \cos \omega t \cdot V_0 + \frac{kP_A^2 \cdot V_0}{2\rho^*(c^*)^2} \sin(2kx) \cos^2 \omega t \quad (3.9)$$

Integration of Eq. (3.9) over one wave cycle, we finally obtain the time-averaged force due to the compressible component

$$F_{compressible} = \frac{kP_A^2 V_0 \sin(2kx)}{4\rho c^2} \left(\frac{1}{\delta \sigma^2} \right) \quad (3.10)$$

where $\delta = \frac{\rho^*}{\rho}$ and $\sigma^2 = \left(\frac{c^*}{c}\right)^2$.

Considering both the rigid and compressible characteristics by adding the force obtained in Eqs. (3.6) and (3.10), the total acoustic radiation force is derived as:

$$F_{acoustic} = F_{rigid} + F_{compressible} = \frac{V_0 P_A^2 k \sin 2kx}{4\rho c^2} \left[\frac{1}{\delta \sigma^2} - \frac{5\delta - 2}{2\delta + 1} \right] \quad (3.11)$$

The fluid compressibility can be defined as $\beta = 1/(\rho c^2)$, and particle compressibility

$\beta^* = 1/(\rho^* c^{*2})$; assuming that $\phi(\beta, \rho) = \frac{5\rho^* - 2\rho}{2\rho^* + \rho} - \frac{\beta^*}{\beta}$, $\varepsilon = \frac{P_A^2}{4\rho c^2}$, the total acoustic

force can be simply written as

$$F_{acoustic} = k\phi V_0 \varepsilon \sin(2kx) \quad (3.12)$$

The Stokes drag force on a spherical particle suspending in fluid is expressed (James and Leslie, 2011):

$$F_{stokes} = -6\pi a \mu v \quad (3.13)$$

The motion of particle includes two phases: accelerating phase followed by a steady phase. At the beginning when the acoustic field is on, the particle starts to move under the acoustic radiation force. Since the Stokes frictional force is proportional to the particle velocity, the initial frictional force is smaller than the acoustic radiation force when the velocity is still low and hence the particle accelerates. After a short period of time when the particle velocity reaches a threshold value, so that the frictional force is equal to the acoustic radiation force, the particle reaches an equilibrium state and moves at a constant

velocity. According to Newton's second law, the dynamic equation of the particle is showed:

$$F_{acous} - F_{stokes} = m_p \frac{dv}{dt} \quad (3.14a)$$

Combining Eqs. (3.14a) with (3.13) and solving the differential equation (3.14a), we get the velocity of particle with releasing velocity u :

$$\mathbf{u}_p = \left(\mathbf{u} + \frac{\mathbf{F}_{acous}}{6\pi a \mu} \right) \left[1 - e^{-(6\pi a \mu / m_p)t} \right] \quad (3.14b)$$

The characteristic time scale for the particle to reach the steady state is defined as

$$\tau_p = \frac{m_p}{f} \sim O\left(\frac{a^2 \rho_p}{\mu}\right) \quad (3.14c)$$

where $f = 6\pi a \mu$, considering a particle of radius $a = 10 \mu m$ and density $\rho_p = 1000 \text{ kg/m}^3$, the characteristic time is approximately $\tau_p = 10^{-4} s$. Since this accelerating phase is significantly short compared to the variation of the external forces as well as the time scale of the experimental observation system, the accelerating phase was ignored and the particle appears to move at an equilibrium velocity:

$$\mathbf{u}_p = \left(\mathbf{u} + \frac{\mathbf{F}_{app}}{f} \right) \quad (3.14d)$$

Letting acoustic radiation force be equal to stokes drag force, the equilibrium velocity can be expressed as:

$$u = \frac{\lambda(ka)^2}{9\pi\mu} \phi \mathcal{E} \sin 2kx \quad (3.15)$$

It is known that $\frac{dx}{dt} = u$, by integrating with respect to time, the total time consuming can be estimated as

Figure 3-2 • The shematic and working principle of surface acoustic device

$$\frac{1}{t} = \frac{\phi \epsilon}{9\pi\mu} \frac{1}{f} \quad (3.16)$$

By Taylor expansion, the time scale factor $f(ka)$ in Eq. (16) can be approximated as:

$$f(ka) = \frac{\ln[\cot(ka)]}{2\pi(ka)^2} = \frac{1}{2\pi} \left[-\frac{\ln(ka)}{(ka)^2} - \frac{1}{3} - \frac{7}{90}(ka)^2 + O[(ka)^4] \right] \quad (3.17)$$

Ignoring the terms higher than second orders, it is approximated to:

$$f'(ka) = \frac{1}{2\pi} \left[-\frac{\ln(ka)}{(ka)^2} - \frac{1}{3} - \frac{7}{90}(ka)^2 \right] \quad (3.18)$$

The scale factors $f(ka)$ and $f'(ka)$ are plotted in Fig.3-3 for different values of ka , which corresponds to the ratio of particle size to the wavelength.

Figure 3-3. the simulation of $f(ka)$ with respect to ka

As showed in Figure 3-3, there is a significant difference between $f(ka)$ and $f'(ka)$ when the particle size is less than 2.2μm, While for laerger particles more than 2.2μm, the $f'(ka)$ shows a good approximation of the original scale factor $f(ka)$. Theoretically, the time for particle aggregation are linearly dependent on the $f(ka)$.

3.3 Experiment

In order to fabricate the interdigital transducers, a LiNbO₃ substrate was firstly patterned by a thin layer of photoresist with the desired negative IDT structure using photolithography. The patterned substrate was then deposited with double metal layers (Cr/Au). After the lift off process, the metal IDTs were patterned on the substrate. The PDMS microchannel was fabricated by standard soft lithography technique. Finally the PDMS microchannel and the LiNbO₃ substrate were aligned and bonded. The detailed fabrication procedures are illustrated in Fig. 3-4.

Figure 3-4 the fabrication procedures of Surface acoustic device and Microfluidic channel.

3.4 Result and discussion

To experimentally investigate the effect of particle size with respect to the time of aggregation, several mono-dispersed fluorescent and non-fluorescent polystyrene particles of different diameters were used in the experiment. The particles size are 0.5 μm , 1 μm , 2.2 μm , 3 μm , 3.5 μm , 4.8 μm , 5.7 μm , respectively. The flow was driven by a syringe pump. The AC voltage at 20 MHz frequency was applied to the two IDTs to induce the identical surface acoustic wave with opposite propagating directions. Fig.3-5 demonstrates the dynamics of particle focusing using 0.5 μm and 2.2 μm particles, respectively. In Fig. 3-5, There are two major reasons for this slightly non-homogeneous alignment of 0.5 μm particles. Firstly, the elliptic IDTs focus the acoustic power in a particular region inside the microchannel, where the maximum focusing occurs at the symmetric center of the elliptic IDTs. This implies that the focusing effect will decay gradually when particles are located away from the acoustic power maxima. Secondly, the 0.5 μm particles are subject to strong brownian motion in the fluid suspension compared to 2.2 μm particles and tend to disperse from the acoustic pressure nodal lines, which causes the fact that 0.5 μm particles are more difficult to focus. Combining these two effects, the 0.5 μm particles are better aligned near the acoustic power maxima, while

they become more dispersed when located far from the power maxima. Actually for larger particles of $2.2\ \mu\text{m}$, this non-homogeneous focusing also occurs, however the region of optimum alignment spans much wider compared to the case of $0.5\ \mu\text{m}$ particles because larger particles are much easier to focus (under the same acoustic power). Although the data were not shown, we did observe this phenomenon in the experiment. This is why $2.2\ \mu\text{m}$ appear to be more uniformly aligned in the same field of view.

Dynamics of particle aggregation

Figure 3-5. the two sizes particle's dynamics in a microfluidic channel under acoustic field. Left image shows the dynamics of diameter $0.5\ \mu\text{m}$ particle, the aggregation time $t = 5.6\ \text{s}$. Right image shows the dynamics of diameter $2.2\ \mu\text{m}$ particle, the aggregation time $t = 2.8\ \text{s}$.

When plotting the result as a function of the parameters, each value is the statistical average of these individual measured result, and the error bars show the related standard deviations. The experimental result of the time of aggregation for particle is displayed in figure 3-6 including fluorescent particles and non-fluorescent particles. The result shows

the time aggregation versus ka shows a similar curve as the parameter versus ka . To experimentally validate the theoretical analysis with reduced error, we take the reciprocal of both measured data and the parameter $f(ka)$ and do the fitting by linear regression. The result shows in Fig. 3-7a, which experimentally validate the linear relationship between $f(ka)$ and time for particles aggregation, which in accordance to the derived equation (3-16). The particle aggregation under different acoustic power was also studied for 3 μm fluorescent particles and 3.5 μm non-fluorescent particles. The experimental results in Fig. 3-7b show that the aggregation time is inversely proportional to the power input, as predicted by (20). This result implies that relatively higher power input is desirable for rapid particle aggregation. However, in reality, one should also consider other side effects under extremely high acoustic power, such as acoustic streaming, heating, and bubbling due to the liquid evaporation. Fortunately, it is found that the microfluidic flow in this SSAW device is free of acoustic streaming or heating under moderate power input in the range of a few mW.

Figure 3-6. (a) The reciprocal of the aggregation time ($1/t$) as a function of the particle aspect ratio ka . (b) The reciprocal of the aggregation time as a function of power input. The dashed and solid lines in (b) represent the linear regression fit of the data

To further study the tunable input power's effect on the time for particles aggregation (3 μm fluorescent particle and 3.5 μm non-fluorescent particle), different acoustic input power were tested. Based on the derived equation (16), the time for aggregation is reciprocally decreasing with the acoustic input power enhances. To experimentally validate the theoretical analysis, the measured data were taken reciprocally as a function of power illustrated in Fig. 3-6(b). By doing linearly regression fitting, the reciprocal value of time for aggregations indicates the linear dependence on the acoustic input power.

The SSAW can be characterized by monitoring the displacement of the substrate material along the surface. Fig.3-7a shows the simulated material displacement along the LiNbO₃ substrate at the IDT region. Fig.3-7b (and the supplementary video in the supporting information), on the other hand, shows the measured surface displacement of the oscillation on the substrate beneath the microfluidic channel using a Scanning Laser Doppler Vibrometer (LDV) system. Both the simulated results and the experimental results indicate that the sinusoidal SSAW has a wavelength of $190\pm5\text{ }\mu\text{m}$, which is consistent with the theoretical analysis in the previous section. However, the simulated magnitude of surface displacement (about 0.25 nm in Fig.3-7a) is greater than the measured magnitude ($0.12\pm0.02\text{ nm}$ in Fig.3-7b). We speculate this discrepancy is mainly due to the attenuation when the SSAW propagates from the IDTs to the channel, and also due to the energy dissipation from the substrate to the loading fluid inside the channel.

Figure 3-7. (a) Simulated material displacement along the surface of LiNbO₃ substrate; (b) Transient displacement on the substrate surface beneath the microchannel measured by a Micro-Scanning Laser Doppler Vibrometer (LDV) system. Two grey bands on the substrate surface represent the location of the PDMS microfluidic channel walls.

Particles also can be manipulated and transported to different location by tuning the channel width or acoustic working wavelength. To further the study particles dynamics under different channel length, we set the acoustic working wavelength at $\lambda = 190\mu m$ and change the width of microfluidic channel W varies from half wavelength to two times acoustic wavelength. The dynamics of the four case are listed in Figure 3-8. Due to the channel width broaden, the anti-nodes located in microchanel densify. Particles assemble at these anti-nodes with different location. On the other hand, we can also fix the channel width and tune the operating acoustic wavelength to achieve the same target. That says, the experiment result indirect demonstrate the adaptive manipulation of particles can be achieved by tuning the operating acoustic wavelength.

Fig.3-8 Particles dynamics in microchannel with different width under the acoustic field with operating wavelength 190 μm .

3.5 Summary

In this chapter, surface standing acoustic wave was applied to dynamically manipulate microparticles on chip. The analytical expression of time cost for particle focusing in a microchannel was derived and validated experimentally. The time cost for particle focusing and alignment under surface acoustic waves depends on the acoustic power of the waves and the particle size. Generally the time cost for particle alignment decreases if the acoustic power is higher or particle size is greater. Furthermore, the particles

alignment dynamics in the channel of different widths are investigated in experiment, which indirectly demonstrates the capability of adaptive manipulation of particles by tuning the operating wavelength inside a fixed channel. The SSAW device has many advantages such as, low cost, easy fabrication, high manipulation efficiency and tunable working wavelength/power. Therefore it has great potential to be applied to develop lab on a chip devices for various biomedical applications.

Chapter 4

DC-Based Microfluidic Cytometer

Portable devices have been introduced to provide companion diagnostics in many applications such as personalized healthcare monitoring since several decades ago. Recently the PDMS-based microfluidic chip enables a cost effective platform for point of care diagnostics. In this **chapter**, we present a systematic theoretical and experimental study of a novel fluidic circuit-based microcytometer. The working principle of this device is based on the characterization of the bandwidth and amplitude of the bias-voltage pulses induced by the microparticle's physical blockage of the sensing aperture. In the simulation, the amplitude and bandwidth of the bias voltage change is simply related to the microparticle translocation time and resistance change in the sensing aperture. In the modeling part, we simulate the two parameters (peak and translocation time) by considering $7\mu\text{m}$ and $16\mu\text{m}$, which is used to approximately characterize the Red Blood Cells (RBCs) and Circulating Tumor Cells (CTCs). In the experimental setup, microparticles of different sizes are used to demonstrate the chip performance. Furthermore, RBCs and CTCs are detected and enumerated by the proposed chip. The microcytometry chip is presented and is expected toward the point of care clinical diagnostics.

4.1 Introduction

The resistive pulse sensor based on Coulter principle has emerged as a cost effective platform for biological particles counting and detection in a completely fluidic environment ([Asghar et al, 2012](#); [Sridhar et al, 2008](#); [Wang et al, 2008](#)). This technique has been widely for the investigations of pollen ([Jagtiani et al, 2006](#)), biological cells ([Zheng et al, 2008](#)), bacteria ([Wu et al, 2012](#)), viruses ([Jeffrey et al, 2010](#)), DNA and other biomolecules ([Lorenz et al, 2010](#); [Bayley et al, 2000](#); [Dekker et al, 2007](#)). The working principle is based on the fact that, when a particle carried by the fluid translocates a micro/nano scale aperture, the electrical impedance of the aperture will significantly increase due to the blockage by the particle, resulting in the significant change of the electrical current (a pulse) across the aperture. Every single pulse corresponds to an event of a single particle; while the higher pulse amplitude implies

larger particle size. By characterizing the amplitude and the bandwidth of the electrical pulses, statistical information of the size and the numbers of the microparticles can be obtained. Recently, with the advance of micro/nanofabrication technology, solid-state micropores have been demonstrated with excellent capability for analysis of whole blood cells and cancer cells (Asghar et al, 2012). The microfluidic impedance cytometry enables a highly cost-effective solution for point of care characterization of CTCs (Choi et al, 2013). It is expected that the next generation of impedance microcytometry will be equipped with multiple microfluidic channels that can achieve much higher throughput for screening tumor cells in whole blood sample.

In this chapter, based on the same Coulter principle, a bias-voltmeter was used for microparticles counting and enumeration. The carrying fluid was driven by a syringe pump and two electrodes were inserted in the microchannel to provide the electrical bias across the sensing aperture. When a microparticle blocks the sensing aperture temporarily during translocation, the electrical impedance of the aperture changes significantly, resulting in a modulation of the electric bias on the fluidic branch. By characterizing the dynamic modulation of the bias pulse, the particle size and translocation time can be analyzed. A statistical analysis on the data for large population of biological cells can categorize all the cells into different phenotypes.

4.2 Theory and Method

The structure of the fluidic circuit-based microcytometer in this chapter is composed of three reservoirs. As Fig.4-1a shows, two electrodes are inserted into both ends of a main fluidic channel, respectively. The side branch channel acts as a conducting electrode. The left section of the main channel is 800 μm in width and 6 mm in length; the right section of the main channel is 800 μm in width and 4.7 mm in length. The branch channel has width of 300 μm . The length and width of the sensing aperture are 50 μm and 20 μm , respectively. The entire channel has the same height of 20 μm . The equivalent electric circuit is shown in Fig.4-1b. In the following part, the theoretical analysis and numerical modeling of the pulse amplitude and translocation time are demonstrated.

Fig. 4-1 (a) design of the microfluidic channel; (b) the equivalent electric circuit of the microfluidic channel.

Since the particle size is much smaller than the channel size in R_1 and R_2 regimes, the electrical impedance modulation of the particle on these two channel sections are negligible. However, when the particle translocates through the narrow sensing aperture R , it will induce a significant modulation on the aperture impedance because its comparable size causes partial blockage of the aperture. We assume ΔR is the resistance change due to the temporary existence of the microparticle in the aperture. The electric current through the aperture without and with a particle inside can be written as:

$$I = \frac{V_0}{R_1 + R_2 + R}; \quad I^* = \frac{V_0}{R_1 + R_2 + R + \Delta R} \quad (4.1)$$

where V_0 is the total bias across the main channel. The corresponding electrical voltages at the side branch are

$$V_x = IR_2; \quad V_x^* = I^*R_2 \quad (4.2)$$

The relative change of branch voltage before and after the particle moves in can be derived from Eqs. (4.1-4.2)

$$\frac{\Delta V_x}{V_x} = \frac{V_x - V_x^*}{V_x} = \frac{\Delta R}{R_1 + R_2 + R + \Delta R} \quad (4.3)$$

Because $\Delta R \ll R_1 + R_2 + R_3$, the relation between the side branch voltage modulation and the resistance modulation in Eq. (4.3) can be simplified as

$$\Delta V_x = \frac{V_x}{R_1 + R_2 + R} \Delta R \quad (4.4)$$

The resistance modulation induced by a spherical particle inside a channel also be related to the particle size as derived by DeBlois and Bean (DeBlois et al, 1977):

$$\Delta R \approx \frac{24\rho}{\pi^2 D^4} V_p \quad (4.5)$$

where V_p is the particle volume; ρ is the fluid resistivity; and D is the channel diameter. From the Eqs.(4.4-4.5), the amplitude of the voltage modulation is linearly proportional to the particle volume.

A 3-D model is developed by using Finite Element Method based commercial software COMSOL Multiphysics ® 4.3 (COMSOL, CA, USA). In the simulation, the particle is released at upstream location before it transports into the small aperture. The carrying liquid flow is pressure-driven, which leads to a hydrodynamic force on the particle. The particle was also subject to both electrophoretic and dielectrophoretic forces under the applied electrical potential across the channel.

4.2.1. Numerical Modeling

A. Electrical Field

In the computational domain in Fig.4-1a, since the liquid is neutral electrolyte solution, the electrical field is governed by (Guo et al, 2013):

$$-\nabla \cdot (\epsilon_0 \epsilon_r \nabla \phi) = 0 \quad (4.6)$$

where ϵ_0 and ϵ_r are absolute permittivity and relative permittivity, respectively; ϕ is the electrical potential. The boundary conditions for Eq. (6) are given as

Electrical potentials at the inlet and the outlet:

$$\phi_{in} = 10 \text{ V} ; \quad \phi_{out} = 0 \text{ V} \quad (4.7a)$$

The particle surface and the channel wall are assumed non-conducting and rigid, therefore

$$\vec{n} \cdot \nabla \varphi = 0 \quad (4.7b)$$

where $\vec{n}$ is the normal vector of the corresponding integration surface. Due to the extremely high input impedance of the measurement equipment (on the order of $G\Omega$), the resistance of the branch channel (on the order of $M\Omega$) can be neglected. Therefore, the voltage that can be measured by the sensing electrode can be approximately evaluated by the average voltage at the intersection between the branch and the main channel:

$$\overline{V}_x = \frac{\int_S \varphi dS}{S} \quad (4.8)$$

where S is the area of the intersection.

B. Flow field

The flow field in the microchannel satisfied the Navier-Stokes equation

$$\rho \left[\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right] = -\nabla p + \mu \nabla^2 \vec{u} \quad (4.9)$$

and the continuity equation

$$\nabla \cdot \vec{u} = 0 \quad (4.10)$$

The boundary condition at the channel wall is given by Smoluchowski formula under the assumption that the electrical double layer (EDL) is very thin (nanoscale) compared to the channel size (microscale) (Guo et al, 2013; Wu et al, 2009):

$$\vec{u} = -\frac{\varepsilon_0 \varepsilon_r \zeta_w}{\mu} \vec{E}, \quad \vec{n} \cdot \nabla p = 0 \quad (4.11a)$$

The boundary condition at the particle surface is given by the summation of the particle velocity and the electroosmotic flow velocity (Wu et al, 2009):

$$\vec{u} = \vec{V}_p - \frac{\varepsilon_0 \varepsilon_r \zeta_p}{\mu} \vec{E}, \quad \vec{n} \cdot \nabla p = 0 \quad (4.11b)$$

$$\text{Channel inlet: } \vec{n} \cdot \nabla \vec{u} = 0, \quad v_{inlet} = v_0 \quad (4.11c)$$

$$\text{Channel outlet: } \vec{n} \cdot \nabla \vec{u} = 0, \quad p = 0 \quad (4.11d)$$

where $\vec{u}$ is flow field; v_0 denotes the inlet flow velocity; ρ is the fluid density; μ is the dynamic viscosity; ζ_p and ζ_w are the zeta potentials at particle surface and at the

channel wall, respectively; $\vec{E}$ is the electrical field and $\vec{V}_p$ is translational velocity of the particle.

C. Particle Motion

When an external potential is applied across the channel, the particle starts to migrate under the hydrodynamic motion and electrophoretic motion. The particle is also subject to dielectrophoretic forces due to the non-uniform electrical field in the sensing aperture. The governing equation of particle motion is given by Newton's second law:

$$m_p \frac{d\vec{V}_p}{dt} = \vec{F}_{net} \quad (4.12)$$

where m_p is the mass of the particle; $\vec{F}_{net}$ is the net force acting on the particle. The net force is composed of three main forces: electrophoretic force $\vec{F}_{EP}$ due to net charge on the particle surface, dielectrophoretic force $\vec{F}_{DEP}$ due to the non-uniform distribution of electrical field near/in the aperture, and the hydrodynamic force $\vec{F}_h$ due to the flow drag. Consequently, the total net force is described by

$$\vec{F}_{net} = \vec{F}_{EP} + \vec{F}_{DEP} + \vec{F}_h \quad (4.13)$$

The Dielectrophoretic force is expressed by (Khoshmanesh et al, 2011):

$$\vec{F}_{DEP} = -2\pi\epsilon_f a^3 (\vec{E} \cdot \nabla) \vec{E} \quad (4.14)$$

where ϵ_f is the permittivity of the fluid. a is the particle radius. Theoretically, the flow field can be separated into an inner region and an outer region. Under the assumption of thin EDL as described by Debye length (Kang et al, 2009), the electroosmotic flow exists close to the particle surface where the local net charge is non-zero. In the outer region, the positive ions are neutralized by the negative ions that results in the local zero charge density. Based upon this assumption, the hydrodynamic force $\vec{F}_h$ includes contributions from flows in both regions:

$$\vec{F}_h = \vec{F}_{ho} + \vec{F}_{hin} \quad (4.15)$$

$\vec{F}_{hin}$ is resulted from the electroosmotic flow in inner region (EDL), while $\vec{F}_{ho}$ is due to the flow field in outer region. $\vec{F}_{hin}$ and the electrophoretic force $\vec{F}_{EP}$ can both be expressed by the same Smoluchowski equation (Wu et al, 2009);, however they have the

opposite directions, which implies that these two forces are counterbalanced (Wu et al, 2009):. Finally, the net force acting on the particle is simplified as

$$\vec{F}_{net} = \vec{F}_{DEP} + \vec{F}_{ho} \quad (4.16)$$

where the hydrodynamic force in the outer region is calculated by integrating the fluidic stress tensor over the particle surface (Guo et al, 2013; Wu et al, 2009):

$$\vec{F}_{ho} = -\oint_S \bar{\bar{\sigma}}_p \cdot \vec{n} dS \quad (4.17)$$

where S is the particle surface, σ_p is the stress tensor described as follows (Guo et al, 2013; Wu et al, 2009).

$$\bar{\bar{\sigma}}_p = -p\bar{I} + \mu[\nabla \vec{u} + (\nabla \vec{u})^T] \quad (4.17a)$$

The initial condition of particle motion is set to zero.

$$\vec{V}_p|_{t=0} = 0, \vec{u}|_{t=0} = 0 \quad (4.17b)$$

In order to simulate the pulse amplitude and translocation time, all the governing equations need to be coupled together during computation. Both COMSOL4.3 and MATLAB R2011b (Mathworks, MA, USA) are correlated to carry out the real time numerical computation. The fine mesh is performed for branch and main channel, the extremely fine mesh was applied for the aperture. The AC/DC module is used for obtaining the electrical field distribution in the microfluidic channel by solving the Possion equations, subsequently the instantaneous flow field is computed by solving the Naiver-Stokes Equation by coupling the electro-osmotic flow which can be related by electrical field and zeta potentials of channel wall as mentioned in equation 11. The computation of electrical field and flow field can be run as the Matlab codes which is generated by interface COMSOL with Matlab. Once the instantaneous flow and electrical field are obtained, the parameters can be imported into Matlab through the interface. The Electrokinetic force and Hydrodynamic force acting on the particle is able to be computed by the surface integration in Matlab. Then the Matlab code can computes the parameters like particle displacement, current etc at next time step and update them in COMSOL. The code contains a loop which can keep the continuous monitor of the particle until it goes out of the sensing aperture. The length of time loop is adaptive, which depends on the particle's velocity. If the velocity is much higher, the time loop is

proportional to $\propto \frac{1}{|u|}$ and therefore behaves much shorter so that the electrical current pulse is better simulated. The particle displacement and velocity are recorded by MATLAB and updated to COMSOL that will update the model and calculate the new flow field and electrical field. The loop is set in MATLAB to control the whole progress. The model was run on a computer with 32GB RAM and Intel Core i-7 Quad processor 3.9 GHz. The major parameters in the numerical simulation are listed in Table 4-1.

Table 4-1 Parameters used in the numerical simulation.

Dielectric constant of water	80
Permittivity of vacuum ϵ_0 (C/Vm)	8.854×10^{-12}
Viscosity μ (kg/ms)	0.9×10^{-3}
Density of fluid ρ (kg/m ³)	998
Zeta potential on particle surface ξ_p (mV) [15]	-40
Zeta potential on channel wall (mV) [16]	-50
Aperture width D (μ m)	20
Left channel length (mm)	6
Right channel length (mm)	4.7
Aperture length L (μ m)	50
Channel height H (μ m)	20
Particle diameter (μ m)	7,16

4.2.2. Method

A. Device fabrication

The microfluidic chip was fabricated by soft lithography. Negative photoresist SU-8 (SU-8 25, Microchem, MA, USA) was spin-coated on a glass slide and subsequently soft baked (65°C for 5 minutes, 95 °C for 15 minutes). After UV light exposure for 20 seconds and post-baking (65°C for 1 minute, 95 °C for 5 minutes), the SU-8 master was developed on the glass slides with the thickness of 20 μ m. The PDMS (Sylgard 184, Dow Corning, MI, USA) and curing agent with a volume ratio of 10:1 were then mixed well and cast onto the SU-8 mold. After degassing in a vacuum box, the PDMS was moved into an

oven and cured at 75 °C for 2 hours. Finally, the PDMS replica was peeled off from the mold and bonded with a clean cover slide by plasma treating for 10 seconds. Fig. 4-2 shows an actual chip and its central structure.

Fig. 4-2 (a) an actual chip with a dollar coin; (b) a microscopic image of the central structure: a sensing aperture that is 20 μm in width and 50 μm in length

4.2.3. Cell culture

MCF-7 (American Type Culture Collection, MD, USA) that is derived from breast adenocarcinoma were cultured in Minimum Essential Media (MEM) (Gibco, cat# 11095-080) supplemented with 10% fetal bovine serum (FBS) (Gibco, cat# 10270106), 1 mM sodium pyruvate (Gibco, cat# 11360-070), 0.1 mM MEM non-essential amino acids (Gibco, cat# 11140-050) and grown at 37 °C under a 5% CO₂ in a T75 flask. Low concentration of MCF-7 cells spiked and dispersed in PBS was stored for use. The whole blood was centrifuged under 2000g for 15 min. The RBCs were obtained at the bottom of the centrifuge tube. Low concentration of RBCs spiked in PBS was stored for use. The concentration of the RBCs are CTCs are diluted around 2000 units/mL

Fig. 4-3 (a) an image of MCF-7 cells under a light microscope; (b) a fluorescent microscopic image of MCF-7 with red staining.

4.2.4. Experiment Measurement setup

Fig. 4-4 shows the typical measurement setup. The fabricated chip was placed under a fluorescent microscope. The device has three reservoirs made for the fluidic interface. The samples were injected from inlet reservoir and the waste was removed from the outlet reservoir. Three platinum wires were inserted into three reservoirs, respectively. Once the PBS solution primed all the channels, the side branch reservoir was sealed. A syringe pump was used to perfuse the sample flow through the main channel. The electrical voltage of the side branch channel was measured under a bias of 1 V across the main channel by using a HEKA amplifier EPC 10 USB Quadro (HEKA Elektronik, Lambrecht, Germany) and the data collecting is by setting the sampling parameters in the Patch Clamp software with sampling rate is 1MHz and then were imported into a homemade MATLAB (Mathworks, MA, USA) program for post-processing.

Fig. 4-4 The system setup for the experiment.

4.3 Result and Discussion

As discussed in the theoretical analysis, the change of the branch voltage is linearly proportional to the particle size. Polystyrene particles of various sizes (7 μm , 10 μm and 16 μm) were used to calibrate the device. A good linearity with regression ($R^2 = 0.998$) was achieved by comparing the sensing voltage pulse peak amplitude (%) to the volume of the particles as shown in Fig. 4-5. As this figure indicates, when particle diameter increases from 7 μm to 10 μm , the current change percentage transits from around 0.25% to 0.72%. It means 3 μm difference in diameter induces three times in current change percentage. While particle diameter increases from 10 μm to 16 μm , the current change percentage transits from around 0.72% to 3.42%, it indicates 6 μm increasing in particle diameter leads to the about 5 times in current change percentage. It is demonstrated that a slight change of the particle diameter leads to a significant increase in the peak amplitude.

Fig. 4-5 (a) the peak amplitude changes linearly with the particle volume. The dotted line is a linear regression of the experimental data; (b-d) presents some individual pulse profiles induced by 7 μm, 10 μm and 16 μm particles.

The modulation of the electrical resistance in a microchannel caused by the particle blockage has been investigated earlier by many researchers (Xu et al, 2012; Byun et al, 2013). However, very limited research has been done for systematic simulation and

characterization of both the peak amplitude and translocation time. These two parameters are of critical importance in designing the impedance microcytometry. In this section, we present the simulation results for the change of peak amplitude and translocation time due to the translocation of microparticles, and compare them with experimental data. During simulation, we use 7 μm and 16 μm microparticles to approximate the RBCs and CTCs, respectively. However, for simplicity, we did not consider the effects due to deformation of the biological cells. In this simulation, we focus on both the pulse peak amplitude that corresponds to the change of sensing voltage, and the pulse bandwidth that corresponds to the total translocation time through the sensing aperture. Under the condition that very low external voltage (10 V in simulation and experiment) is applied cross the microfluidic channel, the translocation time of the particle is mainly determined by the flow rate of the carrying liquid. The flow induced by the electrokinetic flow is much smaller, in terms of magnitude, than the pressure-driven flow. Therefore we can use the hydrodynamic fore for a rough estimation of the translocation time by microparticles of different sizes. The hydrodynamic force is proportional to the surface area of a particle, $F_h \propto r^2$; the particle mass is proportional to the volume, $m \propto r^3$; thus the particle acceleration (F_h / m) is proportional to the scale of r^{-1} , which predicts that a smaller particle accelerates faster than a larger particle during the accelerating motion through the sensing aperture. More accurate predictions by the numerical simulation were summarized in Table 4-2 and compared with experimental data. The corresponding comparison of simulated and experiment result is given in Fig. 4-6 for 7 μm and 16 μm , respectively.

Fig. 4-6 The comparison of simulated and experiment result for 7 μ m and 16 μ m, respectively.

It can be shown that the simulated translocation time agrees with the above rough analysis, which predicts that a 7 μ m particle trends to have shorter translocation time than a 16 μ m particle. The same trend was further verified by the experiment data in Table 4-2. Furthermore, the simulation result can provide a method to predict the distribution of cell groups of different phenotypes using a spectroscopy analysis on the correlation between the peak amplitude and the translocation time, as will be discussed in the next section.

In order to investigate the accuracy of the device, the correlation spectroscopy by two parameters, peak amplitude and the translocation time, were analyzed for determination of different cell types. The experimental data for RBCs and CTCs were shown in Fig. 4-7. As discussed earlier, the RBCs induce smaller peak amplitude than the CTCs, because CTCs' larger size lead to a greater blockage of the sensing aperture. Fig.4-7 shows obvious contrast between these two distinct groups of cells in terms of the correlation between peak amplitude and translocation time. It is indicated that this proposed device is promising to provide a low cost and reliable solution for detection and enumeration CTCs from the complex background of the whole blood, which is highly desirable for point of care diagnosis.

Table 4-2 Summary of the simulation results and the experimental data for different sample tests.

Sample types	Simulation		Experimental Result (Average)	
	Peak Amplitude(%)	Translocation Time(μ s)	Peak Amplitude(%)	Translocation Time (μ s)
Polystyrene particle (7 μ m)	0.182	67	0.21 ± 0.03	52 ± 10
RBC	—	—	0.27 ± 0.25	40 ± 62
Polystyrene particle (16 μ m)	3.27	110	3.51 ± 0.5	132 ± 30
MCF-7 Tumor Cell	—	—	3.93 ± 1.97	150 ± 320

Fig. 4-7 The distribution of the peak amplitude and translocation time for two types of cells independently through the sensing aperture: RBCs and CTCs.

4.4 Summary

In this chapter, we present the systematic design of the fluidic circuit-based microcytometry for circulating tumor cells detection and enumeration toward the point of care diagnosis, which can concurrently characterize both peak amplitude and translocation time. By correlated analysis of these two parameters, cells with different size can be classified. It's demonstrated that RBCs and CTCs can be detected and classified by the proposed device. We also introduce the numerical model to provide the critical guidance for the development of such microcytometry. In a word, this device and design theory can provides critical insight and guidance for developing the cost effective platform for rapid point of care detection and enumeration of rare cells.

Chapter 5

AC-Based Microfluidic Cytometer

Microfluidic impedance sensor has been introduced as a cost effective platform in biological cell sensing and counting since several decades ago. Conventional microfluidic impedance sensor usually requires the patterned gold electrodes directly in contact with the carrying buffer to measure the electrical current change due to the blockage of cells. However, patterning metal electrode probes on the silicon or glass substrate is a non-trivial task, which increases the fabrication cost of the impedance sensor. In this **chapter**, we demonstrate an alternating current (AC) impedance based microfluidic cytometer built on a printed circuit board (PCB) coated with polydimethylsiloxane (PDMS) thin film. In addition, circulating tumor cells (*Hela cells*) are used to successfully demonstrate the feasibility of the microfluidic AC impedance sensor in tumor cell detection. The electrodes pre-deposited PCB costs less than US\$2.00 and is widely available in the market. This device has a good potential for point-of-care diagnosis in resource-poor settings.

5.1 Introduction

The microfluidic cytometer could have a great potential to address the current demands for point-of-care diagnosis, such as for circulating tumor cells (CTCs) due to their advantages of compactness and portability, small sampling size, ultrahigh sensitivity, and flexible choice of detection modalities. Direct current (DC) resistance based microfluidic cytometry and micro-Coulter counters have been widely applied for the detection of pollen ([Jagtiani et al. 2006](#)), biological cells ([Wang et al. 2008](#); [Choi et al. 2013](#); [Asghar et al. 2012](#); [Wu et al. 2012](#); [Guo et al. 2014](#)), viruses ([Damhorst et al., 2012](#)), DNA and other biomolecules ([Lorenz et al. 2010](#); [Bayley and Martin, 2000](#); [Dekker, 2007](#)). The working mechanism of the resistance pulse sensor is based on the Coulter principle. When a particle carried by a polar liquid passes a micro/nano-scale pore, the electrical impedance of the micro/nanopore will significantly increase due to the physical blockage induced by the particle, leading to the notable change of the electrical current (a current pulse) across the pore. Similar working mechanism also applies to the AC impedance based sensing. Both resistance or impedance microcytometry is cost effective due to the

significant reduction of manufacturing cost as compared to the commercial optical flow cytometer such as Beckman-Coulter FC500, which has a very high price tag of about US\$100,000.

However, many available microfluidic cytometers that implement electric impedance or resistance detection modality require patterning the metal electrode probes on a silicon or glass substrate, followed by bonding the microchannel with this functionalized substrate. Obviously it is a non-trivial or cheap task to pattern microelectrodes at desired locations on a silicon or glass substrate, which requires tedious and expensive surface processing to achieve strong bonding between the metal probes and the substrate material (Song et al. 2012). Therefore this challenge significantly increases the fabrication cost of the microfluidic cytometer and limits its wide application for point-of-care diagnosis. To address this problem, in this chapter, we propose a microfluidic AC impedance cytometer that is directly built upon a printed circuit board (PCB) coated with a polydimethylsiloxane (PDMS) thin film as an insulator between the electrode and conducting liquid solution. This PDMS thin film protects the metal electrodes from erosion and electrochemical reactions that are always major concerns for DC resistance based sensing, while allowing alternating current to pass and form an electric circuit. The PCB substrates are commercially available and can be easily customized to include the pre-deposited metal electrodes that are ready to use. The microfluidic channel can be integrated on this PDMS film coated PCB directly through a simple and fast procedure, enabling a highly cost effective platform for detection of biological samples.

5.2 Theory and method

Fig.5-1 illustrates the equivalent circuit model of the proposed AC impedance cytometry. The carrying electrolyte solution can be modelled as a resistance. The parasitic capacitance C_e due to the direct coupling between two electrodes is also considered in this model (Emaminejad et al., 2012). The PDMS film between the carrying fluids and the electrodes acts as an insulator and can be modelled as a capacitance C_s . Therefore, the input impedance without the microparticle can be written as

$$Z_{in} = \frac{1}{j\omega C_e} \cdot \frac{j\omega + \frac{2}{RC_s}}{j\omega + \frac{1}{R} \left(\frac{1}{C_e} + \frac{2}{C_s} \right)} \quad (5.1)$$

where R is the microfluidic channel resistance, ω is the angular frequency of the applied AC field. When a microparticle blocks the constricted aperture temporarily, the impedance variation, ΔR , of the microchannel results in a modulation of the alternating electric current, ΔI , which can be expressed as

$$\frac{\Delta I}{\Delta R} \approx \frac{dI}{dR} = \frac{dI}{dZ} \cdot \frac{dZ}{dR} = -\frac{1}{R^2} \cdot \left(\frac{j\omega}{j\omega + \frac{2}{RC_s}} \right)^2 \cdot V_{in} \quad (5.2)$$

$$|\Delta I| \approx \frac{\Delta R}{R^2} \cdot \left[\frac{\omega^2}{\omega^2 + \left(\frac{2}{RC_s} \right)^2} \right] \cdot V_{in} \quad (5.3)$$

Fig. 5-1 (a) Equivalent circuit model of the proposed AC impedance cytometer without microparticle in the aperture. R is the impedance of carrying fluids, C_e is the

parasitic capacitance between two electrodes. C_s is the equivalent capacitance between electrode and carrying fluids due to the PDMS thin film. (b) The impedance modulation due to the temporary blockage by the microparticle.

5.3 Experiment

The PCB with a pair of pre-deposited electrodes (2 mm apart) was obtained from a commercial supplier (HQPCB, Shen Zhen, China) for a unit price less than US\$2.00. PDMS pre-polymer and the curing agent were mixed at a ratio of 10:1, degassed in a vacuum chamber. The uncured PDMS were then spin-coated on the PCB at 1000 rpm for 1 min. Subsequently, the PDMS coated PCB was cured in an oven for 2 hours at 95 °C. The PDMS film was measured as 30 μm in thickness, which is much thinner than the glass cover slide (150 μm) as used in a previous report ([Emaminejad et al., 2012](#)). Since the capacitance C_s is inversely proportional to the PDMS thickness, thinner coating can lead to greater current modulation and hence the sensitivity according to Eq.(5-3). A 3D microfluidic structure, as shown in Fig.5-2 was created by patterning two layers of photoresist (SU-8 25, Microchem, MA, USA) molds ([Chen et al. 2011](#); [Zheng et al. 2012](#)). Glass slides were cleaned in acetone, methanol, and DI-water and baked on a hot plate at 250°C for 30 min. To create the detection aperture, the first photoresist layer (20 μm in thickness) was spin-coated on a cleaned glass slide at 2250 rpm, soft-baked for 3 min at 65 °C and 7 min at 95 °C. Then the coated slide was exposed under the UV light using the first photomask with the pattern of sensing aperture, and baked on a hot plate for 1 min at 65 °C and 2 min at 95 °C. To create the main channel for cell loading and draining, a second photoresist layer (SU-8 25, 40 μm in thickness) was spin-coated on top of the first layer (as prepared above) at 1200 rpm, soft-baked for 5 min at 65 °C and 15 min at 95 °C. The second photomask with the pattern of main channel was aligned carefully to expose the second layer of photoresist under UV light. The coated wafer was then post-baked for 1 min at 65 °C and 5 min at 95 °C, followed by developing in the SU-8 developer for 4 min, and baked for 2 hours at 250°C. Finally a 3D positive relief of SU-8 mold was created. PDMS pre-polymer and curing agents were mixed at ratio of 10:1. The mixture was cast on the as-prepared SU-8 mold, degassed in a vacuum chamber, and cured in an oven for 2 hours at 75 °C. PDMS channels were sliced and

peeled off from the SU-8 mold and reservoir holes were punched at appropriate locations. The PDMS channel and the PDMS coated PCB were plasma treated for 10 s before they were bonded to form the final chip. The chip fabrication procedure is illustrated in Fig. 5-2. The main channel has dimension of 300 μm in width and 60 μm in height. The sensing aperture has dimension of 20 μm in width and 20 μm in height.

Fig. 5-2 The fabrication procedure flow and the final chip device.

A syringe pump (PHD 22/2000, Harvard Apparatus, MA, USA) was used to provide continuous sample flow in the device. The two electrodes were connected to a function generator (8657A, Agilent Technologies, CA, USA) and a lock-in amplifier (SR850, Stanford Research Systems, CA, USA). The electric current signal was acquired with a data acquisition card and transferred to a PC for post processing. Polystyrene particles of 15 μm in diameter were used to evaluate the sensitivity of the device under different AC frequencies. The microparticles were stripped from the original buffer through multiple dilution steps, and re-suspended in 1X PBS solution. Bovine serum

albumin (BSA) is added in the solution to prevent the agglomeration of the particles. HeLa cell is a type of circulating tumor cell derived from cervical cancer and is a commonly used cell line in cancer research. We used HeLa cells as a model to demonstrate the capability of the proposed AC impedance microcytometer for detecting real biological cells. HeLa cells (American Type Culture Collection, MD, USA) were cultured in the Dulbecco's Modified Eagle Medium (DMEM) supplement with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, and 0.1 mM DMEM nonessential amino acids. The HeLa cells were grown at 37 °C under 5% CO₂ in a T75 flask. The cells were re-suspended in specific concentrations by dilution with 1X PBS solution.

5.4 Results and discussion

It can be easily inferred from Eq.(5-3) that the electric current modulation and hence the sensitivity of this device increases with the excitation frequency. Due to the limit of available equipment setup, we only tested the frequency range from 200 KHz to 1 MHz using the fabricated chip device. Fig. 5-3a illustrates the electric current modulation induced by the passage of 15 µm polystyrene microparticles through the detection aperture under AC excitations at 200 KHz, 400 KHz, 600 KHz, 800 KHz and 1 MHz, respectively. At 200 KHz, no effective signal of current pulse could be detected from the background noise. In contrast, under other four frequencies higher than 400 KHz, each microparticle can be detected with good signal-to-noise (S/N) ratio. The relationship between the magnitude of current modulations and the excitation frequency is shown in Fig. 5-3b. The S/N ratio can reach 2.61 (8.3 dB), 4.05 (12.1 dB), 4.98 (13.9 dB), and 7.25 (17.2 dB) at 400 KHz, 600 KHz, 800 KHz and 1 MHz, respectively. Particularly, the S/N ratio at 800 KHz reached about twice as high as that (2.25 or 7.0 dB) reported in a previous work using a cover glass as the insulating barrier between the electrode and the conducting liquid ([Emaminejad et al., 2012](#)).

Fig. 5-3 (a) Typical electric current modulation at 200KHz, 400KHz, 600KHz, 800KHz and 1 MHz, induced by 15 μ m polystyrene particles translocating through the detection aperture of 20 μ m in height, 20 μ m in width and 40 μ m in length. (b) The current modulation by statistical analysis of 50 detected pulses under different frequencies.

In order to characterize the performance of the fabricated device for practical application in tumor cell detection and counting, HeLa cells with different concentrations were perfused through the chip and the same aliquots were verified by a commercial hemocytometer in parallel. The HeLa cells were cultured following the protocols as described above, and were washed with PBS buffer. Four groups of cells were re-suspended in 1X PBS with specific concentrations ($0.2 \times 10^3 \text{ mL}^{-1}$, $0.8 \times 10^3 \text{ mL}^{-1}$, $1.6 \times 10^3 \text{ mL}^{-1}$, and $4 \times 10^3 \text{ mL}^{-1}$). Each group was further split into two equal aliquots for the chip device and the hemocytometer, respectively. Fig. 5-4a shows a series of typical pulses of the electric current modulation induced by several HeLa cells under excitation frequency of 1 MHz, which is the highest frequency for the available setup. The data measured using the chip were benchmarked with those obtained from a commercial hemocytometer (UIC-BCC01, UNIWISE, Zhejiang, China) in Fig. 5-4b, showing comparable accuracy between the chip and the commercial cytometer. This study showed that the presented AC impedance microfluidic cytometer built on PDMS coated PCB is capable of providing a cost effective (less than US\$2.00 for materials) solution of rapid cell detection for point-of-care diagnosis in resource-poor developing countries. In the presented device, the thickness of the PDMS film and applied signal's frequency plays critical role in the impedance modulation. Therefore we developed a numerical model by using a commercial Finite Element Method package COMSOL Multiphysics 4.3b to investigate the effects on the impedance modulation. As Fig.5-5a shows, the signal significantly decreases as the PDMS film thickness increases which agrees with the qualitative analysis based upon Eq.(5-3), while the signal increases with the frequency enhanced.

Fig. 5-4 (a) The measured electric current modulation at frequency of 1 MHz induced by Hela Cells. (b) Comparison of Hela cell enumeration using the proposed chip device and a hemocytometer at different concentrations. (c) A contrast image of Hela cells under a light microscope. (d) A fluorescent image of Hela cells stained in green.

Fig. 5-5 (a) The simulated impedance modulation induced by 15 μm in the presented model as a function of the PDMS film thickness under frequency 1MHz; (b) The simulated impedance modulation induced by 15 μm in the presented model as a function of frequency applied in the excitation.

5.5 Conclusion

In summary, we reported a cost effective microfluidic device based on AC impedance sensing on a PCB coated with PDMS thin film. The PCB substrate has pre-deposited metal electrodes and is widely available in the market with unit price less than US\$2.00. As a proof-of-concept study, this brief communication has demonstrated that this device has a good potential in detecting and counting microparticles or biological cells, with comparable accuracy as the commercial hemocytometer, for low cost point-of-care diagnosis, especially in the resource-poor developing countries.

Chapter 6

Optofluidics-based Microfluidic Cytometer

We have developed a planar optofluidic chip for circulating tumor cell apoptosis by exciting dual fluorescence through the integrated fibers. In contrast to conventional cell apoptosis study that requires commercial flow cytometer with a very high price tag and bulky optics, our design was realized via a simple single-layer soft lithography fabrication process. The device performance was tested by characterizing the Hela cell apoptosis induced by hydrogen peroxide. The performance of the proposed optofluidic chip was concurrently confirmed by fluorescent microscopy and benchmarked against a commercial flow cytometer. This study demonstrated the capability of the proposed optofluidic chip for cell apoptosis assay. The major advantages of this device include simple fabrication process, compact optical components, reliable performance, and low manufacturing cost, making it a promising platform for future mass-producible, inexpensive, and disposable on-chip investigation of biological samples.

6.1 Introduction

Optofluidics, combining integrated optics and microfluidics, has emerged as an important platform to characterize biological samples (Psaltis et al, 2006; Schmidt et al, 2011; Chen et al, 2013). Such technological combination enables this innovative platform to take advantage of both microfluidics and integrated optics to achieve unprecedented flexibility and capability that cannot be surpassed by any conventional counterpart, such as dynamic reconfiguration, extremely low sample consumption, ultra-high sensitivity, and portability. In the past few years, a number of innovative optofluidic microchips were presented, such as optofluidic laser (Wu et al, 2013), optofluidic switches (Song et al, 2011), on-chip lens (Seow et al, 2008; Liang et al, 2007; Mao et al, 2007; Mao et al, 2010; Huang et al, 2010), and liquid waveguide (Yang et al, 2012). Lapsley et al. demonstrated an optofluidic Mach-Zehnder interferometer for the analysis of liquid samples (Lapsley et al, 2011). Yang et al. designed an optofluidic waveguide for bending and manipulation of lightwave (Yang et al, 2012). Yang et al. reported an optofluidic slot waveguide for manipulation of λ -DNA molecules, following which several other similar devices were developed for manipulation of other bio-nanoparticles (Yang et al, 2009). For example,

Chen et al. developed an optofluidic photonic crystal waveguide to trap and quantify the concentration of the Wilson disease proteins (Chen et al, 2012). Lin and Kenneth demonstrated the on-chip manipulation and evaluation of concentration of green fluorescent proteins by using the photonic crystal resonator (Lin and Crozier, 2013).

One of the important applications of flow cytometry is investigation of the apoptosis of cancer cells, which is a critical study in pharmaceutical engineering. Apoptosis is a genetically regulated process of cell death that occurs as a normal part of its natural growth and development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer (Chabinyk et al, 2001; Barnden et al, 1998; Darzynkiewicz et al, 1997; Kilburn et al, 2006; Kroemer et al, 1995; Majno et al, 1995). The efficient characterization of the anti-cancer factors helps to foster new anti-cancer drug development.

In this chapter, we introduce a PDMS-based optofluidic chip for the investigation of circulating tumor cell (CTC) apoptosis by dual fluorescence excitation with integrated optical fibers. Compared to the regular single fluorescence optofluidic system, this device is able to detect the dual fluorescence emission, which can concurrently provide much more specific and biologically-relevant information of the cell populations. The proposed chip demonstrates its feasibility to evaluate the sub-populations of the CTCs at three different stages (necrotic, apoptotic, dead) with acceptable accuracy as compared to the commercial optical flow cytometry.

6.2 Method

In normal cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment (Engeland et al, 1998). For example, in leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages (Fadok et al, 1992; Fadok et al, 1993). The human anticoagulant, annexin V, is a 35–36 kDa Ca^{2+} -dependent phospholipid-binding protein that has a high affinity for PS (Andree et al, 1990). Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet (Fadok et al, 1993). The Alexa Fluor[®] 488 annexin V/Dead Cell Apoptosis Kit and propidium iodide (PI) for flow cytometry

provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to Alexa Fluor® 488 to provide the maximum sensitivity. In addition, the kit includes a ready-to-use PI solution for red-fluorescent staining of the nucleic acid. PI is impermeant to live cells and apoptotic cells, but is able to stain dead cells with red fluorescence by binding tightly to the nucleic acids in the nucleus. After staining with Alexa Fluor® 488 annexin V and PI in the provided binding buffer, live cells show little or no fluorescence, apoptotic cells show green fluorescence and dead cells show red and green fluorescence as illustrated in Fig. 6-1a. These populations can be easily distinguished using a flow cytometer with 488 nm excitation.

Fig. 6-1 a. Schematic of four stages of CTCs and their corresponding dual fluorescence output: (a.1) neither red tag nor green tag can label the normal CTC, no fluorescent emission; (a.2) the red tag can stain the nucleus of the necrotic cell, only red fluorescent emission; (a.3) the green tag can bind with the receptor on the membrane of the apoptotic cell, only green fluorescent emission; (a.4) the red tag can stain the nucleus and green tag can bind with the receptor located on the

membrane of dead CTC, both red and green fluorescent emission. **b. (b.1) The schematic view of the proposed optofluidic chip; (b.2) configuration of the optical fibers and the flow channel; (b.3) microscopic image of the optofluidic chip with inserted single-mode (middle) and multimode optical fibers (left and right).** **c. (c.1) Schematic illustration of the experimental setup; (c.2) the fabricated optofluidic chip by soft-lithography.**

The schematic configuration of the compact optofluidic cytometer is shown in Fig. 6-1.b The device consists of a flow component (hydrodynamic double sheath flows) and a detection component (integrated optical fiber detection). Inlet A is to introduce the samples, while B and C are used to convey the sheath flow to keep the sample from inlet A focused at the center of the channel. The height and the width of the channel are 128 μm and 200 μm , respectively. An excitation fiber is used to deliver the 488 nm laser to the detection spot of the optofluidic chip. Two detection fibers are used to measure two fluorescent parameters, including red fluorescent light and green fluorescent light, respectively.

The flow component uses hydrodynamic sheath flows to focus the biological cells along the center of the channel. By optimizing the ratio between the sample flow rate and the sheath flow rate, the biological cells can be focused in a thin stream of 20 μm in width along the axis of the microfluidic channel for accurate detection. A finite element-based numerical simulation was performed to optimize the chip design using COMSOL Multiphysics 4.3b (COMSOL Inc., MA, USA). Details of the simulation can be found in the supplementary information. The flow component employs the hydrodynamic sheath flows to focus the microparticles along the axis of the microfluidic channel. The COMSOL Computational Fluids Dynamics (CFD) module can compute the flow field by solving the Navier-Stokes equation:

$$\rho \left[\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right] = -\nabla p + \mu \nabla^2 \vec{u} \quad (6.1)$$

where ρ is the fluid density, $\vec{u}$ is the fluid velocity, p is the pressure, and μ is the fluid viscosity. The diffusion equation is

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R - \vec{u} \cdot \nabla c \quad (6.2)$$

where c is the concentration, D is the diffusion coefficient of the phosphate buffered saline (PBS) solution, and R is the reaction rate. In the simulation, $D=10^{-10}\text{m}^2/\text{s}$, and $R=0$ since there is no chemical reaction that affect the concentration of the reagents. The focusing width can be evaluated through the concentration profile of the PBS solution.

As shown in Fig. 6-1.c, the detection component includes three optical fibers: one incident and two detection fibers. The incident fiber (Thorlabs S405-XP, single-mode, core diameter 5 μm , cladding diameter 125 μm , and NA=1.2) has smaller numerical aperture (NA) and is able to guide the excitation light of 488 nm to the sample flow stream. The small NA reduces the scattering of the incident laser beam so that it can transmit the light to the detection spot without using on-chip lens. The other two detection fibers (Thorlabs AFS105.125Y, multiple-mode, core diameter 105 μm , cladding diameter 125 μm , and NA=0.22) with relatively larger numerical aperture were used to collect the fluorescent emission. The fibers to collect fluorescent emission were buried with receiving angles of 60° (Fig. 6-1.b.2). The height of the optofluidic chamber was 128 μm so that the optical fibers with outer diameter of 125 μm could fit in appropriately. The optical fibers were cut by the high precision cleaver to implement the 90° surface for reducing the optical coupling loss. Ar laser with a central wavelength of 488 nm was coupled into the optical fiber by using the 5-D adjustment of racks and lifts as a coupling device. The two embedded detection fibers were used to collect the green fluorescent (532 nm) signal and red fluorescent signal (580nm), respectively. Band-pass filters with central wavelength at 532 nm and 580 nm were applied to the corresponding channels. Finally, all the filtered optical signals were processed and displayed on a digital oscilloscope through the photoelectric conversion by photomultipliers (PMTs). All the PMTs were driven by a homemade control circuit. The data were collected by a data acquisition board and were analyzed with a program coded in MATLAB (MathWorks, MA, USA).

The microfluidic chip was fabricated using soft-lithography rapid prototyping. Negative photoresist SU-8 (2050, Microchem, MA, USA) was spin-coated on a silicon wafer under speed of 1500 rpm and soft baked (65°C for 5 min, 95°C for 30 min). After UV exposure for 20 s and post-baking (65°C for 5 min, 95°C for 10 min), the coated wafer was submerged in SU-8 developer for 10-15 min. Finally, the patterned SU-8 mold

was created on the silicon wafer with height of 128 μ m. The PDMS (Sylgard 184, Dow Corning, MI, USA) and curing agent were mixed at ratio of 10:1 and cast on the SU-8 mold. After degassing for 30 min, the PDMS was cured in an oven box for 4 h at 75°C. The solidified PDMS chip was peeled off from the mold. The optical fibers were carefully inserted into the fiber groove manually. Finally the chip with embedded optical fibers was plasma-treated and bonded with a clean glass substrate. The final chip with installed tubing is shown in Fig. 6-1.c.3.

Hela cells (American Type Culture Collection, MD, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 0.1 mM MEM nonessential amino acids. The cells were grown at 37 °C under 5% CO₂ atmosphere in a T75 flask. The Hela cells were divided into three groups (I, II, III) of the same concentration and each group was stained by DAPI. Group I was prepared as a negative control by incubating cells in the absence of H₂O₂. Group II and III were subjected to H₂O₂ with low and high concentration, respectively. The cells were harvested after incubation and washed in cold phosphate-buffered saline (PBS). The 1X annexin-binding buffer was prepared by adding 1 mL 5X annexin-binding buffer (Component C) to 4 mL deionized water. The 100 μ g/mL working solution of PI was prepared by diluting 5 μ L of the 1 mg/mL PI stock solution (Component B) in 45 μ L 1X annexin-binding buffer. The washed cells were centrifuged again and re-suspended in 1X annexin-binding buffer at density of $\sim 1 \times 10^6$ cells/mL. 5 μ L Alexa Fluor[®] 488 annexin V (Component A) and 1 μ L 100 μ g/mL PI working solution (as prepared) were added to each 100 μ L of cell suspension for each assay. The cells were incubated at room temperature for 15 min, followed by adding 400 μ L 1X annexin-binding buffer, mixing gently, and storing the samples on ice. Finally, each sample group was divided into two aliquots, in which one was tested by a commercial flow cytometer and the other was analyzed by the optofluidic chip.

6.3 Result and discussion

The position of the biological particle when it moves through the optical detection spot is a critical factor to ensure the detection accuracy. It is desirable that all the cells are excited at the sweet spot, where the light beams are in focus and hence the scattered and fluorescent signals are maximized. The hydrodynamic focusing of sample flow was

employed by introducing the sheath flow, which requires the flow to be laminar with low Reynolds number. The ratio of flow rates between the sheath streams and sample stream needed to be adjusted accordingly in order to achieve a focused sample stream with desired width at the center of the channel. The numerical simulation was done to identify the range of the flow rates to be applied on this chip. The optimized ratio of the flow rates between sheath flow (in each sheath flow channel) and sample flow was found to be through the inlet A at a flow rate of 30 $\mu\text{L}/\text{min}$ and the PBS solution was injected through the inlet B and C as sheath flow with the flow rate of 150 $\mu\text{L}/\text{min}$. The simulation result is shown in Fig. 6-2, demonstrating the concentration profile of the PBS solution (in red) in sheath buffer (DI water, in blue) along the microfluidic channel. In this case, the width of the sample flow was focused around 20 μm , which is an appropriate size to enclose the Hela cells.

Fig6-2. Numerical simulation of the hydrodynamic focusing, which shows the concentration profile of the PBS in water. PBS is introduced through inlet A at flow rate of 30 $\mu\text{L}/\text{min}$ and the DI water is injected through the inlet B and C as sheath flow at 150 $\mu\text{L}/\text{min}$. The width of the focused sample flow is about 20 μm .

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employed by introducing the sheath flow, which requires the flow to be laminar with low Reynolds number. The ratio of flow rates between the sheath streams and sample stream needed to be adjusted accordingly in order to achieve a focused sample stream with desired width at the center of the channel. The numerical simulation was done to identify the range of the flow rates to be applied on this chip. The optimized ratio of the flow rates between the sample flow and sheath flow (in each channel) was found to be 1:5, i.e., sample input through inlet A at a flow rate of 30 $\mu\text{L}/\text{min}$ and the sheath flow through inlet B and C at flow rate of 150 $\mu\text{L}/\text{min}$. The simulation result is shown in Fig.6-4, demonstrating the concentration profile of the PBS solution (in red) in sheath flow (DI water, in blue) along the microfluidic channel. In this case, the width of the sample flow was focused around 20 μm , which is an appropriate size to enclose the HeLa cells.

Fig.5 (a) Continuous monitoring of the red fluorescent and green fluorescent signals for 10s; (b) detailed view of 7 individual cell events, including 3 dead CTCs, 3 apoptotic CTCs, and 1 necrotic CTC.

In the cell counting experiment, the Hela cell suspension were introduced through inlet A at 30 $\mu\text{L}/\text{min}$ and the 1X PBS solution was injected through the inlet B and C as sheath flow at 150 $\mu\text{L}/\text{min}$. Three sample groups were introduced into the chip respectively. For each measurement, data were recorded for 10s and analyzed by the control program coded in MATLAB. The dual fluorescent signals were simultaneously detected and monitored. Fig.6-5a shows the signals of red and green fluorescent emission collected by the corresponding optical fibers in Group III. A total number of 8,659 CTCs were counted within 10s, with a counting rate of 866 CTCs per second. As discussed earlier, normal CTCs shown neither red nor green fluorescent signal; necrotic CTCs show only red fluorescent signal; apoptotic CTCs show only green fluorescence; and dead cells show both red and green fluorescence. All the cell events, except normal CTCs, with fluorescent emissions have been captured by the optofluidic chip (Fig.6-5b), which demonstrates a very high signal-to-noise ratio.

Fig.6 (a) Fluorescence microscopy images of HeLa cells, from top to the bottom row are Group I (without any treatment), Group II and III (treated with 20 and 50 μM H_2O_2 , respectively), respectively; From left to the right are fluorescence images under different channels: DAPI, Annexin V, PI, overlay of DAPI and PI, and overlay of annexin V and PI, respectively. (b) subpopulations (Necrotic CTCs, Apoptotic CTCs, Dead CTCs) percentage comparison of Group I, Group II and Group III by optofluidic chip and a commercial flow cytometer (Beckman-Coulter FC500).

In order to induce apoptosis, two aliquots of cell samples were treated with H_2O_2 at two different concentrations (group II: 20 μM ; group III: 50 μM) for 8h, followed by staining with the apoptosis/death cell kit. DAPI was used to stain the nuclei of all the cells in order to show the whole cell populations, including the live CTCs in the control group without H_2O_2 treatment (group I). In Fig.6-6a, the image panels from left to right demonstrate the fluorescent emissions due to staining by DAPI, Annexin V, PI, combination of DAPI and PI, and combination of annexin V and PI, respectively. For the

control group I, extremely low number of red fluorescent signal (FL 1) and green fluorescent signal (FL 2) were detected, indicating minimal cell death in the control. For groups II and III with H₂O₂ induced apoptosis at different levels, the subpopulations of the necrotic, apoptotic, and dead CTCs can be accurately quantified by counting the corresponding fluorescent signal spikes (as in Fig.6-5). From the statistical results in Fig.6-6b, it is obvious that application of H₂O₂ was able to induce both apoptosis and necrosis of the CTCs significantly, especially under higher dosages of H₂O₂. The subpopulations of necrotic, apoptotic, and dead CTCs measured by the optofluidic chip agreed very well with the data measured by the commercial flow cytometer, indicating the comparable performance of the proposed device for apoptosis assay of the circulating tumor cells.

6.4 Summary

We have shown a proof-of-concept study using a planar optofluidic chip for apoptosis assay of the circulating tumor cells by exciting dual fluorescence through the integrated fibers. Compared to the conventional cell apoptosis study that requires bulky and expensive commercial flow cytometer, our device was developed via a simple soft lithography fabrication process. The device was tested by characterizing the Hela cell apoptosis induced by hydrogen peroxide. The performance of the proposed optofluidic chip was verified by fluorescent microscopy and benchmarked with a commercial flow cytometer. The comparison demonstrated the capability of the proposed optofluidic chip for cell apoptosis assay, which could be useful for a lot of biomedical applications such as the cytotoxicity investigation of the anti-cancer drugs in pharmaceutical industry. The advantages of our device include simple fabrication process, compact optical components, reliable performance, and low manufacturing cost, making it a promising platform for future on chip investigation of biological samples.

Chapter 7 Microfluidic Imaging Cytometer

7.1 Introduction

Microfluidic chip-based Coulter Counter has been introduced as a low-cost and portable platform for the biological particles or cells such as human blood cells ([Asghar et al, 2012](#); [Wu et al, 2013](#); [Chen et al, 2013](#)), and circulating tumor cells ([Guo et al, 2013](#); [Xu et al, 2012](#); [Byun et al, 2013](#)). However, the modulated pulse can provide very limited information of the biological sample as compared to the conventional optical microscopic method, which can provide the straightforward vision of the biological sample pervading many aspects of modern biomedicine and bioscience. However, conventional optical microscope implementation cost is such high and hence limited its applications like point of care diagnosis ([Heng et al, 2006](#); [Xu et al, 2001](#); [Garcia-Sucerqui et al, 2006](#); [Cui et al, 2008](#); [NLindquist et al, 2009](#); [Seo et al, 2009](#); [Schonbrun et al, 2009](#); [Zheng et al, 2010](#); [Schonbrun et al, 2010](#); [Zheng and Yang, 2010](#); [Breslauer et al, 2009](#); [Wu et al, 2010](#)). Nowadays, with the modernization of our society, there is a growing need to develop compact, cost-effective and portable imaging systems for point-of-care diagnosis in personalized healthcare monitor field. For example, on-chip optofluidic microscopy has been introduced as a powerful platform based on the semiconductor foundries and is able to image human blood cells or tumor cells in high resolution ([Zheng et al, 2010](#)). The on-chip optofluidic microscope requires continuously imaging the microfluidic chip, which originates in many images data may burden the signal processing speed of the central controller. This is quite challenging for micro-controller, memory card of the system.

7.2 Working principles and Experiment

In this chapter, we demonstrate a portable resistive pulse activated lens-free cell imaging system. As Fig 7-1 shows, the chip consists of the microfluidic channel and CMOS imaging sensor. The microfluidic channel was fabricated with a constricted aperture at the centre. When biological particle transports through the aperture, the current decreases since the non-conducting particle instead of the conducting electrolytes which leads the resistance significantly increase. A threshold was set to detect such a pulse, when the current returns above the threshold indicating the particle leaves the aperture, a micro-controller will generate a signal to activate the CMOS sensor that is

working in snapshot mode to capture the images of such a particle. Consequently, both the particle modulated pulse profile and images can be concurrently recorded in the system which is very useful for the correlation analysis (peak amplitude & pixel size) of biological cells.

The mold of the PDMS microfluidic channel was fabricated on a glass slide, which were cleaned in acetone, methanol, and de-ionized water, and dried on a hot plate for 30 min at 250°C. SU-8 25 was spin-coated on the glass slide (rpm x2000), soft-baked for 5 min at 65 °C and 15 min at 95 °C, and exposed to UV light irradiation. The glass slide was then baked on a hot plate for 1 min at 65 °C and 5 min at 95°C. After post baking, the mold was developed in SU-8 developer solution for 5 min, then was put on the hotplate and baked for 20mins at 250°C. PDMS pre-polymer and curing agents were mixed at a ratio of 10:1, degassed in a vacuum chamber. The mixture was poured on the SU-8 mold and baked in an oven for 2 hours at 95 °C. PDMS channels were then sliced and peeled off from the SU-8 mold and reservoir holes were punched. The PDMS channel and a clean CMOS image sensor were plasma treated for 10s before they were bonded to form final Lab-on-CMOS chip.

HepG2 cells (American Type Culture Collection, MD, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 0.1 mM MEM nonessential amino acids. The cells were grown at 37 °C under a 5% CO₂ in a T75 flask. Fig.7-3 a shows an image of the Hela cells under an optical microscope and Fig.7-3b presents an image of the Hela cells stained in DAPI under a fluorescent microscope. RBCs were separated from the whole blood using optiprep^{RM} density gradient medium. Whole blood was mixed with optiprep at a ratio of 1:8. Haft ml of Tricine buffered saline (TBS: 0.85% NaCl, 200000 mM Tricine-NaOH, pH 7.4) was layered at the top of the mixture for the protect it from mixing with the blood. The whole blood sample was centrifuged at 2000 g_{av} for 15 minute. RBCs were collected from the bottom of the centrifuge tube, The acquired samples were washed three times with PBS for 10 minute and transferred into PBS solutions.

A power management circuit in the system can provide the voltage bias for the continuously electrokinetic flow in the chip with two electrodes emerged in the inlet and

outlet chamber. The current change in the microfluidic circuit loop is monitored by current module which is implemented by a high-speed precision current-sensor amplifier, low-pass filter and output signal was collected and digitalized by an ADC chip which is controlled by the central controller **FPGA (Altera, USA)**. Once a pulse signal was detected, the FPGA will produce a signal to activate the CMOS image sensor to capture the cell image. CMOS image sensor (Aptina MT9M032, San Jose, CA) measures as small as 11.5mm(W)× 11.5mm(L)×2.3mm(H) with a pixel size of 2.2μm × 2.2μm and a full resolution array size of 1472(H) × 1096(V). The sensor frame rate is 60 frames/second (fps) at 1280(H) × 720(V) resolution. This frame rate can be much further increased when we select region-of-interest (ROI) to readout, which just fits our system as we do not need to image the whole sensor area but only part of the channel area after the constricted aperture at the centre. To ensure enough lensless image contrast, the cover glass of the sensor is removed using a razor blade so that the distance between microfluidic channel and pixel is reduced. The schematic diagram of the proposed system is shown in Fig7-2.a and the practical photo of the system is illustrated in Fig7-2.b.

Polystyrene particles of 6.3 μm and 15.6 μm in diameter were used to evaluate the performance of the proposed system. Microbeads solutions were stripped from their buffer through multiple dilution steps, and suspending in the x1 PBS solution. Bovine serum albumin (BSA) is added in the solution to prevent the microsphere agglomeration. RBCs and CTCs solutions were also collected from their buffer through multiple dilution steps, and suspending in the x1 PBS solution. Bovine serum albumin (BSA) is also added in the solution to prevent the microsphere agglomeration.

Fig. 7-1 the working principles of system; The Imaging sensor stays off-state when no current pulse detected while stays on-state when one current pulse is completely detected.

7.3 Results and discussion

Fig. 7-3 summarizes the analysis result of 6.3 μm , 15.6 μm polystyrene particles and RBCs, CTCs by the pulse-activated lens-free system. First column presents the images of 6.3 μm , 15.6 μm polystyrene particles and RBCs, CTCs under optical microscope, respectively. Second column shows the captured images of these samples. By the pixel size analysis, the diameter of these samples calculated. The corresponding detected pulse peak amplitudes are also recorded in the system. For polystyrene samples, 6.6 μm particles shows the modulated pulse peak amplitude is 3.13 μA and calculated diameter size is 6.6 μm while 15.6 μm particle presents the modulated pulse peak amplitude is 19.57 μA and calculated diameter size is 15.4 μm . For biological samples test, RBC indicates the peak amplitude is 2.56 μA and calculated diameter size is 6.6 μm while CTC shows the peak amplitude is 20.76 μA and calculated diameter size is 22 μm .

Fig. 7-2 a. the schematic view of the proposed system; the FPGA is the central micro-controller to implement the signal detection and processing; the left Analog part realized the continuous monitor of the current pulse modulated by the biological particles or cells; right part shows the CMOS image sensor which is activated by the FPGA after the current pulse detection in the current module; b. the photo of the proposed system consists of FPGA controller, Current Module, and CMOS imaging module. The two electrodes were used to provide continuously electrokinetic flows in the microfluidic chip.

	Microscope images	CMOS images	Diameter pixel size (μm)	Pulse peak amplitude (μA)
6.3 μm (particle)			$3 \times 2.2 = 6.6$	3.13 ± 0.12
15.6 μm (particle)			$7 \times 2.2 = 15.4$	19.57 ± 1.23
RBC			$3 \times 2.2 = 6.6$	2.56 ± 0.79
CTC			$10 \times 2.2 = 22.0$	20.76 ± 3.89

Fig. 7-3 The captured images of 6.3 μm & 15.6 μm polystyrene particles, and RBC & CTC (HepG2) by the proposed system. The diameter calculated from the pixel

size analysis by the CMOS imaging module and pulse peak amplitude recorded by the current module are correspondingly shown.

In order to evaluate the performance of proposed system in differentiating CTCs from the RBCs, the mixed sample by RBCs and CTCs are used to test the chip. Fig 7-4 illustrates the short time records of the current signal cross the microfluidic channel and the corresponding images activated by the current pulse. The pulse of smaller amplitude is judged as the RBC which is concurrently verified by the pixel size. The pulse of higher amplitude is judged as the CTC which shows a significant pixel size. From the correlation analysis (pixel size & current peak amplitude) in Fig 7-4, the CTC is able to be easily differentiated from the RBCs.

Fig. 7-4 the recorded current change across the microfluidic channel by the current module, indicating the passage of 9 biological cells (RBCs mixed with CTCs); each detected pulse activates once imaging of the CMOS imaging module to capture the corresponding images.

7.4 Conclusions

The portable pulse-activated lens-free imaging system provided a correlated analysis (pulse peak amplitude & image) of the biological samples. CTCs are easily distinguished by the proposed system from the mixed sample. It is a very promising platform for point of care detection of rare cells. Future work will focus on the dimension optimization of the system and enhancement of the throughput of biological samples analysis: 1) optimize the system to make sure the reliability 2) add an imaging processing software in the system 3) increase the flow rate and the density of cells to implement the high-throughput for analysing the cells.

Chapter 8 CONCLUSIONS AND FUTURE STUDIES

8.1 Contributions Made by this Study

Since the biological microparticles, such as tumor cells, blood cells, bacteria and macromolecules, exist in fluidic environment, microfluidics-based lab-on-a-chip devices provide the excellent platforms for biomedical assays. The lab-on-a-chip technology develops amazingly rapid and has significant impacts on the healthcare industry in recent years. The current lab-on-a-chip technology can now implement some basic functions, such as loading, focusing, detection and sorting toward even single-cell on-chip analysis, however a lot of research effort remains to be conducted for accurate control and quantitative analysis of the bioparticles. Therefore the overall objective of my dissertation research is to develop the microfluidic cytometry for manipulation and detection of cells and other biological particles. Specifically, the main contribution of my work to the lab-on-a-chip technology is to develop microchip devices for cell detection based upon different mechanisms, such as DC-based cytometry, AC-based cytometry, Optofluidics-based cytometry and image-based cytometry(chapters 3, 4, and 5). DC/AC-based cytometry arises from the Coulter principle, which is when micro-particles or cells translocate through a micro/nano sensing aperture, the aperture is electrically blocked because these non-conducting particles displace the conducting electrolyte solution. This actually results in the significant electrical current change in the aperture. By analyzing the change of electrical pulse amplitude and pulse bandwidth, the information of size and numbers of the micro-particles can be obtained. Optofluidics-based cytometry is based upon the mechanism that when cells transport through a detection region, where an incident light beam is active, optical signals such as forward scattering (FSC), side scattering (SSC), fluorescent light emission (FL) are simultaneously measured to provide important biological information of cells. Forward scattering signal measured at a small angle (0.5° - 20°) is able to provide the information such as cell size and viability. Side scattering signal measured at large angle (15° - 150°) reveals the information such as cellular granularity. Fluorescent signal is measured from the same angle as SSC and can provide the specific biological information which is determined by the type of labeled molecules. Imaging cytometry has been introduced as a powerful platform based on the semiconductor foundries and is able to image human blood cells or tumor cells in high

resolution. It works like a portable CCD to directly image the biological cells or particles.

8.2 Directions for Future Studies

My research will continue to look at how microfluidics can be applied in development of biomedical microdevices. More specifically, in the fundamental perspective, **as a follow-up study, the findings in this study can be useful to further study of the high-throughput manipulation of the micron and submicron sized bioparticles with surface acoustic wave;** in the application perspective, **the findings in this study can be useful to further** develop integrated biochip devices and the corresponding electronic system which can perform multiple bioanalytical functions, such as detection, characterizing, and manipulation, for a variety of biological particles. Based on the results presented in this study, future research will be focused on two aspects as listed in the following:

- 1) In the fundamental perspective, **the findings in this study can be useful to further study of** the high-throughput acoustic manipulation of the micron and submicron sized bioparticles. Conventional manipulation of microparticles by standing surface acoustic wave was achieved by the parallel IDT electrodes. However the design limited the throughput of such device since all the microparticles located at the region under surface acoustic wave were manipulated. I intend to investigate the manipulation of microparticle by the arc IDT electrodes. This design can improve the throughput since the acoustic wave focus at narrow region which enables the manipulation of microparticle at a more constricted region as compared to parallel IDT electrodes.
- 2) In the application perspective, **the findings in this study can be useful to further study of** develop integrated biochip devices which can perform multiple bioanalytical functions, such as separation, detection, and manipulation, for a variety of biological particles. Specifically, **the findings in this study can be useful to further study of** integrate the acoustic focusing, impedance spectroscopy and surface acoustic wave sorting on a single microchip, here we define it as impedance activated cell sorting system. **the findings in this study can be useful to further study of** build the automation system with the aid of electronic control technology.
- 3) Current methods for separation of the bioparticles are mostly based on the phenotype or immunological labeling. However, phenotypic separation is limited to the bioparticles with large size or shape difference. And immunological labeling for bioparticles with

small phenotypic difference is expensive and requires complex optical detection system. I intend to investigate label-free and less expensive methods for characterizing and separation of the bioparticles (ranging from DNA, bacteria, to cells), based on the difference of their membrane or cytoplasmic properties (impedance, dielectric response, images etc). The long-term goal is to develop a portable microdevice with multiple bio-analytical functions for on-site diagnosis, forensic investigation, or bio-defense applications.

In conclusion, I plan to look ahead to issues that will necessarily arise if microfluidics is to be used in development of lab-on-a-chip devices. My particular focus is on the control of the bio-reagent transport and methods for characterizing and manipulation of bioparticles. I plan to advance our understanding of biological transport phenomena by fundamental investigations. The impact of this research will be to broaden the application of the microfluidics by making lab-on-a-chip technology better and more accessible.

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