Single cell refractive index measurement via lab-on-a-chip system

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGLE CELL REFRACTIVE INDEX
MEASUREMENT VIA LAB-ON-A-CHIP SYSTEM

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VIA LAB-ON-A-CHIP SYSTEM**

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SCHOOL OF ELECTRICAL AND ELECTRONIC
ENGINEERING**

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SINGLE CELL REFRACTIVE INDEX MEASUREMENT VIA LAB-ON-A-CHIP SYSTEM

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Chin Lip Ket

SUMMARY

Living cell refractive index can provide physical, biological or chemical insights of cells, such as the current cell state in cell division, the concentration of intracellular constituents etc. Different investigations required different measurement precisions and cell manipulation techniques, which can be solved by Lab-on-a-chip technology. This doctorate thesis focuses on the design, fabrication and experiments of single living cell refractometers using Lab-on-a-chip (LOC) technology. Specifically, three LOC devices have been designed and investigated: the on-chip grating interferometer, the optofluidic microlens Fabry-Pérot refractometer, and the complementary optofluidic Fabry-Pérot resonant system.

In the development of the on-chip grating interferometer based on the fiber Bragg gratings, the various aspects of grating interferometer are studied and modeled. A theoretical study of the frequency response for the variation of the optical path length in the cavity is carried out, and a simplified model based on Fabry-Pérot (FP) resonant cavity is established. The differential measurement scheme is employed for the simultaneous measurement of the cell refractive index and size. Then, a fiber-based optical trapping mechanism is used for single cell trapping in the cavity. Besides, the microfluidic network is designed to facilitate single cell measurement and realize the differential measurement scheme. Hydrodynamic focusing mechanism is designed for single cell alignment, and then multiphase plug generating mechanism for single cell encapsulation. In the cavity, plug mixing mechanism is designed to change the extracellular environment. The on-chip grating interferometer is finally fabricated and packaged with interconnections for external equipment. The measurement of Madin-Darby canine

kidney (MDCK) cell using the fabricated LOC device is presented with a precision of 0.001 refractive index unit (RIU).

Optofluidic microlens and optofluidic grating have been designed and demonstrated, which can be integrated into the on-chip optical systems. The optofluidic microlens is formed by three laminar flow stream of liquids with different refractive indices. Light is refracted at the interfaces between the liquids and the radii of curvature of the interfaces can be tuned by varying the flow rates of the three flow streams. Different microlens profiles with varying focusing powers have been demonstrated by tuning the flow rates of the three flows. The optofluidic grating is realized using multiphase droplet microfluidics. A stream of plugs is formed by two immiscible liquids with refractive index contrast and acted as long-period grating. The grating period, and subsequently the resonant wavelength are tunable by changing the flow rates of the two liquids.

The development of optofluidic microlens FP refractometer begins with the study of the essential criteria for high-finesse resonant cavity. Based on the theoretical study, a novel design of LOC FP refractometer with the integration of a pair of optofluidic microlenses is presented. The optofluidic microlens is simulated to optimize the lens formation process and to match the perfect lens profile. The optofluidic microlenses provide real-time tunability to optimize the finesse of the resonant cavity. Based on the design, the optofluidic FP refractometer is fabricated, packaged and experimented. Compared to the previous LOC device, the fabrication process is relatively simpler which does not require complicated fabrication process such as the Bragg grating writing system. The finesse of the FP resonant cavity is improved by 5 folds to 18.79. The measurement of MDCK cell is presented and a precision of 0.0008 RIU is achieved.

A theoretical study of the intensity variation of the FP resonant cavity with the variation in optical path length at a single wavelength is carried out, which is used to analyze the sensitivity and the measurement range based on different parameters, such as reflectivity, cavity length and sample size. Based on the theoretical analyses, the complementary optofluidic FP resonant system using single-wavelength intensity based measurement scheme is designed, fabricated and experimented. An on-chip pressure driven valve is also integrated to sort out interested cells from the samples. Using this LOC device, a real-time measurement of cell refractive index is achieved without the need of cell trapping mechanism such as those in the previous two devices. The measurement time period is successfully reduced to milliseconds per sample as a high-throughput LOC device.

The successful implementation of the three LOC refractometers has evidenced the effectiveness of employing the LOC technology as biosensors in improving the measurement precision and the integration of diverse functionalities such as cell encapsulation, optical trap, cell sorting etc.

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NOMENCLATURE

LOC	Lab-on-a-chip
FP	Fabry-Pérot
MDCK	Madin-Darby canine kidney
RIU	Refractive index unit
MEMS	Micro-Electro-Mechanical System
DNA	Deoxyribonucleic acid
DEP	Dielectrophoretic
FACS	Fluorescently activated cell sorter
TIRF	Total internal reflection fluorescent
FBG	Fiber Bragg Grating
PDMS	Polydimethylsiloxane
CAD	Computer-aided design
LPG	Long period grating
CaCl₂	Calcium chloride solution
IPA	Isopropanol solution
SLED	Superluminescence light emitting diode
NA	Numerical aperture

CHAPTER 1

INTRODUCTION

1.1 Motivation

This PhD research topic is motivated by the potential of Lab-on-a-chip (LOC) technology in exploring the development of portable, robust and integrated biomedical, chemical, biophysical devices etc. The miniaturization of LOC technology does not only pave the way for technological innovations, but also incubate micro-scale discoveries. Specifically, LOC technology has made integrated chemical-based or optical-based biological detection devices to evolve from large desktop devices into micro-sized ones with low cost, high sensitivity, high reliability and stability.

LOC technology refers to the class of technology with the ability to miniaturize normal laboratory equipment and perform laboratory operations on a micro-scale level in single chips of only millimeters to several square centimeters in size [1]. These chips enable the handling and interaction of low liquid volumes down to picoliters. The LOC technology is emerged from the development of Micro-Electro-Mechanical Systems (MEMS). Based on the well-developed semiconductor microtechnology for realizing integrated semiconductor structures for microelectronic chips, the lithography-based technologies are further advanced to create micrometer sized mechanical structures in silicon wafers, which is known as MEMS. Subsequently in the 1990s, fluid handling devices are developed such as

mixers, valves, pumps and fluidic sensors, which lead to the development of the concepts for integrated fluid treatments for analytical systems. These concepts showed that the simple chip-based sensor functionality could be extended to a complete chemical or biological analytical device by integrating various fluid treatment steps into a single chip, including fluid cleaning and sample separation processes [2].

The basic fabrication technology employed to develop LOC devices is photolithography, in which part of the thin film (photoresist) or the bulk of a substrate (such as silicon wafer) is selectively removed to form the microstructures [3]. Initially, silicon based devices are developed as the fabrication technologies are well-developed. However, the demands for the searching of alternative materials are growing to fit certain criteria in optical characteristics, biocompatibility, chemical compatibility, faster prototyping and lower production costs. Therefore, new chip materials have been used such as glass, polymers (polydimethylsiloxane, polystyrene, polymethyl methacrylate etc) and different fabrication processes have been developed such as glass and metal etching [4], deposition [5], soft lithography [6], chip bonding [7] and fast replication techniques via injection molding and embossing [8 – 9]. Recently, LOC technologies have advanced into nanotechnology to fabricate nano-scaled structures [10].

LOC technology have attracted wide research interests in multi-disciplinary areas, which have great importance in realizing chemical processes such as mixing [11] or biological processes such as cell culturing in a more controllable micro-environment [12]. For example, flash chemistry is a new field in chemical synthesis using LOC technology due to its high controllability [13]. In conventional chemistry, slow reactions are commonly used because fast reactions are difficult to

control and undesired by-products are produced significantly. However, with the innovative LOC technology, fast chemical synthesis processes can be conducted in a highly controlled manner and desired organic compounds with high selectivity can be produced. In stem cell biology, the highly controllability of LOC technology has enabled the highly specificity in differentiation and also the studies of differentiation under different extracellular micro-environment [14]. In drug delivery, LOC technology is employed to facilitate the production of drug encapsulation in various monodispersed nano- to micro-sized polymer droplets [15]. Since LOC technology is promising at its high controllability, it attracts the development of devices for other applications such as cell-based studies, protein and deoxyribonucleic acid (DNA) synthesis and analysis, pathogen or disease diagnostic devices [16 – 20]. For biosample detection and analysis, engineers have contributed much effort in expanding the applications of LOC devices by integrating various manipulations, detections and analytical techniques, specifically optical approaches [21 – 25]. On-chip optical components have been designed and developed such as prisms, lenses, waveguides etc [26 – 30]. Cell sorting and trapping using optical forces have been realized [31]. In addition, optical biosensors have also been presented such as interferometry, scattering measurement, Raman spectroscopy and optical absorbance measurement etc [32 – 35].

In addition to its high controllability, LOC technologies have provided several advantages which lead to the interest of researchers from various fields to develop promising LOC devices. First, low liquid volume is consumed in the devices, which subsequently lower the operational costs, less required sample volumes especially biological samples and less waste [36]. The sample volume can be scaled down from milliliter in laboratory process to nanoliter or even picoliter in

the devices. Second, faster analytical and response times for different chemical or biomedical reactions due to shorter diffusion distances or higher surface to volume ratios as compared to laboratory scaled processes [37]. Third, high-throughput analysis can be performed in the chip device with massive parallelization and integration of different functionalities [38]. For example, thousands of localized chemical reactions can be performed using droplet microfluidics where different combinations and concentrations of chemical solutions can be encapsulated in droplets for chemical reactions or analytical experiments [39]. Fourth, it is a safer platform with a complete closed system and integration of diverse functionalities for chemical and biological studies. Smaller liquid volume and efficient temperature control facilitates the study of chemical reaction which is highly exothermic, such as the transformation of a carbon-hydrogen bond to a carbon fluorine bond [40]. Highly exothermic reactions are vital to have efficient temperature control especially on a large scale. Therefore, LOC platform reduces the risks of performing these chemical reactions. In term of biological studies, several devices have been developed to detect viruses in a closed system even for small sample volumes at low concentrations [41 – 42]. These devices reduce the dependence on laboratory technicians to perform the whole detection procedures in a biosafety cabinet, which will be affected by human errors or risk on virus spreading.

Despite the listed advantages, LOC technology is a novel technology with on-going development. Certain physical and chemical effects which become more dominant on micro-scale are not well understood, such as capillary forces, surface interfacial forces and chemical interactions. This will cause the complexity in designing the device for such applications as compared to conventional laboratory approaches. In addition, certain detection principles require additional techniques

such that the signal-to-noise ratio can be improved to be comparable to the conventional methods. However, these issues have open up opportunities to researchers to investigate and develop more precise and concrete models to fabricate highly reliable and precise detection LOC devices.

Recently, cell biophysicists have employed LOC technology to improve the studies of certain cell biophysical properties. The conventional techniques remain hindrances to the studies due to labor intensive, the lack of controllability and difficulties in incorporation of parameter varying functionalities. For example, chip-based patch clamps are developed to replace conventional patch-clamp experimental setup [43]. High-throughput chip-based patch-clamp array has also been developed and the binding activity of Chinese hamster ovary cells expressing Kv2.1 ion channels is examined [44]. The chip-based patch clamp devices provide rapid intra- and extracellular solution exchange for ion channel recording. Other functionalities such as thin-film platinum heater is integrated to produce rapid temperature change and accurate temperature control to study the effect of temperature change on TRPV1 ion channels transfected human embryonic kidney (HEK293) cells [45]. In the measurement of Young's modulus of single cell, conventional techniques include micropipette aspiration studies, atomic force microscopy, or cell poking [46 – 48]. However, the response of cell to externally applied force is affected by these invasive methods due to deformation. In addition, as the mechanical properties of cells are different at different areas, a large deviation in Young's modulus measurement is found. To overcome these problems, a single chip which integrates a force generating device is designed to measure the Young's modulus of a single living cell in real time using hydrostatic pressure [49].

Other cell biophysical studies include cell mass measurement, cell rheological study, cell adhesion force and cell stress investigation [50 – 53].

Among these cell biophysical properties, cell refractive index study is not widely studied due to the difficulties in setting up the experiments. In 1958, R. Barer has developed immersion refractometry to measure a group of cell refractive index under phase contrast microscopy [54]. The extracellular medium is changed from low refractive index to high refractive index and the brightness of the cell under the microscope will change from dark to bright. At a point when the refractive index of the medium and the cell are matched, the cell will be seen to be invisible in the medium. He has also reported some of the details related to the cell refractive index and its intracellular constituents or extracellular environmental conditions. However, the limitations of the technology at that time include the lack of precise control in the medium changing process, the lack of non-contact cell trapping in which contact-based cell trapping will have noise artifact, and the difficulties to incorporate other biological techniques for more thorough investigation. These limitations can be overcome using LOC technology which provides (1) microfluidic manipulations for cell delivery or medium exchange/mixing possibilities; (2) non-contact cell trapping using fiber-based optical trapping for better stability and accuracy with minimum deviations; (3) single cell measurement with improved accuracy due to low measurement noise; and (4) integration of other functionalities such as cell culture, drug delivery system, virus infection etc to study their effects on cell refractive index.

In summary, LOC technology not only helps to simplify, automate and control the chemical reactions and biological studies, it also facilitates the integration of diverse functionalities to expand the applications of LOC devices.

Overall, the development of LOC devices for biophysical studies has been a goal actively pursued by both academia and industry, and the study of cell refractive index using LOC devices is chosen as the topic of this thesis.

1.2 Objectives

The main objective of this research is to develop integrated LOC system for cell refractive index measurement with high measurement precision and integrated with different cell manipulation techniques to facilitate single cell measurement. These integrated LOC systems can be widely used by biophysicists to investigate the relation of cell refractive index to different cell functions. Three LOC systems to measure cell refractive index using different measurement schemes are developed. These systems are (1) on-chip grating interferometer; (2) optofluidic microlens Fabry-Pérot (FP) refractometer, and (3) complementary optofluidic FP resonant system. The primary target is to develop LOC systems which can provide functionalities of cell manipulation by microfluidic design and cell refractive index measurement in better sensitivity and precision. The research investigations include theoretical analyses, system designs, fabrication process development and experimental studies. The theoretical models and analyses for the three different interferometers guide the designs of the corresponding cell refractometers, including optical designs and detection techniques. Microfluidic designs are integrated into the chip to facilitate cell manipulation such as focusing and trapping and measurement process such as buffer modulation. A soft-lithography process is developed for fabricating the LOC systems in this PhD research. The experimental studies are carried out on the developed LOC systems for characterizations and cell refractive index measurements.

The on-chip grating interferometer consists of a pair of Bragg grating fibers, which is employed for cell trapping by optical force and cell refractive index measurement by monitoring the shift of the resonant peaks. A network of microfluidic channels are designed to provide cell manipulation and buffer

modulation. The soft lithography fabrication process is developed. The experiment results are discussed and compared with the theoretical analyses.

The optofluidic microlens and the optofluidic grating are designed, fabricated and experimented. The optofluidic microlens is formed using three laminar flows in the expansion chamber via the tuning of the flow rates. The optofluidic grating is realized via droplet microfluidics in which a stream of uniform plugs in the microchannel is acted as the grating. The period is tuned by changing the flow rate of the two-phase flow. The two optofluidic components are characterized and the experimental results are discussed.

The optofluidic microlens FP refractometer mainly consists of a microcavity and a pair of liquid microlenses. The microcavity forms part of the microchannel to facilitate cell manipulation. The liquid microlenses are used for efficient light coupling from the optical fiber to the cavity. The design theory of the liquid microlens is studied, and the optimum finesse of the resonant cavity is measured. Then, the measurement of cell refractive index is presented and discussed.

The complementary optofluidic FP resonant system consists of three pairs of optical fibers, in which the first pair of fibers is bare fibers used to measure the intensity attenuation due to absorption/scattering, and the other two pairs are fibers with gold coated facets to form FP resonator A and B. Both FP resonators are employed to measure the intensity variation due to the change in the resonant mode using single-wavelength intensity-based measurement scheme. A pressure driven valve is integrated into the single chip for cell sorting. The experiment results of the characterization of the system and the measurement of single cell are discussed.

1.3 Major contributions

The major contributions of this PhD thesis lie in various aspects of the theoretical analyses, designs, fabrications, and experiments of the three different LOC systems for single cell measurement. The details are listed below:

- 1) A grating interferometer is realized by aligning and separating a pair of Bragg grating fibers with a microchannel. The Bragg grating fibers are acted as a resonant cavity to measure the cell refractive index. The theoretical model is developed and forms the foundation in designing the grating interferometer for cell measurement, which is normally 15 – 20 μm in size. (See Chapter 3)
- 2) Differential measurement scheme is employed to measure the cell refractive index and cell size simultaneously by slightly varying the refractive index of the suspended buffer. The theoretical model is studied and developed. (See Chapter 3)
- 3) A network of microfluidic channels are designed to form a robust platform for single cell manipulation. The design consists of (1) hydrodynamic focusing for cell separation; (2) multiphase flow formation for cell encapsulation; and (3) droplet-based enhanced mixing at the detection area for differential buffer modulation. The theoretical models are developed and all parts are integrated onto a single chip together with the grating interferometer. (See Chapter 3)
- 4) Fabrication process for the LOC system is developed using polydimethylsiloxane as the chip material. SU8 is used as the mold and patterned on a silicon wafer by lithography. Chip holder is designed to

provide a robust platform and interface between the external equipment and the chip. (See Chapter 3)

- 5) An optofluidic microlens is developed together with the design model. Particularly, the shape and expansion ratio of the expansion chamber in which the liquid microlens is formed. (See Chapter 4)
- 6) An optofluidic grating is designed and experimented. Particularly, the tunability of the grating period and the relative optical resonant wavelength are studied. (See Chapter 4)
- 7) An optofluidic microlens FP refractometer integrated with the optofluidic microlens is designed, fabricated, integrated and characterized, with all the chip structures fabricated by the polymer soft-lithography process. The integrated FP resonator has an optimum finesse of 18.79, which is enhanced by 5 fold. (See Chapter 5)
- 8) A complementary optofluidic FP resonant system is designed and fabricated. It integrates two fiber-based FP resonators for cell refractive index measurement and a pressure-driven valve for cell sorting. (See Chapter 5)
- 9) Single-wavelength intensity-based measurement scheme is analyzed theoretically and acted as the guidance for the chip design to measure the cell refractive index using the complementary optofluidic FP resonant system. Concurrently, the measurement of cell size can be performed by the signal modulation measurement scheme. (See Chapter 5)

1.4 Organization of the thesis

The thesis is organized into six chapters. The introduction of the thesis covers the motivation, objective, and major contributions as presented in this chapter. The motivation section explains why and how the PhD research is carried out. The objective states the main focus of this thesis, and the contribution section lists the innovations and important findings in both the theoretical and technological aspects.

In Chapter 2, literatures of LOC technology and cell biophysics study are reviewed. This chapter reviews the significance and implications of cell refractive index and the measurement techniques employed to measure its value. Then, the state-of-art for LOC system, which is the basic technology employed in these thesis to develop the cell refractometer, is discussed. All such important background work provides the guidance and impetus for the research, and also lays the technological foundations on which the work of this thesis is built.

In Chapter 3, the design, fabrication and experiments of on-chip grating interferometer are presented. The optical system focuses primarily on the theoretical study of the grating interferometer, the differential measurement scheme employed for the measurement of the effective refractive index and diameter of single cell, and the optical tweezers used for single cell trapping. The microfluidic system is designed to include the hydrodynamic focusing mechanism for single cell alignment, the multiphase plug generating mechanism for single cell encapsulation and the plug mixing mechanism used to realize the differential method.

In Chapter 4, the design, fabrication and experiments of the optofluidic microlens and optofluidic grating are elaborated. The design of the optofluidic microlens is focused on the structural design to form a microlens with better lens

profile and also the relationship between the flow rates of the liquids and the radii of curvature of the formed microlens. For the optofluidic grating, the droplet microfluidics is studied to guide the design of the grating. The tunability and the optical characteristics of the optofluidic grating are studied and discussed.

In Chapter 5, the design, fabrication and experiments of optofluidic FP refractometers are elaborated. In the optofluidic microlens FP resonator, the liquid microlenses for efficient light coupling from the optical fiber to the cavity are designed and developed to provide real-time tuning for optimum finesse. For the complementary optofluidic FP resonant system, the theoretical models of single-wavelength intensity-based measurement scheme and cell size signal modulation scheme are derived and discussed. The fabrication processes for the optofluidic FP refractometers are illustrated. Finally, the experimental results of the optofluidic refractometers are presented and discussed.

Chapter 6 concludes the major contributions of this thesis, and follows by the recommendations for future work.

CHAPTER 2

LITERATURE SURVEY

This chapter is divided into three parts. The first part presents a review of the cell biophysical studies which consists of the studies of different cell biophysical properties and their significances in the biological community. The review is then focused on the studies of cell refractive index and its applications in cell biology and disease diagnoses, which is the main property focused in this thesis. The second part focuses on the techniques being employed to develop cell refractometer. For each of these techniques, the pros and cons of the cell measurement methods are discussed and compared. The third part presents the state-of-art for Lab-on-a-chip (LOC) system, which is the basic technology employed in these thesis to develop the cell refractometer. All these works lay the foundation for the contributions of this thesis in the development of LOC system for cell biophysical studies, i.e. cell refractive index.

2.1 Cell biophysical studies

Cell is the functional fundamental unit of living organisms or the smallest self-sustaining biological entity. Since cell has been discovered in 1665 by Robert Hooke [55], scientists have done extensive researches on living cell. In cell biology, biologists attempt to study the physiological properties of cells, the structure of cells, its life cycle, its similarities and differences as compared with other cell types, the interactions with the extracellular surrounding or with other cells etc [56 – 60]. The fundamental of cell biology is to understand the structures of cells and how cells work. In biochemistry, chemists attempt to study all the chemical processes that occur in the living organisms such as the chemical functions of different cellular components, namely lipids, nucleic acid, proteins etc [61]. Different chemical processes have been identified such as enzyme-catalyzed reactions [62], cell metabolism [63], protein synthesis, and signal transduction. On the other hand, cell biophysics is also one of the main branches in cell-based research, which employs physical techniques to study cells. Biophysicists have used physical parameters to study cell, such as adhesion force for cell-cell adhesion [64], shear force for cell-substrate adhesion, cortical tension, cell stiffness [65] and viscosity to study the structure of the cell membranes etc. In addition, basic physical quantities have been employed in single cell such as cell size, cell shape and cell mass etc [66 – 68]. These physical quantities can provide insight of the cell, which may uncover serials of important but unknown information. For example, in developmental biology, the shaping of tissues and organs during embryogenesis is a fascinating research area [69]. The study of cell shape changes in development are of central importance. The control of the cell shape relies not only on the intracellular mechanics but also the physical interaction with its environment.

Recently, the advancement in optics and photonics provides biophysicists another means to study cells. Microscopic systems such as electron microscopy or fluorescence microscopy have been employed to study subcellular structures [70]; optical tweezers have been employed for cell manipulation [71]. Lasers are developed for biomedical applications which have low phototoxicity on cells [72]. Similarly, photodetectors and spectrometers are employed to obtain optical information of the cell based on several optical quantities such as scattering, absorption, refractive index etc [73]. All these quantities are associated with the cell shape, intracellular content and size etc. Optical techniques provide the advantage of non-contact measurement methods to avoid influences on the sample cell. In this thesis, the main measurement quantity is cell refractive index, which plays a vital role in biophysics and biomedical fields.

2.1.1 Cell refractive index

Refractive index is an optical parameter used as a measure to quantify how fast light wave travels inside a medium. The larger the value for a certain material, the light is slower as it travels in the material. Refractive index is used as an indicator to identify a particular substance and measure its purity or concentration [74].

In solution, refractive index is used as an indicator to measure the concentration of solute in the solvent [74]. For example, refractive index value has been used to measure the concentration of sugar content in the solution associated with the Brix level. The refractive index of a solution, n , is linearly related to the concentration of the solute, C (in g per 100 ml) as

$$n = n_0 + \alpha C \quad (2.1)$$

where n_0 is the refractive index of the solvent and α is a constant known as the specific refraction increment that depends on the nature of the solute [75].

The interpretation of refractive index value for a non-uniform medium such as living cell is not as simple as uniform medium such as solution. There are different types of biomolecular ingredients in the cell, such as inorganic salts, nucleic acids, proteins, carbohydrates, lipids, and water. However, for most cells, the main intracellular constituents are proteins and cells can be regarded as a capsule filled with protein solution [76]. Based on Eq. 2.1, the refractive index of a solution is depending on the concentration of the solutes and the specific refraction increment. The relationship between the concentration of proteins in cell and the refractive index of the cell can only be formed in term of Eq. 2.1 if different proteins have the same specific refraction increment. The specific refraction

increment, α , for many proteins have been measured because the value of α^2 is required in determining the molecular weight based on light scattering measurement and the value of α is required in calibrating interferometer systems in ultracentrifuges or electrophoresis system. The specific refraction increments of some intracellular constituents are listed in Table 2.1 [54].

Table 2.1 Specific refraction increments of intracellular constituents [54]

Constituents	Specific refraction increment
Human serum albumin	0.00185
Human gamma globulin	0.00188
Human serum globulin	0.00186
Human α_2 globulin	0.00183
Human β_1 globulin	0.00185
Human euglobulin	0.00183
Human pseudoglobulin	0.00181
Lactoglobulin	0.00182
β lactoglobulin	0.00182
Peptone	0.00183
Glycine	0.00179

Based on the table, the mean value of the specific refraction increments of soluble proteins is $0.00185 \pm 2\%$. In addition, nucleic acids, nucleoproteins or the breakdown of protein such as peptones and amino acids have values close to those of proteins. Since the relative amounts of these constituents are much smaller as compared to proteins, the value of the specific refraction increments for cell can be regarded as 0.00185. By substituting this value to Eq. 2.1 and n_o is the refractive index of water or of dilute salt solution depending on the extracellular medium, the

total concentration of organic solids in the cell can be determined when the cell refractive index is known.

From the concentration of the organic solids, the mass density of water (in g per 100 ml) can be determined as

$$\rho_{\text{water}} = 100 - \nu C \quad (2.2)$$

where ν is the specific volume (the volume occupied by 1 g of the solute in the solution) of proteins and has a value of 0.75 [75]. Based on these two parameters, the mass of cell can be determined if the cell volume is known. The cell volume can be determined as

$$V = (4\pi/3)r_c^3 \quad (2.3)$$

where r_c is the cell radius and the cell is assumed to be in spherical shape. The dry mass (mass of organic solids) and water mass (mass of water) in the cell can be determined as

$$M_{\text{dry}} = VC \quad (2.4a)$$

and

$$M_{\text{water}} = V\rho_{\text{water}}. \quad (2.4b)$$

Subsequently, the mass of cell is the summation of the dry mass and water mass of the cell [77].

2.1.2 Applications in cell biology and disease diagnoses

Since cell refractive index is related to the concentration and the mass of the intracellular solids, it is significant as a fundamental biophysical quantity for the investigation of cell state, disease diagnoses and oncology.

In cell biology, the dynamics of cell growth is studied to understand the mechanisms of DNA replication or cell division etc. The measurement of cell refractive index provides a non-invasive technique for long-term measurement of cell growth in culture medium [78]. The cell cycle consists of four major phases. The first phase is the G_1 phase, in which the synthesis of several enzymes occurs. Enzymes are mainly proteins, which act as catalyses in chemical reactions. In this case, the enzymes catalyze the replication of DNA. Subsequently, the second phase is known as S phase in which DNA replication occurs. Two identical sets of chromosomes are produced. The third phase is known as the G_2 phase, in which significant protein synthesis occurs during this phase. Microtubules are produced to support cell division in the fourth phase, which is known as M phase. In M phase, two dramatic events occur, one is the division of nucleus, which is known as karyokinesis, and another is the splitting of cell, which is known as cytokinesis. Fluorescent labeling is the common technique used to study cell division [79]. However, the drawback of fluorescent labeling is the complexity in intracellular labeling and the decay of fluorescent emission. By monitoring the cell refractive index, the changes of solid matters in the cell can be quantified continuously throughout the cell cycle. It can be used as an indicator to study and control the cell state in the division cycle and the effects of extracellular surrounding conditions in a controlled system. It is a non-invasive and real-time measurement technique, which provides long-term cell monitoring.

Living cells are very sensitive to the changes of extracellular environment, especially the osmotic pressure. The normal osmotic pressure of the medium for cell to survive in is ranging from 300 to 350 milliosmoles (the number of moles of a chemical compound that contribute to a solution's osmotic pressure). Any deviation from this range of osmotic pressure will causes the cells to shrink or swell. Cell biologists are interested to obtain the relationship between cell volume and the osmotic pressure; and also the viability of the cells in media of different osmotic pressures. Refractometers are able to play an important role for osmotic reaction analyses [80].

In hematology, the refractometry of red blood cells is an important technique to measure the concentration of hemoglobin [81]. The major constituent of mammalian red blood cell is hemoglobin which account for more than 97% of total solids [82]. Therefore, refractometry is an effective tool in hemoglobinometry which can relate refractive index of an individual red blood cell with its hemoglobin concentration. The distribution curves showing the relative number of cell with certain hemoglobin concentration can be plotted. For iron-deficiency anemia or Thalassemia, the population of the red blood cells can rarely reach the concentration of the normal median. As a result, even mild cases of iron deficiency with hemoglobin as high as 80% can be rapidly detected.

Finally, refractometry can play an important role in oncology [83]. The typical RI of normal cell falls within the range of 1.35 to 1.37. However, certain cancer cells such as HeLa cells are measured to have slightly larger RI of 1.392. This may be an important parameter used to diagnose early development of cancer cell. When cancer cell becomes malignant, the nucleus of the cell will be bigger and

the solid concentration is relatively higher. Therefore, it may result in the higher refractive index of cancer cell.

2.2 Development of cell refractometer

Researchers have developed or designed different optical systems to measure the cell refractive index, such as microscopic systems and optical fiber systems. In microscopic system, the phase shift due to cell or tissue on the transmitted wavefront is measured and the cell refractive index is decoupled using image algorithms. On the other hand, optical fiber systems are used to measure single living cell based on different optical signals, such as microscopic phase measurement, light scattering intensity, localized surface plasmon resonance or resonance condition variation in optical cavity. In this section, all these techniques are reviewed based on their pros and cons as cell refractometers.

2.2.1 Microscopic techniques

Digital holographic microscopy is a technique that demonstrates phase measurements of living cells with interferometric resolutions [84 – 85]. The measured phase shift consists of the information of the cell refractive index and the cell thickness. From the quantitative phase images of living cells, the integral refractive index and the cellular thickness can be measured separately by the decoupling protocol. In this protocol, two holograms of the living cell are obtained by having two buffers with different refractive indices but with the same osmolarity. Digital holographic microscopy provides the advantages of low acquisition time up to 20 μ s and the measurements of a group of cells in a single hologram. However, digital holographic microscopy has only a resolution of 0.01 in refractive index measurement and 1 μ m in size measurement.

Hilbert Phase Microscopy is similar to the digital holographic microscopic technique but using different phase retrieval procedure [86]. The phase distribution associated with the microscope image is encoded using a plane-wave reference field that creates spatial modulation, and a quantitative phase image is then obtained via a spatial Hilbert transform. For cell measurement, living cell is squeezed and confined in the microchannel. To obtain the cell refractive index without the knowledge of cell size or microchannel height, a relative phase measurement is performed on the culture medium ($n = 1.337$) and the living cell. This technique has demonstrated a precision of 0.005 refractive index unit (RIU) and low acquisition time of 3 ms. However, the main drawbacks are the requirement of cell confinement that may affect the cells and the absence of the cell size measurement.

Confocal microscopy is also employed together with other microscopic technique to measure the cell refractive index at higher accuracy [87]. Tissue

sample has been measured using immersion refractometry in which the tissue sample and the immersion liquid are squeezed between the glass slides. The tissue sample has exact thickness as the one of the immersion liquid. The thickness is measured using the confocal microscope with an accuracy of 0.625 μm . The refractive index contrast between the tissue sample and the immersion liquid causes an axial scaling factor and can be determined with the known thickness. From the scaling factor, the tissue refractive index is calculated at a precision of 0.004. Another research group has employed confocal microscopy together with the quantitative phase-amplitude microscopy [88]. The measurement technique is similar to the digital holographic technique. The phase image of the cell is obtained and the tissue refractive index is decoupled with the known tissue thickness. Using confocal microscopy can improve the precision of the tissue refractive index but sample confinement is essential and usually is employed for tissue samples. In addition, confocal microscopy is relatively expensive equipment.

2.2.2 Surface plasmon techniques

In addition to complicated holographic and phase-retrieval techniques, surface plasmon resonance on metallic surface is an alternative technique to measure cell refractive index precisely [89]. Surface plasmons are the surface electromagnetic waves, which propagate in parallel to the metallic-dielectric interface. These plasmonic oscillations are very sensitive to the surrounding of the boundary of the metal and the external medium, which can be used for biosensing. The excitation of surface plasmons for planar metallic surfaces is known as surface plasmon resonance and for nano-sized metallic structures is known as localized surface plasmon resonance.

A virtual near-field probe formed by the optical excitation of localized surface plasmon using radial polarization beams for the measurement of refractive index map of the cell-substrate contacts is demonstrated [90]. The glass cover slip coated with 50 nm of gold is used as the substrate in which cell sample is attached on the gold layer. A total internal reflection fluorescence microscopic system is used to focus the laser beam for spatial localization of the surface plasmon for better resolution. Based on the reflected beam captured on the CCD camera, a set of annular rings are observed. The normalized radius of the ring can be converted into the corresponding surface plasmon resonance angle, and subsequently the local effective refractive index at the point of focus is obtained. Therefore, a 2D refractive index map can be measured by scanning the virtual probe across the sample within the area of interest.

In another work, a nano-optical probe for real-time cell refractive index measurement is developed using a tapered fiber tip coated with 13-nm gold nanoparticles [91]. The evanescent wave at the tip excites the localized surface

Plasmon resonance of gold nanoparticles. When the tip is dipped in solutions with different refractive indices, the measured reflected intensity varied. This is the basic principle for refractive index measurement. The sensitivity of the developed fiber tip reaches 5400% in intensity change per RIU, which leads to a resolution of 3.7×10^{-5} . However, complicated scanning mechanism integrated on the microscope is essential to obtain the complete refractive index map of a single cell because the tip size is 80 nm. In addition, the tip has to be dipped into a reference medium to record the intensity level before measurement on real sample can be done. This complicates the measurement scheme by having a movable probe on an attached sample.

2.2.3 Light scattering techniques

Another optical parameter that can be used to measure refractive index of a sample is light scattering intensity. Light scattering is the deflection of light rays in random directions due to the irregularities in the propagating medium or at the interface between two media. Therefore, the scattering pattern of light at certain wavelength on a particular sample medium as a function of scattering angle provides the structural, morphological or optical information on the sample medium. For the study of living cell which is normally spherical in shape, Mie scattering solution is employed as an analytical solution of Maxwell's equations to determine the scattering pattern of the spherical object [92].

In order to obtain the size and refractive index of a spherical sample, the scattering data has to be inverted empirically by setting up a model and adjusting the parameters to obtain the least-square fit to the scattering data [93]. Another empirical method proposed is the optical strip-map technique [94]. This technique is faster in yielding the size and refractive index value from the scattering data, which is less than 1 ms. This technique has been employed to characterize bacterial spore and *Escherichia coli* with a precision of 0.01 in RIU and 0.6% in size. However, for both methods, the scattering intensities at several scattering angle have to be measured. This has imposed complexity in the measurement system, which requires multiple light sources and detectors.

To avoid the requirement of the scattering data for multiple scattering angles, the two-beam optical trap based on forward scattering intensity measurement is developed to obtain the cell size and refractive index [95]. The counter propagating optical trap measurement system uses a two-beam optical trap to hold a single cell in the microfluidic channel. The cell refractive index and size is

measured through the forward scattered light at different position by tuning the power ratio between the two laser beams. The system has a resolution of 0.013 RIU and 3.3% for size measurement which is relatively low as compared to microscopic techniques.

2.2.4 Optical resonant techniques

Optical resonators are another class of optical devices widely developed and exploited as highly sensitive biosensors for biomolecule or single living cell measurements. Optical resonators can be classified into two basic types, namely standing wave resonator such as Fabry-Pérot (FP) resonator and traveling wave resonator such as ring resonator. FP resonator is an arrangement of reflecting surfaces that forms a standing wave cavity for light waves. The ring resonator consists of a waveguide in a closed loop in which traveling light waves are coupled to the loop and built up in intensity due to constructive interference over multiple round trips. The resonant conditions for the light waves are affected by the refractive index of the medium in the FP cavity or surrounding the ring resonator. This optical property of the optical resonators can be employed for sensing applications.

In sensing applications, optical resonators can be classified into two classes, i.e. surface sensor and volume sensor. Surface sensors are based on the Whispering Gallery resonant mode of ring cavity or spherical cavity etc [96 – 99]. Biomolecules are attached on the surface and interact with the evanescent wave to cause the change in the resonant mode for measurement. The interaction depth is typically less than 1 μm , which is suitable for protein or DNA mass measurements. However, for single cell measurement, which requires longer interaction depth, volume sensors are preferred. Volume sensors use the variation in light propagation path on micro-optical cavities as the sensing element [100 – 101]. Cell is delivered into the microcavity, such as laser cavity or FP cavity etc, and cause the change in the resonant mode due to the difference in the optical path.

A microfluidic system has been built for cell refractive index measurement based on the laser cavity [83]. Single cell is delivered into the analysis region in which a laser beam keeps scanning the cell stream flowing through the external laser cavity. The presence of the cell causes the change in the optical path length of the laser cavity. Based on the theory of semiconductor laser, the variation in the external cavity length causes the frequency shift and the intensity modulation. Therefore, by monitoring these two optical signals, the cell refractive index can be subsequently determined. The developed system is used to measure several kinds of cells with a demonstrated precision of 0.004 RIU.

Another microfluidic system using Fabry-Pérot resonator for cell refractive index measurement has been developed [102]. A single cell is captured physically and fixed in the resonant cavity. Differential buffer method is used to record the spectral shift in response to the presence/absence of the cell with two different buffers. Based on these recorded spectral shift, the cell refractive index and size can be determined. The developed microfluidic system is employed to measure Madin Darby canine kidney cell at a demonstrated precision of 0.003 RIU. The precision of the measurement can be increased by improving the finesse of the resonant cavity.

2.2.5 Summary and comparisons of existing techniques

Table 2.2 Summary and comparisons between the existing optical techniques

	Microscopic system	Surface plasmon	Light scattering	Optical resonator
Refractive index measurement	Cultured cell or tissue sample	Cultured cell or tissue sample	Single cell	Single cell
Size measurement	Yes	No	Yes	Yes
Precision in refractive index	0.004	0.0004	0.01	0.003
Manipulation	Confinement	Attachment	Optical Trap	Microfluidics
Merits	Detailed index profile	Highest precision	LOC integratable	LOC integratable
Drawbacks	Expensive	Long acquisition time	Low precision	Low precision

Different optical techniques have been presented for the measurement of cell refractive index and cell size. A summary of the comparisons between these existing techniques is shown in Table 2.2. Microscopic systems and surface plasmon techniques are commonly used to measure cultured cell or tissue sample, which require the confinement or attachment of cells for higher accuracy in determining the cell refractive index. The detailed index profile of the cells can be measured and plotted using these techniques. In microscopic systems, the cells are confined and the phase shift information is captured and processed to decouple the refractive index information. The measurement may affect the cell properties because cell deformation occurs. In addition, microscopic systems are expensive and not suitable to develop portable systems. In surface plasmon technique, the

refractive index map of cells is scanned with an optical probe based on localized surface plasmon resonance. It is more convenient for localized refractive index measurement. Surface plasmon techniques have demonstrated the highest precision in cell refractive index measurement. However, they do not have cell size measurement and is also limited by the long acquisition time. To avoid the expensive and bulky microscopic system, light scattering techniques and optical resonators are more suitable. In addition, cells are not required to be confined or attached in these techniques. Light scattering techniques and optical resonators are focused in single cell measurement. Unlike other techniques in which the cell/tissue sample can only be used for the refractive index measurement, different functionalities can be performed by integrating the light scattering techniques and optical resonators in the lab-on-a-chip system, such as single cell manipulation, measurement, sorting or further analyses. However, by comparing both techniques, optical resonators generally demonstrate higher precision as compared to the light scattering techniques. In addition, the precision of optical resonators has room for improvement.

The required precision or accuracy of a cell refractometer depends on the investigating subject. For example, a precision of 0.005 is sufficient to differentiate normal red blood cells and the one from anemia patients but a precision smaller than 0.001 is required to monitor the small refractive index change of a single cell such as that induced by antigen. In this thesis, optical resonators are investigated for single cell refractive index and size measurements using lab-on-chip system. Optical resonators are designed to achieve higher finesse and subsequently increase the measurement resolution and precision. Lab-on-chip system is designed to provide better cell manipulation and sorting.

2.3 State-of-the-art of Lab-on-a-chip system

In recent decade, LOC technology has been widely innovated, advanced and multi-functionalized to fulfill the requirements and applications in multi-disciplinary area, such as biology, chemistry, physics, engineering and medical field. There are several distinct advantages of LOC technology which have led to the exploration in the research community. First, volume consumption of buffer solutions, biomaterial samples and chemical solutions is greatly reduced to micro- or nano-scale. This can reduce the costs and the wastes generated per experiment. Small volumes of samples reduce the time for complete chemical synthesis or analysis. In addition, micro-scale liquids present unique characteristics such as surface tension, shear stress, solute concentration and interaction, which allow physicists to investigate the liquid behaviors in this regime. Second, LOC technology facilitates the development of compact devices, which are portable and allow analyses to be carried out at the point of need but not in a centralized laboratory. This criterion has attracted researchers in developing promising point-of-care devices in medical field. Third, highly integrated compact devices with interesting functionalities can be developed which in turn reduces start-up costs and operating costs. Different functionalities can be integrated into a single chip. For example, the complete cell analytical process can be integrated into a microfluidic device, which can perform cell culture, treatment, selection, lysis, separation and analysis. By having semi or full-automation, the risk of operating error can be minimized. Since LOC technology is a wide multi-disciplinary area, in this thesis, the following areas are discussed, i.e. microfluidic design for sample manipulation, multiphase flow, cell on chips and integrated optical devices.

2.3.1 Sample manipulation via microfluidic design

Microfluidic platforms provide different approaches in manipulating buffers or samples to suit a wide range of applications and sample compatibilities. Microchannels are not only served as pipes for fluids, but also form valves, mixers and pumps by structural design [103 – 105]. Different generic components are essential: (1) technique for injecting fluid into the chip, (2) technique for driving and regulating the fluid around the chip and (3) technique for combining and mixing fluids. The common techniques applied for manipulating fluids or samples in fluids include pressure-driven flow, electrokinetics and centrifugal force [106].

For pressure driven flow, the fluids are injected and driven in the microchannel due to pressure gradients and will lead to hydrodynamically stable laminar flow profiles. Different pressure sources are used such as syringe pump, peristaltic pump, micropumps and integrated pumps using pneumatic displacement of membranes or bubble-actuated micronozzle-diffuser pump. Pressure driven laminar flow provides several distinct properties such as predictable and controllable flow profiles and stable interface for co-flowing streams. Two flow streams can be injected into a single microchannel and lead to controllable diffusion mixing at the liquid interface. To enhance the mixing process, structural designs are proposed to create chaotic mixing at different Reynolds numbers such as Zigzag-shaped channel, three-dimensional L-shaped channel, staggered-herringbone grooves etc [107 – 109]. The most commonly used application is hydrodynamic focusing technology for sample manipulation [110]. It consists of three microchannels being connected at a junction and formed a common outlet. The flow width of the central flow stream can be changed by varying the ratio of the three flow rates. Therefore, cells or micro-particles can be focused and aligned in the

central flow stream [111 – 112]. 3D focusing which is commonly used in flow cytometry can be realized by having 3D structures or using microfluidic drifting [113 - 118]. In addition to particle focusing, particle separation can also be realized using pressure driven flow by the pinched flow fractionation [119 – 120]. Particles were separated perpendicular to the flow direction based on their sizes by the parabolic flow profile in the pinched segment of the microchannel. Particle and cell trapping is also feasible by simple structural design such as the meander-shaped main channel or Pachinko-style hydrodynamic trapping array [121 – 122].

Electro-osmotic flow is used to transport liquid along the microchannel with induced electric double layer. When electric potential is applied along the microchannel, the charged liquid molecules are attracted and move towards the opposite charged electrode. The liquid has a planar velocity profile due to viscous coupling. Liquid mixing can also be achieved using two co-flowing streams and by applying an AC voltage [123]. With the applied voltage field, charged molecules or particles are attracted or repelled by the electrodes, which is known as electrophoresis. The separation of charged molecules or particles is enabled which depends on the charge and hydrodynamic radius of the molecules or particles. Trapping of submicron bioparticles can be achieved by applying oscillating electric field which is known as dielectrophoresis [124 – 127].

Centrifugal force is the basic principle used in disc microfluidics, where by the microfluidic disc is rotated in a disc drive and synchronized using computer to perform different steps of operations [128 – 131]. Liquid transport is initiated by the force directed outwards in the radial direction, which can be scaled from nL s^{-1} to 1 mL s^{-1} . Liquid mixing can be accomplished within a radially directed rotating

channel based on the Coriolis stirring effect [132]. The main limitation is the limited applications in particle separation and trapping.

2.3.2 Multiphase flow

In addition to single phase liquid handling, microfluidic technology can also provide the handling of multiphase fluids, such as immiscible liquids or liquid with gas [133 - 134]. Multiphase flow has widened the possibilities and the applications of microfluidic technology from complicated chemical analyses and reactions to cell manipulation and analyses [40]. Multiphase flow is the basic principle for droplet microfluidics, which has additional advantages as compared to continuous single phase flow. First, it focuses on the formation of discrete nano-liter volumes, which has the ability to perform a large number of reactions or parallel experiments while maintaining the simplicity of the chip design and compactness of the chip [135]. Second, as micro-sized droplets have high surface area to volume ratios, faster chemical reactions are achieved with faster heat and mass transfer times and shorter diffusion distances [136]. Third, each single droplet is a microreactor which can be individually manipulated, mixed, split and analyzed [137 – 138]. Parallel processing can be achieved efficiently and cross-contamination can be avoided significantly.

In droplet formation, either using two immiscible liquids (water and oil) or air-liquid, the parameters governed include viscous forces, interfacial tensions and microchannel geometry. The two basic geometries used to control the generation of droplets are T-junction and flow-focusing [139 – 140]. Different geometrical modifications have been designed to achieve better stability and uniformity or more

complex geometries have been demonstrated to achieve complicated functionalities such as double-layer emulsions or chemical mixing [141 – 144]. The formation of water-in-oil or oil-in-water droplets are mainly depends on the wettability of the two phases with the microchannel material [145]. For example, water droplets are formed in hydrophobic microchannel but oil droplets are formed in hydrophilic microchannel. In addition to pressure driven control, droplet generation can also be achieved using dielectrophoresis or electrowetting [146 – 147]. These techniques will not discussed in details as it is beyond the scope of this thesis.

Different operational units are demonstrated for droplet handling such as droplet fission, droplet fusion and droplet sorting. Passive droplet fission is controlled by shear forces created by geometrical designs such as T-junction [148], channel obstructions [149] or branching microchannels [150]. On the other hand, active droplet fission can be performed with the presence of external power or electrical control [151 – 152]. Droplet fusion initiates when two or more droplets are closed to each other with a thin film of fluid forms at the interfaces. The film will rupture and the droplets will coalesce due to imbalance surface tension or increased pressure on the film. For droplet fusion, different mechanisms have been employed such as synchronized droplet generators, microchannel expansion, microchannel obstruction, interfacial tension-mediated fusion, or selective surface treatment [153 – 159]. Droplet sorting is essential for individual control of single droplets, such as droplet isolation, sample purification and droplet segregation. Passive droplet sorting by size is based on gravitational force and channel geometry [160 – 161].

With the development of these operational units, droplet microfluidics provides a robust platform for fluid and particle/cell manipulations and is capable in

performing complicated chemical reactions and analyses. In chemical reactions, droplet microfluidics is employed in the titration of acid and anti-coagulants, particle syntheses, crystal growth and chemical precipitations [162 – 167]. Droplet microfluidics offers greater control in quantity and faster reaction time with higher yield. In drug delivery, droplet microfluidics offer the possibility of using microparticles and microcapsules to alter drug release profiles with improved site specific targeting and distribution characteristics [168 – 169]. Drugs, dyes and enzymes are encapsulated in a microdroplet with controllable size and volume and release rate. In cell analysis, single cell or multiple cells can be easily encapsulated in a culture medium droplet and manipulated in the microchannel [170 – 173]. Droplet isolates cells from external harsh environment and viability of cells in droplets are monitored. Different drugs or enzymes can also be injected to the cell droplet by droplet fusion and analyzed to obtain significant experimental quantitative data [174].

In short, droplet microfluidics has provided better control in fluid or sample handling and as a more superior platform for complex and stringent biological and chemical applications with precise concentrations and kinetic conditions.

2.3.3 Cell on chips

Many integrated microfluidic devices have been demonstrated to perform cell handling and analyses [175]. Microfluidic system serves as a platform for cell culture, cell treatment, cell selection, cell lysis and cell analyses.

In vitro cell culture is an important area in modern biology but the difficulties in environmental controls to mimic in vivo environment have limited the biological research exploration. Microfluidic system offers a feasible approach with better environmental controls. Tissue organization and cell culture can be performed on chip by integrating cell delivery mechanism to on-chip cell seeding, scaffolds or extracellular matrix for cell growth, culture media and soluble factors injection system for cell stimulation [176 – 178]. Microfabrication techniques allow nano- to micro-sized surface patterning, including soft-lithography, photolithography and E-beam patterning etc. Nano- to micro-sized features confine and control the ensembles of cells and also the interaction between cells and the extracellular matrix. In addition, surface modifications using surface chemistry techniques enhance and control the attachment of cells within the defined geometrical patterns [179 – 180]. For example, lysine which is commonly used in coated glass slide for better cell attachment can be easily patterned and coated on the surface of the microfluidic chip. Mechanical strain in the physiological range can also be created by microfluidics through shear and enable the studies of the efficiency and strength of cell attachment on the substrates [181]. The precise control of the cell seeding environment enables the studies of the cell growth behaviors affected by the shape and size of the cell ensembles. Moreover, the transportation of culture media and soluble factors by the designed microfluidic system can be controlled precisely in spatial and temporal patterns. This enables the

study of cell phenotype and behavioral control by fine tuning the perturbations in the extracellular surrounding in the microfluidic system. For example, an endothelial cell is being exposed to two flows of mitochondrial dyes and the movement of organelles inside the cell can be observed [182]. In addition, rapid cellular responses such as calcium flux can also be monitored by rapid fluidic switching in a two-channel reagent delivery system [183]. Different microfluidic systems are designed to integrate other functionalities. For example, an on-chip force measurement technique has been developed based on the deflection of micro-sized elastomer post array to accurately measure the force exerted by the cell on the extracellular matrix [184]. Biosensors are integrated to monitor key biological indicators such as pH, calcium ion concentration, dissolved oxygen and biomass etc [185 – 186].

Cultured cell populations can then be fractionated or a single cell can be selected via the microsystem technologies such that particular cell types can be analyzed separately. Adherent cell ensembles can be treated by injecting Trypsin solution into the culture sites to resuspend the cells for harvesting [187]. Suspended cells are then flowing through a cell sorting mechanism to select a particular interested cell type. Different sorting mechanisms have been developed in microfluidic chip, including dielectrophoretic (DEP) cell sorter, fluorescently activated cell sorter (FACS), surface affinity-based trapping technique and etc [188 – 190]. The presence of a cell with induced dipoles in a non-uniform electrical field will give rise to the DEP force being exerted on the cell. The induced DEP force on the cell depends on the intrinsic electrical properties of the cell, which is different with cell type or cell activation. By exploiting the DEP force in a microfluidic system, the particular interested cell can be trapped, sorted and manipulated. For

example, MDA231 cancer cells are separated from blood samples and rare cells tagged with specific marker particles are sorted with enrichment factors > 200 [188]. FACS exploits the fluorescent properties of the particle tagged onto the cell to identify the interested cell type and divert them to the collection chamber using integrated pumps and valves. Fluorescently activated cell sorters are commonly used in flow cytometer and a microfluidic system is able to miniaturize a lab-scale flow cytometer to a portable system. For surface affinity-based trapping technique, proteins such as ligands are immobilized on the surface of the microfluidic channels. Interested cells with compatible receptors are adhered transiently due to biochemical binding and the cell movement along the microchannel is retarded. For example, the separation of HL-60 cells from the U937 cells are demonstrated using selectin as the surface ligand [190]. However, the separation resolution is relatively low.

Cell lysis is a common used method to break the cell membrane without denaturing or degrading sensitive macromolecules, such as DNA and proteins. Cell lysis is accomplished using enzymic, osmotic, chemical or mechanical mechanisms. Enzymic mechanisms are commonly used to lyse bacteria such as lysozyme [191]. Cytolysis is caused by the suspension of cells in the hypotonic environment, in which the cell membrane explodes because it cannot withstand the osmotic pressure of the water in the cell [192]. Chemical lysis has been demonstrated in micro-environment using Triton X-100 and sodium dodecyl sulphate [193 - 194]. Mechanical lysis exploits shear stress on the cell membrane and causes it to rupture [195]. The release of proteins and DNA from the lysed cell can be analyzed subsequently. As the sample separation and analytical techniques for proteins and

DNA are beyond the scope of this thesis, this area of research is not further discussed.

For cell-based microfluidic devices, different biosensors have been developed for drug screening, single-cell analysis and cell-cell interaction etc. The readouts of biosensors are commonly based-on electrical or optical signals. Electrical signals generated by the chemical-dependent electrophysiological activity of the cell are monitored and recorded in microfluidic devices integrated with microelectrode arrays. Conventional patch clamping technique is miniaturized into a microfluidic device integrated with cell manipulation and external chemical environment control for nano-liter reagent volume injection. For optical approach, image-based techniques and intracellular fluorescent probes are commonly employed for single cell analysis. The integration of optical elements into the microfluidic devices for optical manipulation and detection are being extensively studied to avoid the limitation in depending on free-space optical elements or microscopic systems. This area of research is discussed in details in the next section.

2.3.4 Optofluidics

In a typical optical detection system, the three main components include light sources, light guiding elements and light detectors. To realize a compact, robust and portable microfluidic system, it is essential to miniaturize the optical components.

One of the common light sources in microfluidic devices is laser sources, which are essential for fluorescent related analysis. The laboratory laser sources are bulky and require high power. To replace the laboratory laser sources, different on-chip integrated laser sources are developed using liquid dyes as the gain media. Micro-cavity fluidic dye lasers are developed using a pair of metallic mirrors or a ring resonator to form the laser cavity [196]. The laser dye used is Rhodamine 6G, with the excitation wavelength at 532 nm and the emission wavelengths ranging from 550 nm to 620 nm. Microfluidic dye laser is also integrated with liquid-liquid waveguide to provide low threshold in lasing using long optical cavity [197]. Real-time direct control of the lasing specifications can be easily achieved, such as lasing wavelength, numerical aperture and geometry etc. The on-chip dye lasers do not provide mode selection, which leads to wide emission linewidth. To obtain single spatial mode operation dye lasers, Bragg gratings or distributed feedback structure are employed to cause high losses in the high order spatial modes [198]. The lasing wavelength (λ) is determined by the Bragg condition, i.e.

$$\lambda = \frac{2n\Lambda}{m} \quad (2.5)$$

where n is the effective refractive index of the m -th guided mode and Λ is the grating period. It can be seen that the lasing wavelength is tunable either by changing the refractive index of the laser dye solution or by compressing/stretching

the polymer chip to vary the grating period. Dye laser can also be realized using droplet microfluidics which is capable of switching the lasing wavelength between 580 nm to 680 nm at frequencies up to 3.6 kHz [199]. The fast-switching dye lasers are based on a stream of droplets with different laser dyes, which are capable of lasing at different wavelength. The dye droplets are suspended in a fluorocarbon carrier with lower refractive index than that of the dyes, in which the emitted light from the excited dyes is well confined in the droplet cavity by total internal reflection. Lasing occurs when the emitted light has a wavelength in which coincides with the whispering gallery mode of the droplet cavity. In addition to dye lasers, other light sources have also been integrated into microfluidic chip such as fluorescent light source or organic light emitting diode [200 – 201].

In order to integrate optical detection systems into the microfluidic system, it is essential to replace the free space optical elements, such as prisms, waveguides, microlenses and gratings, with integrated optical elements. Integrated optical elements that have been demonstrated can be divided into three phases, i. e. solid-air phase, solid-liquid phase and liquid-liquid phase. Initially, optical elements are designed using the interface of the chip material and air. For example, solid lenses are patterned and fabricated using soft lithography [202]. Different radii of curvature of interfaces can be easily fabricated. In-plane microlenses are fabricated to focus or collimate the incident light and increase the excitation light intensity in the neighboring microfluidic channels or chambers. Subsequently, solid prism can be easily realized by controlling the incident angle of the light on the solid-air interface [203]. Similarly, waveguide is developed with the core made of solid material that has higher refractive index than the one of the cladding, which is normally air [204]. Although the integration of optical elements is solved, the main

limitation of the solid-air optical elements is the lack of tunability with fixed geometries. To provide some tunability in optical properties, tunable optical elements are demonstrated based on the flexible solid geometries or the solid-liquid interface. Microlenses fabricated with flexible and deformable membranes are capable to tune its radii of curvature in a limited range [205]. For optical elements based on solid-liquid interface, the tunability in optical properties is mainly based on the variation of refractive index of the liquid phase. The index contrast at the interface is varied with different solutions and subsequently the angle of refraction or reflection, the transmissibility or the reflectivity at the interface are changed. For example, the focusing power of the microlens can be tuned by injecting different solutions to form a liquid-air or solid-liquid microlens [206]. Similarly, the tunability in prism or waveguide is achieved based on the same principle [207 – 208]. In order to achieve greater tunability, the latest integrated optical elements are developed based on liquid-liquid interface. This new class of optical element, which is known as optofluidics, employs the unique properties of liquids to synthesize novel functionalities. In addition to the ability to change the optical properties by replacing the liquids, optofluidics has the advantages of having optical smooth interfaces to minimize scattering loss and refractive index gradient between several streams of miscible liquids due to diffusion [209]. The first optofluidic element developed is liquid waveguide, which consists of three laminar flow streams [210]. The central flow stream is filled with liquid with higher refractive index as compared to the one in the side flow streams. Liquid waveguide provides greater flexibility in tuning from a step-index waveguide to a gradient index waveguide or from a narrow single mode waveguide to a wide multi-mode waveguide by changing the flow rates. With the liquid waveguide as the basic element, a tunable

splitter is demonstrated [211]. The optical splitter is controlled by the diffusion between two parallel liquid waveguides, in which the light is injected into the diffusive mixing end and split into two separate core flows of the liquid waveguides. In addition, an optofluidic prism has also been realized based on the liquid waveguide structure in a sector-shape chamber [212]. The apex angle of the prism can be tuned from 20° to 70° with a maximum deviation angle of 11.4° . Similarly, Liquid microlens is also demonstrated using the liquid waveguide structure but operated in extremely low flow rate to achieve the desired refractive index gradient [213]. With the transversely variable refractive index, a liquid Gradient Refractive Index lens is formed. Light travelling along the optical axis of the gradient index lens is deviated gradually toward the optical axis to become a focused or collimated beam.

With the development of a wide range of integrated optical elements, several optofluidic devices have been developed, e.g. interferometers, gratings and optical attenuator etc. These optofluidic devices are essential to integrate optical detection and analytical techniques into the microfluidic system for biological and biomedical research such as on-chip spectrometer, refractometer and biomaterial concentration sensor. In addition, optofluidic microscope has also been demonstrated, which successfully replace a bulky laboratory microscopic system with a compact and portable imaging chip [214]. The image of the sample flowing in the microchannel is obtained by compiling the time-dependent changes of the optical transmission across the microchannel. Another microscopic system demonstrated is the versatile microfluidic total internal reflection fluorescent (TIRF) microscopy [215]. The microfluidic chip is integrated with optical fiber for light illumination, cylindrical microlens and prism for light guiding to achieve total internal reflection. The

developed microfluidic chip allows researcher to perform TIRF microscopy easily using a normal inverted or upright microscope without the need of expensive TIRF system.

With the on-going research advancement in developing robust integrated optical systems into the microfluidic system, Lab-on-chip system will be employed extensively in biomedical research to develop robust and portable optical detection system for numerous chemical detection and biomedical diagnostic applications.

2.4 Summary

Based on the literature survey presented, cell refractive index is an important cell biophysical parameter, which plays a vital role in biophysics and biomedical fields. Based on the refractive index value, the concentration of solid mass in the cell, the dry mass and water mass of the cell can be determined consequently. Since cell refractive index is related to the concentration and the mass of the intracellular solids, it is significant as a fundamental biophysical quantity for the investigation of cell state in cell cycle, disease diagnoses such as thalassemia and external environment analysis such as osmotic pressure. With the current development in LOC technology, it is capable to develop an integrated optical system based on optical resonator for single cell refractive index measurement, which also provides other functionalities such as buffer injection and exchange, cell sorting, encapsulation and trapping.

CHAPTER 3

ON-CHIP GRATING INTERFEROMETER

This chapter presents the design, fabrication and experiment of the on-chip grating interferometer for the effective refractive index measurement of single living cell. The optical designs of the on-chip grating interferometer are presented, including the theoretical analysis of the grating interferometer, the differential measurement scheme employed for the measurement of the effective refractive index and diameter of single cell, and the optical tweezers used for single cell trapping. Subsequently, the microfluidic designs of the grating interferometer are discussed, including the hydrodynamic focusing mechanism for single cell alignment, the multiphase plug generating mechanism for single cell encapsulation and the plug mixing mechanism used to realize the differential measurement scheme. Then, the fabrication processes for the development of the on-chip grating interferometer are illustrated. Finally, the experimental results are presented and discussed.

3.1 Architecture of the on-chip grating interferometer

Fig 3.1 Schematic illustrations of (a) the overview of on-chip grating interferometer, and (b) the zoom view of the buffer mixing for differential measurement scheme.

An on-chip grating interferometer is proposed as a refractometer for single cell measurement. The schematic illustration of the on-chip grating interferometer is shown in Fig. 3.1 (a). In the on-chip grating interferometer, cells are loaded through the cell inlet. The cell inlet and a buffer inlet are connected by a T-junction to realize hydrofocusing for single cell alignment. The combined microchannel is

connected to another oil inlet by another T-junction downstream to form multiphase flow for single cell isolation. The cell inside the buffer plug is isolated from other cells and transferred to the measurement area. At the measurement area, a pair of Bragg grating fibers is used for cell trapping and measurement. The grating interferometer is realized by aligning and separating the two grating fibers with a microchannel that acts as the resonant cavity. Differential measurement scheme is employed to measure the effective refractive index and the diameter of single cell simultaneously. During measurement, optical tweezers is employed to trap the single cell within the resonant cavity. Another buffer plug is mixed with the cell plug to change the concentration of buffer in the cell plug as shown in Fig. 3.1 (b). The effective refractive index and the diameter of the cell can be determined based on the resonant spectra. In the next section, the working principles of the optical systems employed in the on-chip grating interferometer are discussed in detail.

3.2 Optical system for cell trapping and measurement

In the on-chip grating interferometer, the optical systems employed consist of fiber Bragg grating interferometer, differential measurement scheme and optical tweezers. This section of the chapter presents the theoretical analyses of the optical systems used for the on-chip grating interferometer.

3.2.1 Fiber Bragg Grating interferometer

Fiber Bragg Grating (FBG) is a type of distributed Bragg reflector constructed in a short segment of optical fiber that reflects particular wavelengths of light and transmits all the others. This is achieved by having a periodic perturbation of the refractive index along the fiber length, which is formed by UV exposure of the core to an intense optical interference pattern [216]. The change in refractive index is permanent if it is annealed at a temperature of 50°C above its maximum operating temperature for hours [217]. The magnitude of the refractive index change (Δn) obtained depends on several different factors, such as the irradiation conditions (wavelength, intensity and total dosage of irradiance light), the composition of glassy material forming the fiber core, and any processing of the fiber prior to irradiation. For example, hydrogen loading technique is used to enhance the refractive index change obtained on irradiation [218]. By using hydrogen loading, a Δn as high as 10^{-2} can be obtained. The most distinguishing feature of fiber grating is the flexibility they offer for achieving desired spectral characteristics. FBG can therefore be used as an inline optical filter to block certain wavelengths [219], or as a wavelength-specific reflector [220].

Fig. 3.2 Variations of the induced index change. (a) Uniform, (b) chirped, and (c) Gaussian-apodized.

There are different types of FBGs classified by the variation of the induced refractive index change along the fiber axis, such as uniform, chirped and Gaussian-apodized as shown in Fig. 3.2. However, the uniform Bragg grating is chosen for the simplicity in designing the interferometer. A uniform Bragg grating consists of a constant period Λ and a continuous sinusoidal refractive index modulation Δn as shown in Fig. 3.2 (a).

A uniform Bragg grating is characterized by the physical length L , the period Λ and the refractive index modulation Δn superimposed on the mean refractive index of the fiber's core, n . The reflected wavelength is known as the Bragg wavelength λ_B , which is defined by

$$\lambda_B = 2n_B\Lambda \quad (3.1)$$

where n_B is the mean effective refractive index at λ_B [221]. The output spectrum characteristics can be described by the transmission matrix based on the couple mode theory [222].

Fig. 3.3 Transmission matrix representation of the uniform FBG. (a) Refractive index distribution along the fiber, and (b) T-matrix model.

The uniform FBG can be represented by a transmission matrix as shown in Fig. 3.3, in which the complex amplitude of the waves at the input ($z = 0$) and the output ($z = L$) can be expressed as

$$\begin{bmatrix} E_l(0) \\ E_r(0) \end{bmatrix} = \begin{bmatrix} T(1,1) & T(1,2) \\ T(2,1) & T(2,2) \end{bmatrix} \begin{bmatrix} E_l(L) \\ E_r(L) \end{bmatrix} \quad (3.2)$$

where the coefficients of the transmission matrix are

$$T(1,1) = T(2,2)^* = \left[\cosh(\gamma L) - j \frac{\Delta\beta}{\gamma} \sinh(\gamma L) \right] \exp(-j\pi L / \Lambda) \quad (3.3a)$$

and

$$T(1,2) = T(2,1)^* = -j \frac{\kappa}{\gamma} \sinh(\gamma L) \exp(j\pi L / \Lambda) \quad (3.3b)$$

Fig 3.4 Simulation result of the transmittance based on the parameters: $L = 1$ cm,

$\lambda_B = 1550$ nm, $n = 1.45$, and $\Delta n = 1 \times 10^{-4}$.

where the coupling coefficient κ of the grating, the detuning $\Delta\beta$ and γ are defined as

$$\kappa = \frac{\Delta n \pi}{\lambda} \quad (3.4a)$$

$$\Delta\beta = \frac{2\pi}{\lambda} n_o - \frac{\pi}{\Lambda} \quad (3.4b)$$

and

$$\gamma^2 = \kappa^2 - \Delta\beta^2. \quad (3.4c)$$

The transmissivity of the grating is determined by

$$|t| = \left| \frac{E_t(L)}{E_t(0)} \right| = \left| \frac{1}{T(1,1)} \right| = \frac{\gamma}{\left[\gamma^2 \cosh^2(\gamma L) + \Delta\beta^2 \sinh^2(\gamma L) \right]^{1/2}}. \quad (3.5)$$

Therefore, the transmittance of the grating is simplified as $|t|^2$. Based on the

Fig. 3.5 Transmission matrix representation of the uniform FBG with resonant cavity. (a) Refractive index along the fiber core, and (b) T-matrix model.

transmission matrix, a typical output spectrum can be simulated as shown in Fig. 3.4. A single Bragg grating can be acted as a band stop filter with wavelengths around λ_B (1550 nm) to be reflected back. However, in order to develop the FBG-based interferometer, a pair of FBGs is employed and separated by a certain gap to form the resonant cavity [223]. The equivalent transmission matrix model is shown in Fig. 3.5, in which the complex amplitude of the waves at the input and output can be expressed as

$$\begin{bmatrix} E_l(0) \\ E_r(0) \end{bmatrix} = T_1 T_2 T_3 \begin{bmatrix} E_l(2L+D) \\ E_r(2L+D) \end{bmatrix} \quad (3.6)$$

where $T_1 = T_3 = T$ and T_2 is expressed as

$$T_2 = -\left(\frac{1}{t_{fc}}\right)^2 \begin{bmatrix} 1 & r_{fc} \\ r_{fc} & 1 \end{bmatrix} \begin{bmatrix} \exp(-j\frac{2\pi}{\lambda} n_c D) & 0 \\ 0 & \exp(j\frac{2\pi}{\lambda} n_c D) \end{bmatrix} \begin{bmatrix} 1 & r_{fc} \\ r_{fc} & 1 \end{bmatrix} \quad (3.7)$$

where r_{fc} and t_{fc} are the reflectivity and the transmissivity between the fiber/air cavity, respectively.

Fig 3.6 Simulation result of the transmittance based on the parameters: $L = 2$ mm, $D = 50 \mu\text{m}$, $\lambda_B = 1550$ nm, $n = 1.45$, and $\Delta n = 5 \times 10^{-4}$.

Figure 3.6 illustrates the typical transmittance spectrum of the FBGs with a resonant cavity. It can be clearly seen that several peaks are obtained within the bandstop region. The position of the peaks depends on the optical path length in the cavity. Subsequently, the relationship between the optical path length and the position of the peaks in the transmittance spectrum is investigated. Based on the FP formula [224], the power transmittance of the cavity with two FBGs can be written as

$$T = \frac{(1 - R)^2}{(1 - R)^2 + 4R^2 \sin^2(\beta D + \varphi)} \quad (3.8)$$

where R is the reflection coefficient for the gratings, D is the length of the cavity formed between the gratings, β is the propagation constant and φ is the phase of the FBG's reflection coefficient. To verify the effectiveness of Eq. (3.8), the calculated

Fig 3.7 Comparison of different theoretical models for FBG interferometer.

transmittance spectrum is compared with the one simulated by the standard T-matrix method described as shown in Fig. 3.7. The simulation results show that both methods match well with each other within the band-stop region and give identical peaks. Since the FP cavity formulation can be used to describe the resonant cavity formed by the two FBGs, the FP cavity formulation is used to study the relation between the optical path length and the positions of the resonant peaks.

When the optical path length of the cavity is changed slightly, the shifted resonant peaks can be determined by

$$\frac{\Delta\lambda}{\lambda} = \frac{\Delta\text{OPL}}{\text{OPL}_{\text{eff}}} = \frac{2\Delta n_{\text{cav}} D}{2n_{\text{eff}} L_{\text{eff}}} \quad (3.9)$$

where $\Delta\lambda$ is the wavelength shift, λ is the resonant wavelength, Δn_{cav} is the change of refractive index, n_{eff} and L_{eff} are the effective refractive index and effective length of the resonant cavity formed by the FBG fibers, respectively. ΔOPL is the change of the optical path length in the cavity and OPL_{eff} indicates the effective optical path length. As the change of OPL_{eff} due to the slight change in refractive index is

Fig 3.8 Shift of resonant peaks with the change of refractive index within the cavity.

negligible, a linear relation between the wavelength shift ($\Delta\lambda$) and the change of optical path length (ΔOPL) can be derived as

$$\Delta\lambda = k\Delta\text{OPL} \quad (3.10)$$

where k is a dimensionless variable defined as the resonant peak shift per unit change of optical path length. Fig. 3.8 shows the shift of resonant peaks with the change of refractive index within the cavity. It shows that the peaks will be right shifted with the increasing refractive index. Therefore, the shift of peaks due to the refractive index change in the cavity is the fundamental property used in the design of the interferometer for cell refractive index measurement.

3.2.2 Differential measurement scheme

Fig 3.9 Differential measurement scheme. (a) Surrounding medium's refractive index is n_1 , and (b) surrounding medium's refractive index is n_2 .

The basic principle of cell refractive index measurement is based on the differential measurement scheme employed in the on-chip grating interferometer [225]. The interferometric technique presented is based on the measurement of the optical path length of the cavity, i.e. the sum of the products of the refractive indices and the associated thicknesses. In order to extract the information of the refractive index from the associated thickness, the surrounding medium is changed with different refractive indices. As a little variation of the surrounding medium's refractive index is introduced, the total optical path length is varied. Hence, the cell refractive index and diameter can be determined. To make this method reasonable, the cell is assumed to have no variation in refractive index and diameter during the differential measurement process. With the change in the surrounding buffer, osmolarity effect on the cell cannot be avoided. However, with the osmolarity ratio of the buffer approximately 1.2 times, the change in the cell size is less than 1% [226]. In addition, the osmolarity effect can be reduced by taking the measurements immediately after the change in the external buffer. The differential measurement scheme is illustrated in Fig. 3.9.

The optical path length, L_1 with the cell in the cavity and surrounding medium's refractive index as n_1 is expressed as

$$L_1 = (a+b)n_1 + l_c n_c \quad (3.11)$$

where n_c is the cell refractive index, l_c is the cell diameter, a and b are the path lengths of the surrounding medium in the cavity. When the surrounding medium is changed to n_2 , the optical path length, L_2 is varied and expressed as

$$L_2 = (a+b)n_2 + l_c n_c \quad (3.12)$$

With the absence of the cell, the optical path lengths are changed to

$$L_{n_1} = (a+b+l_c)n_1 \quad (3.13a)$$

or

$$L_{n_2} = (a+b+l_c)n_2 \quad (3.13b)$$

By substituting Eq. (3.13) into Eqs. (3.11) and (3.12), the cell refractive index and diameter can be determined by

$$n_c = \frac{L_1 n_2 - L_2 n_1}{l_c (n_2 - n_1)} \quad (3.14)$$

and

$$l_c = \frac{(L_1 - L_2) - (L_{n_1} - L_{n_2})}{n_2 - n_1} \quad (3.15)$$

The application of differential method is not only to separate the information of the cell refractive index and diameter, but also to eliminate uncertainties in experimental setup and during measurements, such as noise factor.

Fig 3.10 Schematic of FBG interferometer for cell refractive index measurement using differential measurement scheme.

Figure 3.10 illustrates the schematic for the FBG interferometer used for cell refractive index measurement. The cavity length, D , is set to be $20\ \mu\text{m}$ to optimize the detection range for cell refractive index within one free spectral range but at the same time is limited by the size of the cell ($< 20\ \mu\text{m}$). With the defined k , differential measurement scheme can now be used to determine the refractive index and cell of a single cell. With two buffers of different refractive indices injected alternatively into the cavity, four transmission spectra can be obtained with two spectra for each buffer, i.e. with the absence or presence of the single cell in the cavity. The wavelength shifts can be expressed as

$$\Delta\lambda_1 = k(n_c - n_1)l_c \quad (3.16a)$$

and

$$\Delta\lambda_2 = k(n_c - n_2)l_c \quad (3.16b)$$

where k is the dimensionless constant, n_1 is the refractive index of buffer 1 and n_2 is the refractive index of buffer 2, $\Delta\lambda_1$ and $\Delta\lambda_2$ are the wavelength shift in buffer 1 and

buffer 2, respectively. Subsequently, the cell refractive index and diameter can be determined as

$$n_c = \frac{n_2 \Delta\lambda_1 - n_1 \Delta\lambda_2}{\Delta\lambda_1 - \Delta\lambda_2} \quad (3.17)$$

and

$$l_c = \frac{\Delta\lambda_1 - \Delta\lambda_2}{k(n_2 - n_1)} . \quad (3.18)$$

Based on the equations (3.17) and (3.18), the cell refractive index and diameter can be obtained by monitoring the output spectral shifts.

3.2.3 Fiber optical trapping

Fig 3.11 Schematic illustrations of (a) the laser optical force and analogous to spring system, and the net force acted on particle either (b) it is displaced from the center, or (c) it is located at the center.

Optical force for cell trapping is a non-contact technique using a focused laser beam to provide attractive or repulsive force [227 – 228]. Depending on the index mismatch, the optical force can temporarily hold or move a microscopic dielectric particle. The laser Gaussian beam is typically focused by a microscope objective. It has a narrowest point, known as the beam waist which contains a strong electric field gradient, as shown in Fig. 3.11 (a). The dielectric particles are attracted along the gradient to the point with the strongest electric field, i.e. the center of the beam. Moreover, along the direction of beam propagation, a light scattering force is acted on the particle and causes the particle to be displaced slightly downstream from the beam waist. The force applied on the particle is linearly depending on its displacement (X) from the trap center and is analogous to a spring system as shown in Fig. 3.11 (a). Ray optics can be used to explain the trapping phenomenon when the diameter of the particle is significantly greater than the wavelength of the light. The laser light is refracted as it enters and exits the

dielectric particle as shown in Fig. 3.11 (b) and (c). The change of direction is associated with the change of its momentum and there is an equal and opposite momentum change on the particle based on Newton's third law. If the particle is displaced away from the beam's center (Fig. 3.11 (b)), the particle has a net force acted on it to return back to the center. This is because more intense beams at the center will impart a larger momentum change towards the center than less intense beams do. For the case where the particle is located at the center as shown in Fig 3.11 (c), there is no net lateral force; instead the net force is only along the axial direction of the trap. As a result, the particle is stably trapped slightly downstream of the beam waist.

Optical trapping can also be realized by optical fiber [229]. Compared to common microscopic optical trapping, the fiber optical trap has two advantages. Firstly, the implementation of fiber optical trap is simple, robust and inexpensive, which does not need parallel focusing, or other optical components such as filters, telescopes or beam splitters. Next, the trap is decoupled from the microscope, which provides great flexibility in viewing and manipulating samples.

Two types of commonly used fiber optical traps are the lens fiber to generate Gaussian beam (similar to microscopic optical tweezers) and dual fiber trap that uses two counter propagating laser beams [230 – 231]. The type of fiber optical traps used in this report is the latter one for easier integration with the existing optical detection system.

The dual fiber optical trap was demonstrated whereby the laser light is introduced directly into the sample from side by two easily aligned single-mode optical fibers as shown in Fig. 3.12 (b) [231]. The optical trapping forces can be resolved into two components as shown in Fig. 3.12 (a), i.e. the gradient force and

Fig 3.12 Schematic illustrations of (a) the forces of the light beam emitted from the fiber, and (b) the total forces when the fibers are perfectly aligned.

the scattering force. The gradient force pushes the particle in the direction of increasing intensity which provides the restoring force in all the directions perpendicular to the beam propagation. On the other hand, the scattering force pushes the particle in the direction of propagation is counter-balanced by the one from another fiber. Assume that one trapping beam, designated by the subscript l is propagating in the $+\vec{z}$ direction (from left to right) and the counter propagating beam is designated by r , i.e. in the $-\vec{z}$ direction. When P is the power transmitted through the fiber and the light from the fiber is represented by a Gaussian beam with a beam waist of w_0 at the fiber facet, then the intensity at the fiber facet is given by

$$I_0 = \frac{2P}{\pi w_0^2} \quad (3.19)$$

With the scattering force of a plane wave on a spherical particle of radius R is given by $(R\pi^2)IQ_{pr}/c$ and let $z = 0$ at the center point between the fibers, the total scattering force acted on the particle is defined as

Fig 3.13 Schematic illustrations of fiber misalignment. (a) Translational misaligned, and (b) rotational misaligned.

$$F_s = \frac{2R^2 P_l Q_{pr,l}}{cw_{ol}^2 [1 + (\lambda / \pi w_{ol}^2)^{-2} (S/2 + z)^2]} - \frac{2R^2 P_r Q_{pr,r}}{cw_{or}^2 [1 + (\lambda / \pi w_{or}^2)^{-2} (S/2 - z)^2]} \quad (3.20)$$

where c is the speed of light, Q_{pr} is the radiation pressure coefficient defined in [231], λ is the wavelength and S is the distance between the fiber facets. It is assumed that the two trapping beams are exactly counter propagating and is under the approximation in which the intensity and the phase of the trapping beams are constant over the area of the sphere.

There are two possible types of fiber misalignment that will lead to the failure of particle trapping, as illustrated in Fig. 3.13. The first type is translational misalignment in which the beams are propagating in the z direction but the fibers are translationally displaced. The second type is rotational misalignment in which both fiber facets still have their centers in the z axis but are at skewed angles to each other's facets and not counter propagating. The alignment of the two counterpropagating beams is critical for optimal trapping. For instance, when the fibers are translationally misaligned as shown in Fig. 3.13 (a) the trapped particle can oscillate back and forth between the fiber facets without a stable trapping

Fig 3.14 Schematic illustration of the two-fiber system integrated with grating interferometer and optical trapping.

position. On the other hand, when the fibers are rotationally misaligned, the particle will not be trapped by the optical force because of the absence of a stable trapping position as shown in Fig. 3.13 (b).

To integrate the fiber optical trap with the optical detection system, a two-fiber system is designed in this on-chip grating interferometer, which provides the functions of cell trapping and cell measurement. The schematic diagram of the two-fiber system is shown in Fig. 3.14. The trapping laser beam and the detection beam are combined into a single fiber through the 50/50 fiber splitter. The two-fiber system provides several advantages compared to the four-fiber system. First, the cell is perfectly trapped within the cavity along the fiber core in which detection can always be carried out whenever the cell is trapped. It also avoids the need to adjust the power ratio to keep the cell in the center of detection area. Second, the fiber optical system is integrated with microfluidic system which provides ease manipulation of cells and liquids. Third, with the two fibers being positioned along the sides of the microchannel, the flow of cells and buffers is not disturbed. Fourth,

the alignment and packaging issues are easier with two-fiber system as compared to four-fiber system.

3.3 Microfluidics for cell manipulation and buffer mixing

Fig 3.15 Schematic illustration of microfluidic system for single cell manipulation, encapsulation and detection.

The analysis of single living cells presents a variety of challenges in manipulation and handling. Eukaryotic cells are generally in the order of 5 to 20 μm in diameter. Therefore, techniques which can handle such small size are necessary. Moreover, cells must be handled gently prior to and during the analysis so as not to perturb the cell's bio-processes. To tackle these problems, microfluidic devices are developed, and further offer a unique platform for rapid analysis of single cells. Several different single-cell manipulation techniques have been reported, which include hydrodynamic flow and focusing, on-chip valves and pumps to direct cell transport, the incorporation of cells into microdroplets and dielectrophoretic manipulation etc [232 – 235]. Although hydrodynamic focusing can successfully separate the cells, it cannot ensure single cell detection without isolation. The application of on-chip valves and pumps or dielectrophoretic manipulation however increases the complexity in fabrication process. Therefore, in order to successfully separate single cell from a group of cells and also to isolate the single cell from others in the detection area, multiphase flow manipulation based on hydrodynamic focusing mechanism is proposed as shown in Fig. 3.15. The design consists of three main features, namely hydrodynamic focusing for cell separation, multiphase flow

formation for cell encapsulation and buffer modulation at detection area. These three main features will be elaborated in theory and design in the next section of this chapter.

3.3.1 Hydrodynamic focusing for single cell alignment

Cell alignment can be achieved using microchannel with comparable width. However, this imposes stringent requirements in fabrication and the cells may affect the microfluidic flow. To overcome this, hydrodynamic focusing is employed for cell alignment such that a larger microchannel can be used. Hydrodynamic focusing is a common technique used in microfluidic device, not only to confine the sample flow within a certain dimension, but also to separate and align the cells/particles one-by-one to facilitate the measurement process in the detection area [232]. The continuity equation of the fluidic motion, which is consistent with the law of conservation of mass, is expressed as

$$\Sigma Q_{in} = \Sigma Q_{out} \quad (3.21)$$

where Q is the volume flow rate. Based on the continuity equation, Navier-Stokes equations for the dynamic flow is expressed as [236]

$$\rho \left(\frac{\partial v}{\partial t} + v \cdot \nabla v \right) = -\nabla p + \nabla \cdot T + f \quad (3.22)$$

where v is the flow velocity, ρ is the fluid density, p is the pressure, T is the stress tensor and f is the body forces acting on the fluid. Although Navier-Stokes equations describe almost all flows, it is too complicated to be solved in many cases. For better understanding on the hydrodynamic focusing design, an analogical electrical circuit model has been introduced [237 - 238]. According to the

Fig 3.16 (a) T-junction microchannel design, and (b) equivalent circuit model.

equivalent circuit model, the fluid flow can be mapped to a resistors network under two conditions. First, the flow is laminar without turbulence or bubbles inside. Second, the width of the intersection must be negligibly small compared to the length of the channels. The proposed design satisfies both conditions. In this model, the applied pressure is mapped to the voltage source, the fluidic resistance is mapped to the electric resistance and finally the volume flow rate is mapped to the electric current. By then, the relationship among the fluid fluxes is governed by Ohm's law.

For simplicity, a T-junction microchannel design is used for hydrodynamic focusing as shown in Fig 3.16 (a). The corresponding equivalent circuit model is shown in Fig 3.16 (b). The parameters and definitions are listed in Table 3.1. In the design, the rectangular microchannels have width of $40\ \mu\text{m}$ (W_o) and the same driving pressure is used for both inlet and side channels. Therefore, based on Kirchhoff's current law, the outlet current is

Table 3.1 Terminologies used in microchannel and the equivalent circuit model.

Microchannel		Equivalent Circuit	
Parameter	Description	Parameter	Description
Q_i	Flow rate of inlet channel	I_i	Current of inlet brunch
Q_o	Flow rate of outlet	I_o	Current of outlet brunch
Q_s	Flow rate of side channel	I_s	Current of side brunch
P_i	Inlet pressure	V_i	Driving voltage on inlet
P_s	Sheath pressure	V_x	Potential at the intersection
W	Width of channel/flow	I/R	Respective resistance

Fig 3.17 The change of the width of the focus flow under different focusing ratios with constant W_o .

$$I_o = I_i + I_s = 2I_i \quad . \quad (3.22)$$

Thus, the focusing ratio, $\beta = W_o/W = I_o/I_i = 2$, i.e. the focus flow has a width W of 20 μm . The tuning of W by changing the focusing ratio is simulated using Comsol Multiphysics and shown in Fig. 3.17. By increasing the focusing ratio, the width of focus flow is decreased. To achieve a width of 20 μm , a focusing ratio of 2 is required, which is matched with the electrical equivalent model.

3.3.2 Two-phase plug generating for single cell encapsulation

Fig 3.18 Serial compartmentalization of multiphase flow with stationary detector.

The two-phase flow formation is designed to perform isolation for single cells. As the detection method is stationary, serial compartmentalization scheme is used in which droplets are formed and transported by flow to the detection area in a microchannel as shown in Fig. 3.18 [239]. Two-phase flow means the formation of plugs when two immiscible fluids are used in the microfluidic channel. Plugs are defined as droplets that block the channel but do not wet the walls. This method can solve two common microfluidic problems, i.e. Taylor dispersion and mixing. While mixing will be discussed at the latter part of this section, Taylor dispersion is the problem in which solutes are left behind along the channel during pressure-driven flow [240]. Dispersion will cause dilution of sample concentration and also lead to cross-contamination. Therefore, when sample solution forms a series of plugs in a continuous oil flow stream, the samples are confined within the plugs and no longer dispersing along the microchannel.

In order to obtain a clean transport of plug without cross-contamination, the carrier fluid (oil) must wet the walls of the channels preferentially compared to the aqueous phase (cell in buffer) [241]. By satisfying this condition, the sample plugs

are not in contact with the walls, but are stable without leaving any residue behind when they are transported through the microchannel. The wettability of the fluid with the channel's wall is solely depends on the surface tension at the fluid/wall interface, i.e. the surface tension of water/PDMS interface has to be higher than the oil/PDMS interface. However, the surface tension of olive oil/PDMS interface is slightly higher than that of water/PDMS interface (38 mN/m) [242]. In order to achieve the condition, surfactant (Span 80) [243] is added into the olive oil to reduce its surface tension with PDMS. Moreover, the surface tension at the water/PDMS interface must be sufficiently high so that the destruction of plugs by shear can be avoided.

The formation of plugs is characterized by the dimensionless Capillary number,

$$Ca = U \mu / \gamma \quad (3.23)$$

where U is the flow velocity, γ is the water/oil surface tension and μ is the viscosity of the fluid. When Ca is high (> 1), droplets of diameter smaller than the channel's width can be formed and the diameter is inversely proportional to the flow velocity. In order to form a plug at the size of the channel's width, the Ca number has to be low (< 1) [244]. Under this condition, the length of the plug is independent of the total flow rate or the Ca number. The plug's length, l can be estimated as

$$l = p \frac{V_{aq}}{V_{aq} + V_{oil}}. \quad (3.24)$$

where p is the plug's period, V_{aq} and V_{oil} are the sample and the oil flow velocities, respectively as shown in Fig 3.13. The length of the plugs can be easily controlled by varying the velocities of the sample and the oil flow [245].

3.3.3 Buffer plug mixing for differential measurement scheme

Fig 3.19 Schematic illustration of the microfluidic design in the detection area.

The final microfluidic design is the buffer modulation process in the detection area as illustrated in Fig. 3.19. When sample plug reaches the detection area, it is stretched due the reduction in the width of the channel. The increased length of the sample plug is to facilitate the detection and mixing process. In order to employ the differential method as discussed in Section 3.1.3, the content in the buffer plug has to be changed. After a measurement is taken when the cell is trapped in the fiber cavity, buffer modulation process is performed by varying the refractive index of the surrounding buffer. One way of realizing buffer modulation is by mixing the buffer plug with another smaller reagent plug.

Mixing is one of the problems associated with the laminar flow. Two streams injected into a channel at low Reynold's number are flowing side-by-side, with mixing by diffusion. Rapid mixing of fluids in microchannel is hardly achieved. Methods to enhance mixing include hydrodynamic focusing, multiple laminar stream injection and the application of beads in channels. All methods of mixing is based on the condition to reduce the striation length l_{st} , i.e. the distance over which mixing occurs by diffusion. The time for diffusion (t_{diff}) is given by

Fig 3.20 Schematic illustration of the mixing mechanism in a plug.

$$t_{diff} = l_{st}^2 / (2D) \quad (3.25)$$

where D is the diffusion constant. By reducing the striation length from meter to millimeter, the diffusion time is reduced from second to microsecond. The striation length can be shortened by employing plug flow because recirculating flow is developed when plug moves in the channel as shown in Fig. 3.20. The development of recirculating flow is caused by the shearing interactions of the liquid inside the plug with the stationary wall [234]. In ideal initial conditions, the recirculating flow reduces the striation length, which is expressed as

$$l_{st}(d) = l_{st}(0) \frac{l}{d} \quad (3.26)$$

where d is the distance traveled by the plug, l is the plug's length, $l_{st}(0)$ and $l_{st}(d)$ are the initial striation length and the striation length after traveled a distance of d , respectively. Based on Eq. (3.26), the striation length is inversely proportional to the distance traveled and greatly reduces the diffusion time. In addition, shorter plug length is preferred to achieve faster diffusion time. However, to enable single cell encapsulation, the preferred plug size is the one comparable or at the size of the cell, i.e. about 20 - 50 μm .

3.4 Fabrication and packaging

3.4.1 Polymer soft lithography fabrication processes

Fig 3.21 Process flow of PDMS soft-lithography fabrication process.

The fabrication of the on-chip grating interferometer is primarily based on the polymer soft lithography fabrication technique [6]. This technique provides a simple and rapid molding process. The polymer commonly used in the fabrication of microfluidic chip is known as polydimethylsiloxane (PDMS), which has material properties of optically transparent especially in visible and infra-red regions [246]. Therefore, the use of the PDMS material can minimize the propagation loss which is critical and also suitable for optofluidic devices and biomedical applications [247]. The fabrication process flow is shown in Fig. 3.21.

The design of the on-chip grating interferometer is schematically drawn using a computer-aided design (CAD) program (L-Edit 12.0). A dark field clear feature sodalime glass mask is commercially fabricated based on the CAD drawing. To fabricate the master for molding, a 6-inch silicon wafer is cleaned using a piranha clean ($\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$) and rinsed with DI water before the spin coating process. A layer of SU-8 photoresist (MicroChem, SU-8 50) is spun coated on the wafer. In order to have a thickness of 50 μm SU-8 layer, the photoresist is spun

coated at 2000 rpm for 30 s (Spin coater: CEE, 200). After spin coating, the substrate is soft baked using a hot plate at 65 °C for 6 min and 95 °C for 20 min to evaporate the solvent and densify the film of the SU-8 layer. Next, the substrate is exposed to UV light for 40 s under the soda lime glass mask (Mask aligner: OAI, J500-IR/VIS). After exposure, a post expose bake is performed at 65 °C for 1 min and 95 °C for 5 min to selectively cross-link the exposed portions of the SU-8 film. Then, the SU-8 layer is developed using the SU-8 developer (MicroChem) for 6 min. The substrate is rinsed briefly with isopropyl alcohol and dried with a gentle stream of nitrogen. Finally, the substrate is hard baked at 120 °C in a convection oven for 10 min to further cross-link the SU-8 layer and the master for rapid molding is fabricated.

With the fabricated master, the microfluidic chip can be fabricated using PDMS replica molding. Replica molding is the casting of PDMS prepolymer against a master and obtaining the negative replica of the master. PDMS elastomer (Dow Corning, Sylgard 184) is a two-component heat curing system, i.e. it consists of a base part and a curing agent part. The PDMS prepolymer mixture is prepared by mixing the base part with the curing agent at a ratio of 10:1. The mixture is then degassed in the desiccators under low vacuum for 1 hour. After that, the mixture is simply pour onto the master and cured for 1 hour at 75 °C. The curing temperature and the ratio of the curing agent determine the stiffness and optical properties of the PDMS. Moreover, at higher temperature, the PDMS shrinks more. Therefore, a lower temperature and longer baking time are required. After curing, the PDMS replica is cut and peeled off from the master. A through hole is punched at each inlets and outlets with a 0.75 mm diameter puncher (Harris Uni-core, 0.75). Fig. 3.22 shows the micrograph of the microstructure in the PDMS replica taken by

Fig 3.22 SEM micrograph of the PDMS replica showing a microchannel connecting to the inlet reservoir.

scanning electron microscope (SEM). Smooth and vertical sidewalls can be easily obtained using the PDMS soft lithography. Finally, the PDMS replica has to be bonded with a glass slide. Before bonding, the glass slide is coated with a flat layer of PDMS polymer. To perform irreversible PDMS bonding, a handheld corona treater (Electro-Technic Products, BD-20AC) is used for plasma bonding [248]. Cured PDMS is hydrophobic and has a very low surface energy with repeating units of $-\text{O}-\text{Si}(\text{CH}_3)_2-$. It is difficult for chemical species to interact with the PDMS surface and has low adhesion strength. Changing the surface characteristics of the PDMS to achieve a higher surface energy can enhance the adhesion characteristics of the PDMS. Therefore, the PDMS surface can be oxidized by plasma treatment. The corona discharge can electrically break down the chemicals comprising the air. Some of the chemical species can react with the PDMS surface to oxidize it and the methyl groups ($\text{Si}-\text{CH}_3$) are replaced with silanol groups ($\text{Si}-\text{OH}$), i.e. repeating

units of $-\text{O}-\text{Si}(\text{OH})_2-$ are formed. After surface treatment, both surfaces are brought into conformal contact and $\text{Si}-\text{O}-\text{Si}$ bonds are formed with the removal of H_2O molecules. These covalent bonds form the basis of the irreversible seal of the PDMS replica and the glass slide. In addition, the plasma treatment facilitates the flow of fluid inside the PDMS channel because the hydrophobic property is changed to be hydrophilic.

3.4.2 Fiber Bragg grating fabrication

Fig 3.23 Transmission spectrum of single 2-mm long fiber Bragg grating with Bragg wavelength at 1258.55.

Single mode hydrogen-doped photosensitive fibers are used to be inscribed the Bragg grating due to its advantage in yielding higher refractive index changes up to 0.03, in excess of 0.011 in standard optical fiber. The Bragg grating is inscribed on the fiber using standard phase mask exposure technique. Each grating is 2 mm long with Bragg wavelength at approximately 1258.55 nm and coupling strength of -8.5 dB as shown in Fig. 3.23. Based on the simulation, the refractive index change is approximately 3.4×10^{-4} . After fabrication, the optical fibers are put into the convection oven at 100 °C for 24 hours to stabilize the Bragg wavelength.

To package the optical elements, the fibers were cleaved with the edge away from the FBG by 2 mm and then were inserted into the microfluidic chip through the patterned fiber grooves. To monitor the alignment state, one fiber is connected to a tunable laser source (Advantest Inc, AQ4321D) and the other fiber is connected to the optical power meter (Newport, 1835-C). The fibers are aligned to obtain the maximum possible power output. Then, the fibers are fixed by UV curable epoxy

Fig 3.24 Transmission spectrum of the on-chip grating interferometer.

(Ablestik) and then exposed to a UV source (Novacure 2100) for 10 min. The output spectrum of the packaged grating interferometer with a resonant cavity length of 40 μm is shown in Fig. 3.24.

3.4.3 Chip Packaging

Fig 3.25 Packaging of PDMS chip with a chip holder.

As external syringe pumps are used to realize pressure-driven flow in the microchannel, it is important to have robust microfluidic interconnections between the pumps and the chip. The microfluidic connectors used between the silicon tubings and the inlets/outlets are precision tips (EFD, BD-90). However, the tips may be spoilt due to the bending by the tubings or leakage from the tips may occur during experiment. To solve this problem, a slab of poly(methyl methacrylate) is fixed on top of the PDMS chip to improve the rigidity of the tips on the PDMS chip as shown in Fig. 3.25.

3.5 Experimental results and discussions

3.5.1 Preparation of Madin-Darby canine kidney cell

Madin-Darby canine kidney (MDCK) cell is epithelial cell derived from the dog's kidney, which has a nominal size of 16 – 20 μm . The cell seed consists of a monolayer of MDCK cells attached on the inner layer of the cell culture flask. To retrieve MDCK cells for experiment, the cell monolayer in the flask is washed with Trypsin-EDTA. The Trypsin-EDTA causes the cell monolayer to be detached from the flask layer and MDCK cells are separated from one another. Portions of the cells are transferred into a new flask and 10 mL cell culture medium (DMEM + 10% FBS) is added into the flask. The flask is stored in the incubator for the MDCK cell to grow into monolayer in about 3 – 4 days. The portion of cells which are used for experiment is added into a centrifuge tube. The MDCK cells undergo centrifugation to segregate the cells into the bottom layer of the medium. The cell culture medium is pipetted out and $10 \times$ phosphate-buffered saline (PBS) is replaced to form a concentration of about 1.2×10^5 cells mL^{-1} . The MDCK cells are viable in PBS solution for 5 – 6 hours.

3.5.2 Experimental setup

Fig 3.26 Schematic illustration of the experimental setup.

The experiment setup of the on-chip grating interferometer consists of two parts: the microfluidic system and the optical system. The microfluidic system provides the injection of buffer solutions into the microfluidic chip. On the other hand, the fibers are connected to the external optical system for optical measurement. The schematic illustration of the experiment setup is shown in Fig. 3.26.

The microfluidic system consists of 5 sets of syringe pumps (New Era, NE1000). Each pump is filled with different solutions, i.e. MDCK cells in PBS solution (P1), PBS solution (P2), immersion oil (P3 & P5) and higher concentration of PBS solution (P4). The hydrofocusing effect is performed by driving together P1

and P2. Cell plugs are formed by driving P3 to inject immersion oil into the microchannel. The reagent plugs are formed by driving P4 and P5.

The optical system consists of a pair of FBGs, high-power laser, superluminescent light emitting diode (SLED) and optical spectrum analyzer. Firstly, a high-power laser with wavelength 1550 nm and maximum power of 45 mW (Ultrale, CL16e) is used to realize the fiber optical trap. Then, SLED is worked as the broadband light source with the center wavelength of 1275 nm and a bandwidth of 70 nm (Denselight, DL-CS2079A). Finally, the output spectrum is measured by the optical spectrum analyzer (Advantest, Q8384). It has a measuring wavelength range of 600 – 1600 nm with an accuracy of 0.001 nm. Fiber splitters/couplers (50/50) are used to allow both the detection beam and optical trapping beam to be multiplexed into the fibers. It ensures the high transmission efficiency for the trapping laser light while keeping the transmission power of the FBGs to be detectable.

The experiments are carried out for two main objectives. Firstly, single living cell is isolated within a plug and a reagent plug is mixed to the sample plug at the detection area. These results are presented in section 3.5.3. Secondly, the optical measurement of the MDCK cell refractive index and diameter. These results are presented in section 3.5.4.

3.5.3 MDCK cell encapsulation and mixing

Fig 3.27 Microphotos of the focused flow with a width of (a) 1/3, and (b) 1/2 of the width of the outlet.

Fig 3.28 Width of focused flow vs focusing ratio.

Figure 3.27 shows the experiment results for hydrodynamic focusing. The widths of inlet and side channels are both 40 μm . Initially, when the DI water has a velocity of twice as high as the dye buffer, the focused dye buffer flow stream is 1/3 of the width of the outlet as shown in Fig. 3.27

Fig 3.29 Microphotos of the formation of buffer plug with (a) $t = 0$ s, (b) $t = 1$ s, and (c) $t = 1.5$ s.

(a). When the velocity of the dye buffer is increased to be the same as the one of the DI water, the focused dye buffer flow stream is half of the width of the outlet, i.e. $20\ \mu\text{m}$ as shown in Fig. 3.27 (b). This is the width to be achieved for single cell separation as the diameter of MDCK cell is around $15\text{--}18\ \mu\text{m}$. The control of the width of the focused dye buffer flow stream by tuning the flow rate ratio is shown in Fig. 3.28. The flow rate of the DI water flow stream is fixed at $20\ \mu\text{L min}^{-1}$ and the flow rate of the dye buffer flow stream is changed from 5 to $40\ \mu\text{L min}^{-1}$ to achieve focusing ratio of 1.5 to 5 . The width of the focused flow stream is tuned from 7.5 to $27\ \mu\text{m}$. The experimental results agreed well with the theoretical analyses. In order to achieve a focus width of $20\ \mu\text{m}$ for cell focusing, a focusing ratio of 2 is required ($Q_{\text{water}} : Q_{\text{cell}} = 1 : 1$).

Figure 3.29 shows the buffer plug formation process. Immersion oil and standard $10\times$ PBS solution are used in the experiment. The PBS solution is injected from the side channel while immersion oil is injected from the main channel as a carrier flow. The flow rate of the carrier flow is adjusted to investigate the effect of the flow rate ratio between the buffer and carrier flows to the length of plug. Fig. 3.30 shows the length of the buffer plug varied by changing the flow rate of the carrier flow. A buffer plug of $150\ \mu\text{m}$ is formed with a flow rate ratio of 1 . When the flow rate ratio is increased to 3 , a plug of length $75\ \mu\text{m}$ is formed. When the flow rate ratio is further increased to 5 , the plug's length is $50\ \mu\text{m}$. This can ensure single MDCK cell encapsulation. On the other hand, a larger plug length can be

Fig 3.30 Length of PBS plug vs flow rate ratio.

Fig 3.31 Microphotos of single MDCK cell encapsulation process with (a) $t = 0$ s, and (b) $t = 1$ s.

formed with a lower concentration of MDCK cell solution. Figure 3.31 shows the encapsulation of single MDCK cell in a buffer plug. A stable plug is formed in approximately 2 s. The cell plug is then transferred to the measurement area to perform optical measurements and mixing process.

Figure 3.32 shows the mixing process of the dye plug into the buffer plug to investigate the effectiveness of the mixing process. When the plug reaches the

Fig 3.32 Microphotos of (a) the buffer plug being elongated by structural design, and (b) the dye plug being mixed into the buffer plug.

detection area, it is first elongated as it passes through the smaller channel's width, as shown in Fig. 3.32 (a). Afterward, it is mixed with a dye plug as shown in Fig. 3.32 (b). The mixing process is effectively fast in which the cell trapped within the cavity can be measured for the second time after mixing is completed. The mixing process is ensured to be completed when the wavelength shift is stabled.

3.5.4 Measurement of single MDCK cell

Fig 3.33 Transmission spectra of the interferometer with different buffers in the resonant cavity.

Before the grating interferometer can be used for the measurement of single MDCK cell, the interferometer has to be characterized to determine the dimensionless constant k as stated in Eq. 3.16. Initially, the deionized water ($n = 1.3337$) is used as the reference buffer in the microchannel. Then, saline solutions with different concentrations are used to obtain the peak shifts with respect to the RIs of the solutions. Fig. 3.33 shows the shift of resonant peaks as a comparison between the 13% saline solution and the deionized water. The refractive indices of the solutions are measured by a handheld refractometer with a sensitivity of 0.0001 (Reichert, AR200). The relationship between the peak shift and the refractive index change in the cavity is shown in Fig. 3.34. Based on the measured results, the value of k for the system is determined to be 1.56×10^{-4} . For one free spectral range, the refractometer is sufficient to measure the range of change of optical path length

Fig 3.34 Characterization of the wavelength shift with buffers of different refractive indices.

(Δ OPL) up to 640 nm.

After the characterization, the system is then employed for Madin-Darby Canine Kidney (MDCK) cell measurement. Optical trap is employed to trap the single MDCK cell during measurement, which is verified by microscope image. Fig. 3.35 shows the trapping of MDCK cell using optical force. The cell is deviated from the center of the fiber gap when the light intensity of either fiber is reduced. The zoom view of the cell under optical force is shown in the insets of Fig. 3.35, which show that the optical force is high enough to trap the cell but not high enough to cause cell distortion on the shape as the cell is maintained in spherical shape. The MDCK cell is optically trapped within the fiber cavity and is almost perfectly aligned along the core of the optical fiber which greatly improves the stability of the system. In addition, with the presence of optical trapping, the flow is halted to avoid its effect on the cell. The optical trap is applied on the cell less than 5 sec to minimize the phototoxicity and the output spectrum is obtained after the peaks are

Fig. 3.35 Microphotos of the optically trapped MDCK cell, in which the position is changed by varying the optical force of the fibers

Fig 3.36 Microphoto of the MDCK cell being optically trapped in the resonant cavity while a PBS plug is added into the cell plug for buffer modulation.

stabilized. Then, a higher concentration of PBS solution is mixed into the plug to change the refractive index (buffer 2) as shown in Fig. 3.36. The refractive indices of the buffers before and after mixing are determined by the characterized graph obtained in Fig. 3.35, i.e. $n_1 = 1.355$ and $n_2 = 1.360$, respectively.

Figure 3.37 shows the measurement results of the MDCK cell. Based on Fig. 3.37 (a) and 3.37 (b), the peak shifts are determined as $\Delta\lambda_1 = 0.071$ nm and $\Delta\lambda_2 = 0.058$

Fig 3.37 Output spectra of the resonant cavity (a) without/with MDCK cell in buffer 1, and (b) without/with MDCK cell in buffer 2.

nm (resolution of optical spectrum analyzer is set as 0.001). The MDCK cell refractive index and diameter can then be determined, as listed in Table 3.2. Two MDCK cells are measured for four times. The first measured MDCK cell has an effective refractive index of 1.383 ± 0.001 and diameter of $16.7 \pm 1.0 \mu\text{m}$. Similarly

for the second measured cell, the effective refractive index is 1.383 ± 0.001 and the diameter is $16.1 \pm 0.8 \mu\text{m}$.

Table 3.2 Measurement results of MDCK cell effective refractive index and diameter.

	1 st cell		2 nd cell	
	Refractive index	Size (μm)	Refractive index	Size
Measurement 1	1.382	16.7	1.384	15.4
Measurement 2	1.383	16.7	1.384	15.4
Measurement 3	1.382	17.9	1.382	16.7
Measurement 4	1.384	15.4	1.382	16.7
Arithmetic mean	1.383	16.7	1.383	16.0
Standard deviation	0.001	1.0	0.001	0.8

Based on the experimental results, the standard deviation of measured cell refractive index is 0.001 which is improved in precision as compared with the FP method, i.e. 0.002. [102] For the cell size, the standard deviation is larger due to the fact that the cell size varies dynamically in the measurement environment. With the change in the surrounding buffer, osmolarity effect on the cell cannot be avoided. However, with the osmolarity ratio of the buffer approximately 1.2 times, the change in the cell size is less than 1% [226]. In addition, the osmolarity effect can be reduced by taking the measurements immediately after the change in the external buffer.

Cell viability test is performed using Trypan blue. After cell trypsinization, half of the cells are used to perform viability test and half of the cell are used in the experiment. Another viability test is performed after the refractive index measurement by extracting the cells from the microfluidic chip. Trypan blue

Fig 3.38 Microphoto of (a) the viability test of MDCK cells (scale bar represents 100 μm), and (b) dead cell stained by Trypan blue (scale bar represents 10 μm).

selectively stained dead cells in blue color as shown in Fig. 3.38. Based on the viability test, 98 % of the cells (sample size: 200) are remained viable without being stained by Trypan blue before the refractive index measurement. After the refractive index measurement, 93 % of the cells are remained viable (sample size: 30). This shows that the on-chip grating refractometer has no significant effect on the cell viability.

Several issues and challenges of the developed on-chip grating interferometer for single cell measurement are discussed as follow,

- a) During cell encapsulation, it is unable to ensure single cell being encapsulated in a plug. However, several approaches can be employed to improve the efficiency. First, the trypsinized cells in the culture flask are shook to separate cell clusters as much as possible. Second, the encapsulation experiment has to be carried out as soon as possible after the trypsinized cells are resuspended in PBS solution to avoid cell aggregation. Third, the cell density is diluted to 10^5 cells per ml to

improve the single cell encapsulation. In the experiment, plugs with single encapsulated cell are chosen for RI and size measurements. Other plugs without any cell or with more than one cell are ignored.

- b) One of the advantages of multiphase flow is the isolation of cells from external surrounding environment. Cell stiction on microchannel does not occurred as the cells are isolated by the oil phase. This can prolong the usage of the system in which less residual is found in the microchannel after measurements.
- c) For real-time monitoring the viability of the cells in the microchannel, the cells can be pre-stained with Calcein AM, which is transported into live cell and emitting green fluorescence. This can provide an insight on the viability of the cell during refractive index measurement.
- d) For each measurement, the time period with the cell in the cavity was ranging from 20 to 30 s. With the function of compartmentalization, sequential measurement is possible by integrating a full microfluidic control network.

3.6 Summary

This chapter focuses on the theoretical analysis, optical designs, microfluidic designs, chip fabrication and experiments of the on-chip grating interferometer. The theoretical analysis explores the optical grating interferometer and the differential measurement scheme, which guides the design of the grating interferometer as the refractometer for single cell refractive index and size measurement. The on-chip refractometer is integrated with optical trap to manipulate the cell during measurement in a non-contact mean. Subsequently, the microfluidic system is studied and designed, including the hydrodynamic focusing mechanism for single cell alignment, the multiphase plug generating mechanism for single cell encapsulation and the plug mixing mechanism used to realize the differential measurement scheme. In addition, chip fabrication processes are developed for the fabrication of the on-chip grating interferometer using polymer PDMS material. Experiments show that the developed on-chip grating interferometer has a k of 1.56×10^{-4} , which is characterized using saline buffer solutions. It has a detection range of 640 nm in the change of optical path length and a sensitivity of 2.63 nm per RIU for a sample with 16 μm diameter. The interferometer is employed to measure the refractive index and size of MDCK cell with a precision of 0.001 RIU.

The innovation of the developed on-chip grating interferometer is summarized as follow,

- a) This is the first effort demonstrated to design a cell refractometer using the Fiber Bragg grating interferometer combined with differential measurement scheme. The accuracy of refractive index measurement is

improved. The precision of the measurement is improved to 0.001 as compared to the one with FP resonator [102]. The precision of the measurement can be further improved by increasing the finesse of the grating interferometer. This can be done by reducing the light propagation loss in the microchannel using microlenses or improving the Q-factor of the fiber grating.

- b) A single pair of optical fibers is employed for cell trapping and cell refractive index measurement, which avoid the alignment problem in measurement and reduce the complexity in integration and packaging. In previous demonstrated FP resonator, the pair of fibers cannot be used for optical trapping as they are gold-coated. An external micro-capillary tube is used to fix cell in the cavity, which complicates the packaging process and may leads to cell distortion [102]. In this system, optical trapping is used as non-contact trapping technique. The optical power can be easily tuned not only to efficiently trap the cell within the micro-cavity, but also to avoid cell distortion.
- c) A droplet microfluidic system is designed, fabricated and demonstrated to realize differential measurement scheme with enhance mixing. It has the capability to isolate suspended cells into a series of plugs for single cell measurement with the designed microfluidic networks. Further biological analytic or handling processes can be easily integrated into the microfluidic networks.

CHAPTER 4

OPTOFLUIDIC MICROLENS AND GRATING

This chapter presents the design, the fabrication and the experiment of the optofluidic components, which include microlens and grating. The optofluidic microlens is formed by three laminar flow stream of liquids with different refractive indices. Light is refracted at the interfaces between the liquids and the radii of curvature of the interfaces can be tuned by varying the flow rates of the three flow streams. The optofluidic grating is realized using multiphase droplet microfluidics. A stream of plugs is formed by two immiscible liquids with refractive index contrast and acted as long-period grating (LPG). The grating period is tunable by changing the flow rates of the two liquids. First, the working principles and the design criteria of the optofluidic microlens and grating are presented. Second, the fabrication processes and the experimental setups for both optofluidic components are illustrated. Finally, the experimental results are discussed in details.

4.1 Optofluidic microlens

Fig 4.1 Schematic illustration of the overview of the on-chip optofluidic microlens.

The integration of tunable optical components into an adaptive optical system is essential in realizing Lab-on-a-chip (LOC) system. Many research efforts have been put into combining integrated micro-optical components with microfluidic systems. The ultimate goal is to integrate different optical components such as lenses, gratings, splitters, waveguides etc onto a single chip that encapsulates different optical capabilities especially for single cell analysis and biochemical sensing. LOC provides the tunabilities in microfluidics to vary the optical properties.

Microlenses are one of the most important components in optical system, which are commonly employed to refract and change the optical light path. For example, diverging light from an optical fiber can be collimated and propagates into the FP resonator for efficient light coupling. Light can also be focused into a point

for localized fluorescent excitation. Many research efforts have been put into developing different fluid actuation technique in realizing optofluidic microlenses, such as hydraulic pressure [249], redox surfactants [250], electrowetting [251] and stimuli-responsive hydrogels [252] etc. However, these techniques have limited tunable range in the radius of curvature of the microlens, require complicated fabrication processes, and also focus light perpendicular to the plane of the device. To overcome these limitations, an on-chip optofluidic microlens, which employs three laminar flows to form the microlens in an expansion chamber is designed [253 – 254]. The overview of the on-chip optofluidic microlens is shown in Fig. 4.1. The curvatures of the microlenses can be easily tuned by controlling the flow rates of the liquids. An optical fiber is integrated in-plane with the microlens. In the next section, the working principles and the design criteria for the optofluidic microlenses are discussed.

4.1.1 Optofluidic microlens via three laminar flows

Fig 4.2 Schematic illustration of the streamlines for the flow entering and exiting the expansion chamber.

The optofluidic microlens is formed based on the hydrodynamic focusing flow with an expansion chamber. The flow field is governed by the incompressible Navier-Stokes equations, together with the governing mass and momentum equations, which can be expressed as

$$\nabla_i u_i = 0 \quad (4.1)$$

and

$$\rho \frac{\partial u_i}{\partial t} + \rho (u_j \times \nabla_j) u_i = -\nabla_i p + \mu \nabla^2 u_i \quad (4.2)$$

where u_i denotes the liquid velocity, ρ and P are the density and the pressure, respectively. When three flow streams are injected into the focusing junction, the straight liquid interface is formed between two neighboring flows, in which the

position of the interface is depending on the flow rate ratio between the flows. However, when the three flow streams are flowing into the expansion chamber, the interfaces between two neighboring flows are gradually deviated from the original position as shown in Fig 4.2. Similarly, when the three flow streams are flowing out of the expansion chamber, the interfaces are gradually repositioned to the original position. As a result, the curve interfaces are formed in the expansion chamber, and a microlens is formed when the liquids have different refractive indices.

The curving direction and the degree of curvature of the interface are depending on the position of the interface. For instance, when the interface is positioned at the center of the microchannel, it remains as a straight interface as highlighted in Fig. 4.2. If the interface is positioned at the left of the microchannel, it is left deviated in the expansion chamber, and vice versa. The degree of curvature of an interface is higher for the one that is further from the center of the microchannel. Based on these phenomena, microlenses with different lens profile and different radii of curvature can be realized by tuning the flow rates of the flow streams as shown in Fig. 4.3. A biconvex microlens is realized by having higher flow rate in the central flow stream and maintaining the same flow rate for both the side flow streams ($Q_{\text{center}} > Q_{\text{left}} = Q_{\text{right}}$) as shown in Fig. 4.3 (a). By gradually increasing the flow rate of the right flow stream, the degree of curvature is reduced; and up to a point when the right interface is positioned at the center of the microchannel, the right interface remains as straight in the expansion chamber such that a planar convex microlens is realized as shown in Fig. 4.3 (b). By further increasing Q_{right} , the position of the right interface is further shifted to the left of the center of the microchannel. Therefore, the right interface is left deviated in the expansion chamber and a concave convex microlens is formed as in Fig. 4.3 (c).

Fig 4.3 Formation of optofluidic microlens: (a) biconvex microlens, (b) planar convex microlens, and (c) concave convex microlens.

The positions of the interfaces can be theoretically determined based on the hydrodynamic focusing model as shown in Fig. 4.4 [255]. Assuming the central flow stream is injected with liquid A and the side flow streams are injected with liquid B, the viscosity ratios and the flow rate ratios between the side flow streams and the central flow stream are defined as

$$\beta = \frac{\eta_B}{\eta_A} \quad (4.3)$$

and

$$\kappa_1 = \frac{Q_{\text{right}}}{Q_{\text{center}}}, \quad \kappa_2 = \frac{Q_{\text{left}}}{Q_{\text{center}}} \quad (4.4)$$

where η_A and η_B are the viscosities of liquid A and liquid B, respectively. The relative position of the centerline of the core flow stream can be estimated as

$$W_{\text{right}} = \frac{W}{1 + (1 + 2\beta\kappa_2) / (1 + 2\beta\kappa_1)}. \quad (4.5)$$

Fig 4.4 Analytical model of the interfaces of the three flow streams in hydrodynamic focusing.

The interface positions of the core flow stream measured from the centerline of the core flow stream can be estimated as

$$r_1 W_{\text{right}} = \frac{W_{\text{right}}}{1 + 2\beta\kappa_1} \quad (4.6a)$$

and

$$r_2 W_{\text{left}} = \frac{W_{\text{left}}}{1 + 2\beta\kappa_2} \quad (4.6b)$$

These equations are the approximations with the condition that the microchannel has a low aspect ratio.

In the experiment, the expansion chamber has a dimension of $525 \times 525 \mu\text{m}$. Calcium chloride solution (6.22 mol L^{-1} , $\eta \approx 8 \text{ mPa s}$) and isopropanol solution ($\eta = 1.96 \text{ mPa s}$) are used as the core and side flow streams, respectively. Based on these experimental conditions, the flow rate conditions of the three flow streams in forming the optofluidic microlens with the respective lens profiles are analyzed.

First, the flow conditions in realizing biconvex microlens are discussed. From Eqs. 4.5 and 4.6, it can be clearly seen that a symmetrical biconvex microlens can only be formed when the flow rate ratios $\kappa_1 = \kappa_2$. Under this flow condition, the

centerline of the core flow stream is positioned at the center of the microchannel ($W/2$). The width of the core flow stream or the thickness of the microlens is estimated as

$$W_{\text{lens}} = \frac{525}{1 + 0.49\kappa_1}. \quad (4.7)$$

By maintaining the flow rates of the side flow stream to be 50 $\mu\text{l}/\text{min}$, the flow rate of the core flow stream is varied from 5 to 50 $\mu\text{l}/\text{min}$. The simulated microlens thickness based on Eq. 4.7 is shown in Fig. 4.5. Based on the simulated streamline of the microlens using Comsol Multiphysics, the respective radius of curvature of the interface is determined and shown in Fig. 4.5. The radius of curvature is decreasing while the thickness of the microlens is increasing when the central flow rate is increased. The smallest achievable radius of curvature is limited to 270 μm , which is approximately half of the width of the expansion chamber.

Next, the flow conditions to realize a planar convex microlens is discussed. For a planar convex microlens, one of the interfaces has to be straight in the expansion chamber, which means that the interface is positioned at the center of the microchannel such that

$$W_{\text{right}} - r_1 W_{\text{right}} = \frac{W}{2}. \quad (4.8)$$

By substituting Eqs. 4.5 and 4.6a into Eq. 4.8, the flow conditions can be defined as

$$\kappa_1 = \frac{1}{\beta} + \kappa_2. \quad (4.9)$$

With the combination of CaCl_2 solution and IPA solution ($\beta = 0.245$), the relation between κ_1 and κ_2 in realizing planar convex microlens is shown in Fig. 4.6. To realize planar convex microlens, the flow rate of the right flow stream has to be higher

Fig 4.5 Simulated thickness and radii of curvature of the optofluidic biconvex microlens at different flow rate ratios.

than the one of the left flow stream such that the core flow stream can be squeeze to the left of the centerline of the microchannel. In addition, higher the flow rate ratio leads to the formation of planar convex microlens with smaller thickness and larger radius of curvature.

Based on Eq. 4.9, it can be easily defined the flow conditions in forming interfaces with different curvatures, which are expressed as

$$\left\{ \begin{array}{ll} \kappa_1 < \frac{1}{\beta} + \kappa_2 & \text{Convex} \\ \kappa_1 = \frac{1}{\beta} + \kappa_2 & \text{Planar} \\ \kappa_1 > \frac{1}{\beta} + \kappa_2 & \text{Concave} \end{array} \right. \quad \text{for right interface} \quad (4.10a)$$

and

Fig 4.6 Simulated thickness of the optofluidic planar convex microlens at different flow rate ratios.

$$\begin{cases} \kappa_2 < \frac{1}{\beta} + \kappa_1 & \text{Convex} \\ \kappa_2 = \frac{1}{\beta} + \kappa_1 & \text{Planar} \\ \kappa_2 > \frac{1}{\beta} + \kappa_1 & \text{Concave} \end{cases} \quad \text{for left interface} \quad (4.10b)$$

The transformation of the lens profile from biconvex to concave convex can be investigated by maintaining the flow rate ratio κ_2 to be constant and gradually increasing the flow rate ratio κ_1 . The simulated lens profiles with the radii of curvature for both interfaces of the microlens at different flow rate ratios are shown in Fig. 4.7. By gradually increasing the flow rate of the right flow stream, the radius of curvature of the right interface is gradually increased. At the point when the interface becomes flat with infinite radius of curvature, a planar convex lens is formed. When the right cladding flow rate is further increased, the interface forms a concave curvature and a concave convex lens is formed. The radius of curvature is

Fig 4.7 Simulated transformation from biconvex to concave convex microlens by varying the flow rate ratio κ_1 .

gradually decreased. For the left interface, the radius of curvature is decreased because the interface is squeezed by the right interface. Similar effects can be observed on the left interface by increasing the flow rate of the left flow stream and keeping the other two flow streams to be constant.

The optofluidic microlenses consist of attractive characteristics in which other microlenses do not possess. First, the microlens can be reconfigurable dynamically with different lens profiles by adjusting the flow rates of the three flow streams in real time. Second, different combinations of liquids can be used to achieve different refractive index contrast across the core and the side flow streams, and subsequently different operating focal distance. Third, the liquid interfaces between the three laminar flows are optically smoothed when the optofluidic microlenses are formed in the low Reynolds number regime. Fourth, the fabrication

process of the chip involves only a single step fabrication which greatly reduces the complexity in fabrication and integration. The fiber grooves, the optofluidic microlenses and the FP cavity are pre-aligned during fabrication.

4.1.2 Design of the expansion chamber

Fig 4.8 Simulated flows in the expansion chambers with width-to-length aspect ratio of 1 : 1, 2 : 1 and 1 : 2, respectively.

In order to form an optofluidic microlens with optimum optical precision, which is affected by the lens curvature and the liquid stability, the physical structure of the expansion chamber has to be designed. The shape of the optofluidic microlens is studied by simulating the flows of the three laminar flow streams using Comsol Multiphysics based on two parameters, i. e. the width-to-length aspect ratio and the injection angle of the chamber.

For the optofluidic microlens, the optical aberration is prominent in the case where the deviation of the curvatures of the core-cladding interfaces from the ideal lens' curvature is significant. To reduce optical aberration, the flows of the three laminar flow streams in expansion chambers with aspect ratio of 1 : 1, 2 : 1 and 1 :

Fig 4.9 The simulated flows in the expansion chambers with injection angle of 90°, 70° and 60°, respectively.

2 are simulated, respectively as shown in Fig. 4.8. For the expansion chamber with an aspect ratio of 2 : 1, the shape of the interface at the central region is perfectly parallel to the chamber wall (the radius of curvature, $R = \infty$), which is the worst deviation from the ideal lens' profile. On the other hand, if the aspect ratio of the expansion chamber is 1 : 2, the shapes of the interfaces are comparable to the ideal lens' shape. However, two recirculation zones are formed at both ends of the chamber walls. The presence of the recirculation zones causes the instability in the liquid flows and the undesired asymmetry in the core-cladding interfaces. In short, the aspect ratio of the expansion chamber has to be 1 : 1 to minimize the deviation of the interfaces from the ideal lens' shape and to avoid the instability caused by the recirculation zones in the expansion chamber.

The sudden expansion of the microchannel's width in the expansion chamber causes the abrupt change in the flow rates of the liquid flow streams. This induces turbulence flow and causes recirculation zones at all edges of the chamber with a 90° sudden expansion angles as shown in Fig. 4.6. The existence of the recirculation zones at the edges in the expansion chamber can be avoided by designing the chamber with an injection angle smaller than 90°, such as 70° and 60° as shown in Fig. 4.9. By comparing the two cases, the shape of the interfaces for 70°

injection angle has a better match with the ideal lens. Therefore, an aspect ratio of 1 : 1 and an injection angle of 70° are chosen in the design of the expansion chamber.

4.1.3 Focusing power of optofluidic microlenses

Fig 4.10 Simulated focal distance of the optofluidic biconvex microlens with varying radius of curvature.

In Lab-on-a-chip application, microlenses are commonly used to collimate or focus diverging light such as the one emitted from the optical fiber. The focusing power of the optofluidic microlens can be determined by the lensmaker's equation,

$$\frac{1}{f} = (n-1) \left[\frac{1}{R_1} - \frac{1}{R_2} + \frac{(n-1)t}{nR_1R_2} \right] \quad (4.11)$$

where n is the relative refractive index, t is the thickness of the lens and R_1 and R_2 are the radii of curvature of the first and second interface, respectively. The focusing power of the simulated microlenses in Fig. 4.5 and 4.7 are illustrated in Fig. 4.10 and 4.11, respectively. For biconvex microlens, the focal distance is reduced with the decrease of the radius of curvature. To reduce propagation losses, a short focal distance is essential such that the distance between the light source or detector and the optofluidic microlens is shorter. Based on the lensmaker's equation, the focal distance (f) for symmetrical biconvex microlenses ($R_1 = -R_2 = R$) can be determined as

$$\frac{1}{f} = (n-1) \left[\frac{2}{R} + \frac{(n-1)t}{nR^2} \right] \quad (4.12)$$

It can be clearly seen that shorter focal distance can be achieved by higher relative refractive index or shorter radius of curvature. To achieve higher relative refractive index, different combination of liquids can be employed as the central and the side flow streams. In the simulation, CaCl_2 solution with refractive index of 1.462 and IPA with refractive index of 1.382 are used, respectively. Therefore, a contrast of 0.08 in refractive index value is achieved. Shorter focal distance can be achieved by designing a smaller chamber as the minimum radius of curvature is limited by the width of the chamber ($R_{\min} = \frac{1}{2}$ width). However, smaller chamber will induce difficulty in fabrication because the microchannel is at least 5 times smaller than the chamber and also the beam width is limited by the length of the chamber (aspect ratio 1 : 1). Therefore, one has to consider the requirements for the optical system in the design.

The focusing powers of the microlens with different lens profiles are shown in Fig. 4.11. The focal distance is increased when the radius of curvature of the right

Fig 4.11 Simulated focal distance of the optofluidic microlens with different lens profiles.

interface changed from biconvex to planar convex. The focal distance decreased near the region where a planar convex microlens is formed because the radius of curvature of the left interface is reduced when the right flow stream squeezed to the left. The focusing power is improved by the left interface. However, the focal distance is increased when a concave interface is formed in the right interface and no significant reduction in the radius of curvature of the left interface.

In short, the focusing power of the optofluidic microlens is limited to > 2 mm with the chosen liquid combination. Using other liquid combination with a contrast of 0.27, the focusing power can be enhanced to approximately $800 \mu\text{m}$. Another approach to effectively shorten the focal distance is by the compound biconvex microlenses. The effective focal distance of the compound biconvex microlenses is estimated as

$$\frac{1}{f} = \frac{1}{f_1} + \frac{1}{f_2} - \frac{d}{f_1 f_2} \quad (4.13)$$

where d is the distance between the two microlenses, f_1 and f_2 are the focal length of the first and second microlens, respectively. For microlenses with focal length of 2 mm and a separation of 625 μm , the effective focal length is shortened to 1.185 mm. In the experiment, two expansion chambers with a center-to-center separation of 625 μm are employed to demonstrate the focusing power of the compound biconvex microlenses.

4.2 Experimental results and discussions of optofluidic microlens

4.2.1 Fabrication processes and experimental setup

Fig 4.12 Schematic illustration of the experimental setup of the optofluidic microlens.

The fabrication of the optofluidic components are primarily based on the polymer soft lithography fabrication technique as discussed in Chapter 3. PDMS is transparent at visible and infrared wavelengths which makes it suitable for optofluidic devices. The use of the PDMS material minimizes propagation loss. The experimental setup of the optofluidic microlens is illustrated in Fig. 4.12. A continuous-wave argon ion laser (Melles Griot, 35-MAP-321) with wavelength 514 nm and intensity of 150 mW is coupled into the optofluidic chip via a single-mode optical fiber. The input fiber is inserted and 1 mm away from the center of the

expansion chamber. The liquids are injected into the optofluidic chip via syringe pumps (New Era, NE-1000). Microphotos of the microlens in the expansion chamber are captured using a charge-coupled digital camera (Olympus, DP70) under the inverted microscope (Nikon, TE2000-E). The radii of curvature of the microlens' interfaces are processed digitally (MATLAB R2008A) using the captured microphoto. Each microphoto is processed by selecting three points on the interface to measure the radius of curvature of the microlens. The process is repeated by 20 times and the average value is recorded. For the optofluidic microlens, the experiments are carried out for two main objectives. First, the varying microlens profile via the tuning of the flow rate of the liquids is investigated. Second, the focusing power of the optofluidic microlens is measured. These results are presented in section 4.3.2.

4.2.2 Formation and tuning of optofluidic microlenses

The formation of biconvex microlenses with different radii of curvature is shown in Fig. 4.13. In the experiment, two types of expansion chambers are used, i.e. 375 μm and 525 μm to visualize the tunability in the radius of curvature of the microlens. The flow rate of the side flow streams is maintained at 50 $\mu\text{L}/\text{min}$ and the flow rate ratio ($\kappa_1 = \kappa_2$) is varying from 1 to 10. By gradually increasing the flow rate ratio, the radius of curvature is gradually increased and the thickness of the microlens is decreased. The diffusive broadening effect is not significant with the selected liquids and flow rate as shown in the insets. The smallest radius of curvature achieved by the expansion chamber with dimension of 375 μm and 525 μm are 190 and 275 μm , respectively. By comparing these results, it can be

Fig 4.13 Tuning of the radius of curvature with respect to the change of the flow rate ratio.

concluded that smaller expansion chamber can lead to larger tunable radius of curvature because the minimum radius of curvature is limited by the dimension of the expansion chamber (approximately $\frac{1}{2}$ of the width of the expansion chamber).

Fig. 4.14 shows the experimental results of realizing three microlenses with different lens profiles by simply tuning the flow rate of the right flow stream. In the experiment, the expansion chamber has a dimension of 525 μm . By maintaining the flow rate ratio between the left flow stream and the core flow stream at $\kappa_2 = 7$, the flow rate ratio between the right flow stream and the core flow stream is increased from 7 to 13. The tuning of the optofluidic microlens from biconvex lens and planar convex lens to concave convex lens is obtained. Initially, the biconvex lens with radius of curvature of 375 μm is formed in the chamber when both side flow streams have the same flow rate ratio. When the flow rate of the right flow stream is increased, the radius of curvature of the right interface is increased and reached

Fig 4.14 Tuning of the lens profile with respect to the change of the flow rate ratio κ_1 .

infinity when the flow rate ratio κ_2 reaches 11. Consequently, a planar convex lens is formed in the chamber. By further increasing the flow rate of the right flow stream, the right interface is bent toward the left and a concave convex lens is formed. Meanwhile, the left interface is squeezed by the right interface during the whole process. The radius of curvature of the left interface is reduced from 405 μm to 205 μm when the flow rate ratio κ_2 reaches 13. In summary, the formation of optofluidic microlens using three laminar flows in the expansion chamber provides the flexibility in tuning the radius of curvature of the interfaces and also the shape of the microlens. In the next section, the focusing power of the optofluidic microlens is studied and discussed.

4.2.3 Focusing power of optofluidic microlenses

Fig 4.15 Optical micrographs of (a) diverging light emitted from the optical fiber, and (b) focused light with the presence of the optofluidic microlens.

The tuning of the lens profile and the radius of curvature of the optofluidic microlens has been demonstrated. In this section, the focal distances for different microlenses are investigated. Fig. 4.15 (a) shows that the light emerged from the optical fiber is diverging without the formation of the optofluidic microlens. When the biconvex microlens is formed in the expansion chamber, the light is focused after propagated across the microlens as shown in Fig. 4.15 (b). The focal distance is approximately 3.3 mm from the centerline of the expansion chamber. By analyzing the pixel intensity across the cross section, which is highlighted by the dotted lines, the focused light has a higher intensity of approximately 3-fold.

The tunability of the focal distance via the change of the flow rate ratio is investigated with the formation of optofluidic biconvex microlens in the expansion

Fig 4.16 Change in focal distance of the biconvex microlens with the variation in the radius of curvature.

chamber. The radius of curvature of the biconvex microlens is varied from 275 to 813 μm . Fig. 4.16 shows the tuning of the focal distance as a function of the radius of curvature of the biconvex microlens. The focal distance is measured from the centerline of the expansion chamber to the focused point of the light. The change of the focal distance matches with the one predicted using the lensmaker formula, in which microlens with smaller radius of curvature has higher focusing power. In the experiment, the expansion chamber has a dimension of $525 \mu\text{m} \times 525 \mu\text{m}$ and the formed microlens has a minimum radius of curvature of 275 μm . Therefore, the focusing power of the formed biconvex microlens is limited to $> 2 \text{ mm}$.

There are three feasible ways to improve the focusing power by reducing the focal distance. First, the achievable radius of curvature is smaller for a smaller dimension of expansion chamber and subsequently the focal distance is reduced. Second, the liquid combination between the three flow streams can be changed to

Fig 4.17 Microphoto of the compound optofluidic microlenses.

achieve higher refractive index contrast and subsequently higher focusing power can be achieved. Third, the focal distance can be shortened by employing compound microlenses as shown in Fig. 4.17. The two optofluidic microlenses are separated with a 100- μm gap of PDMS between the expansion chambers. With two optofluidic microlenses, the light ray passing through the microlenses undergoes two refractions and subsequently focused on the focal point, which is relatively shorter than the one with single microlens.

In the experiment of the compound microlenses, the dimension of the expansion chamber and the liquid selection are the same as the single microlens. The second microlens is maintained with a radius of curvature of 300 μm . The radius of curvature of the first microlens is tuned from 275 μm to 370 μm by changing the flow rate ratio. The focal distance is defined as the distance between the focal point to the centerline of the first expansion chamber. Similar to the single microlens, the change of the focal distance matches with the one predicted using the

Fig 4.18 Change in focal distance of the combinational microlenses with the variation in the radius of curvature of the first microlens.

lensmaker formula, in which microlens with smaller radius of curvature has higher focusing power as shown in Fig. 4.18. The focal distance has been shortened from 2.5 mm to 1.4 mm. To further reduce the focal distance, the second microlens can be tuned to achieve smaller radius of curvature.

In summary, a real-time tuning of the optofluidic microlens has been demonstrated and the focusing power is investigated. The combinational microlenses have achieved shorter focal distance and provide a wider tuning range in focal distance. The optofluidic microlenses provide several advantages as compared to others. First, the fabrication process of LOC chip only involves a single step fabrication. Second, the interface between two laminar flows, which forms the curvature of the optofluidic microlens, remains optically smooth. Light scattering or intensity losses are reduced. The diffusion at the liquid interfaces due to the concentration difference between the liquid layers is minimized using faster

flow rate because the diffusion distance of Ca^{2+} and Cl^- ions is reduced [256]. In addition, the designed injection angle (70°) has further reduced the diffusion effect by avoiding the presence of the circulation vortex in the expansion chamber. Third, the optical tunability is relied on the tunability of the optofluidic microlens and the dynamic modification of the optical properties through local variations of the refractive indices. In the experiment, the refractive index contrast achieved is 0.08, which is ten to a hundred times higher than the one of the thermal effect and the electro-optic [251].

4.3 Optofluidic grating

Long-period grating (LPG) has been developed with promising applications in optical sensors. The LPG consists of a periodic perturbation of refractive index with a typical period of several hundred micrometers and a length of several millimeters to centimeters. It acts to couple propagating light from core mode to cladding modes, which leads to a series of attenuation bands centered at discrete wavelengths in transmission spectrum. The central wavelength and the attenuation strength of the attenuation bands depend on the index variation, the period and the length of the gratings. For sensor applications, fiber LPGs are used as temperature, strain and refractive index sensors [257] etc. Despite the broad applications of the fiber LPGs, optical fibers significantly limit the performance of the LPG due to its constraints in geometry and material. To overcome these limitations, the realization of the LPGs in planar optical waveguide structures have been explored [258]. An optical waveguide can be fabricated using different materials and into different geometries, which provide the possibilities in design and application. However, the realization of the LPG in solid waveguide has limitations in tunability. For example, the tunability of the central wavelengths of the attenuation bands can only be performed by tuning the temperature. It might not be suitable for applications with very stringent requirement in temperature such as biosensor. Therefore, a liquid waveguide can be employed to realize a widely tunable LPG using droplet microfluidics.

Recently, droplet microfluidics has been widely used in biochemical engineering, such as microreactors and microsorters. It has also been used in LOC [259] in sensing applications due to its wide tunability in the optical characteristics [260 – 261]. In order to improve the optical performance and the stability of these

Fig 4.19 Schematic illustration of the overview of the on-chip optofluidic grating.

MOFS devices, research works focus on the stability and the monodispersity of the plug formation have also been tremendously developed [262]. These results further suggest the potential of droplet microfluidics as a promising technology in LOC.

The overview of the on-chip optofluidic grating is shown in Fig. 4.19. The optofluidic grating consists of T-junction with two inlets being injected with two immiscible liquids for plug formation. The stream of plugs in the microchannel and the chip material (PDMS) are acted as the core and the cladding layer of the optofluidic grating. Air gaps are included at both sides of the microchannel, which play the role of the external layer to improve the optical properties of the optofluidic grating. Two fibers are aligned between the two ends of the microchannel as the optical input and output. In the next section, the working principles and the design criteria for the optofluidic microlenses and gratings are discussed.

4.3.1 Optofluidic grating via droplet microfluidics

Fig 4.20 Working principle of optofluidic grating.

An optofluidic grating is designed and shown in Fig. 4.20. In this optofluidic grating design, the two-phase plugs in the microchannel is the core layer and the chip material is the cladding layer. With the refractive index of the oil phase and the aqueous phase to be higher than that of the chip material (PDMS), a microfluidic waveguide is formed by droplet microfluidics for the light propagation inside the microchannel. Two fibers are aligned between the two ends of the microchannel. One fiber is connected to a light source and the other fiber is connected to an optical detector or analyzer. With a broadband input, the output spectrum has an attenuation band at a discrete wavelength. As light propagates in the core and encounters the optofluidic grating, a portion of the light with certain distinct wavelengths from the core are coupled with the cladding modes, and propagate in the same direction. Since the light in the cladding layer cannot be coupled back to the core due to the high attenuation rate in the cladding modes, resonant attenuation bands in the transmission spectrum is generated as shown in Fig. 4.20. The central wavelengths of the attenuation bands (λ_i) are derived using the phase-matching condition of coupled-mode theory as

$$\lambda_i = (n_{\text{core}} - n_{\text{clad}}^i) \Lambda \quad (4.14)$$

where Λ is the grating period, and n_{core} and n_{clad} are the effective refractive indices of the core mode and i^{th} cladding mode, respectively. In the optofluidic grating, the central wavelength can be easily tuned by varying the period of the droplet grating using different ratios of the flow rates of the two immiscible liquids. On the other hand, the attenuation strength depends on the refractive index variation in the core (the refractive index difference between the oil phase and the aqueous phase) and the grating length.

4.3.2 Droplet microfluidics for plug formation

In the formation of the plugs, the immersion oil is injected into the main microchannel and known as the carrier liquid. CaCl_2 solution is injected into the side branch and known as the dispersed liquid. Plugs of CaCl_2 are formed at the T-junction when the dispersed liquid is squeezed into the carrier liquid in the main microchannel. In fluid dynamics, the capillary number shows the relative effect of the viscous forces such as shear stress to the interfacial tension acting across the interface between the two immiscible liquids. The capillary number is expressed as

$$Ca = \frac{\mu v}{\gamma} \quad (4.15)$$

where μ is the viscosity, v is the mean velocity of the carrier fluid and γ is the interfacial tension.

With the chosen liquids, the capillary number is only dependable to the mean velocity of the carrier liquid. For the flows at low values of capillary number (typically $< 10^{-2}$), the breakup of the two immiscible threads at the T-junction is dominated by the interfacial forces and not the shear stresses. In this regime, the shear stress does not have significant effect on distorting the interface when the

dispersed liquid enters the main microchannel. Subsequently, the dispersed liquid blocks almost the whole cross-section of the main microchannel. As a result, the flow of the carrier liquid is confined within the thin wetting films on the sidewalls of the microchannel and pressure is built up upstream of the emerging droplet [245]. This leads to the reduction of the neck of the immiscible tread and eventually a plug is formed at the T-junction. The size of the plug (L) is determined solely by the ratio of the flow rates of the two immiscible liquids and is expressed as

$$L = w \left(1 + a \frac{Q_d}{Q_c} \right) \quad (4.16)$$

where w is the width of the microchannel, a is a constant of order one, Q_d and Q_c are the flow rates of the dispersed and carrier fluids, respectively [245]. The constant a is determined experimentally and is depending on the structure of the microchannel including width and height. The minimum size of the plug is limited by the width of the microchannel, and droplets with size smaller than w cannot be formed under this regime. In summary, different plug lengths and grating periods can be realized using different flow rates of the two liquids.

4.3.3 Liquid selection

In order to realize a high optical performing liquid tunable grating, the liquids used must have low light absorption or high transmission in the detection wavelength. The absorptions of the selected liquids are measured and the results are listed in Table 4.1. The concentrations of CaCl_2 solutions are 1.60 mol L^{-1} ($n = 1.372$) and 4.16 mol L^{-1} ($n = 1.426$), respectively. The result shows that nearly 90 - 99% of the light energy is absorbed in DI water and CaCl_2 solution at 1550 nm. For the other case, the absorption is reduced from 99% to 50% when 1275 nm light source is used.

TABLE 4.1. Light absorption of different liquids ($\lambda = 1275 \text{ nm}$ and 1550 nm)

	DI water	CaCl_2 ($n = 1.372$)	CaCl_2 ($n = 1.426$)	Oil ($n = 1.462$)
1275 nm	0.52	0.49	0.48	0.05
1550 nm	0.99	0.93	0.91	0.34

4.3.4 The effect of shape profile of the plugs

Fig 4.21 Light propagation in microchannel with different types of plugs. (a) Flat curvature, (b) contact angle of 60° , (c) contact angle of 45° and (d) rear concave curvature.

The efficiency of light confinement in the microchannel is investigated against different shape profiles of the plugs formed. Four different shape profiles of the plugs are simulated as shown in Fig. 4.21. In the simulation, the length of the microchannel is 7 mm, the period is $100\ \mu\text{m}$ and the refractive indices of the carrier and dispersed flows are 1.462 and 1.430, respectively. The ideal shape profile is a plug with 90° contact angle, which is same as solid grating, as shown in Fig. 4.21 (a). In this ideal condition, light is perfectly confined within the microchannel and the grating effect is not degraded or affected. When the contact angle is reduced to 120° , the confinement of light within the microchannel is still preserved as shown in Fig. 4.21 (b). However, the confinement of light is degraded when the plug formed has a contact angle of 135° . The light diverges out to the cladding layer after it propagates through 5 plugs as shown in Fig. 4.21 (c). It leads to optical loss and light cannot be detected after it is reflected back. Fig. 4.21 (d) shows another shape

Fig 4.22 Force diagram of the surface tension forces and the adhesive force acted on the boundary of the three phases.

profile where the rear interface has a concave curvature. This kind of shape profile is also unable to confine the light in the core, which is same as the one in Fig. 4.21 (c). Therefore, to preserve the confinement of light in the microchannel, the plugs formed should have a lower contact angle of approximately 90° .

The contact angle of the CaCl_2 plug in the PDMS microchannel filled with immersion oil is determined by the surface tensions exist at the interfaces between the three phases. When the CaCl_2 plug form a boundary with the immersion oil, the geometry of the plug is reformed to balance all the forces acted at the interface. By then, the plug is stabilized with a contact angle of θ . The forces acted on the interface are illustrated in Fig. 4.22. F_{LS} , F_{LO} and F_{OS} are the surface tension forces between the liquid-solid interface, liquid-oil interface and the oil-solid interface. F_A is the adhesive force between the liquid and solid surface. By balancing the forces in the horizontal direction, a relation between the contact angle and the surface tension forces can be defined as

$$F_{LS} - F_{OS} = -F_{LO} \cos \theta. \quad (4.17)$$

Since the surface tension forces are in direct proportion to their respective surface tensions, the relation can be expressed as

$$\gamma_{LS} - \gamma_{OS} = -\gamma_{LO} \cos \theta. \quad (4.18)$$

In order to form a convex interface with contact angle larger than 90° ,

$$\gamma_{LO} > \gamma_{LS} - \gamma_{OS} > 0. \quad (4.19)$$

On the other hand, in order to form a concave interface with contact angle less than 90° ,

$$\gamma_{LO} > 0 > \gamma_{LS} - \gamma_{OS}. \quad (4.20)$$

To form an ideal grating with flat curvature (contact angle of 90°), the liquid-solid surface tension has to be matched with the oil-solid surface tension ($\gamma_{LS} = \gamma_{OS}$). This means that the PDMS surface should neither be too hydrophilic nor be too hydrophobic, which is partially wet against CaCl_2 phase. There are several factors that can vary the hydrophobicity of PDMS wall. First, the surface roughness of the developed PDMS wall normally increases the hydrophobicity [263]. Second, the PDMS wall becomes more hydrophilic after oxygen plasma treatment applied for chip bonding [264]. The hydrophobicity of the PDMS is gradually restored by leaving the bonded chip in open air for days. In addition, the contact angle can also be lower by adding surfactants to lower the surface tension of the CaCl_2 phase [265].

4.4 Experimental results and discussions of optofluidic grating

Fig 4.23 Schematic illustration of the experimental setup of the optofluidic grating.

The experimental setup of the optofluidic grating is illustrated in Fig. 4.23. A broadband superluminescence light emitting diode (Denselight, DL-CS2079A) with a central wavelength of 1275 nm and a bandwidth of 70 nm is used as the light source and coupled into the optofluidic chip via a single-mode optical fiber. The transmitted light is coupled into the output optical fiber and the transmission spectrum measurement is detected via an optical spectrum analyzer (Advantest, Q8384). Similarly, the liquids are injected into the optofluidic chip via syringe pumps (New Era, NE-1000). The physical measurements (size, period and contact angle) are measured (ImageJ) based on the microphotos captured with the charge-coupled digital camera (Olympus, DP70) under the inverted microscope (Nikon,

TE2000-E). Each measurement is repeated by 20 times and the average value is recorded.

For the optofluidic grating, the experiments are focused on the tunability in the physical parameters of the plugs (size, period and contact angle). Subsequently, the optical properties of the optofluidic grating are characterized. These results are presented in the next section.

4.4.1 Formation and tuning of optofluidic grating

Fig 4.24 Microphotos of (a) CaCl_2 plug formation, and (b) optofluidic grating in the microchannel.

Figure 4.24 shows the formation of optofluidic grating in the microchannel. The immersion oil and CaCl_2 solution injected into the microchannel act as the carrier flow and the dispersed flow, respectively. When the CaCl_2 solution is dispersed into the carrier flow, it blocks the flow of the immersion oil substantially and pressure is built up at the junction. A CaCl_2 plug is formed when the pressure is sufficiently high. This process is repeated and a long stream of plugs with constant period and length is formed as shown in Fig. 4.24 (b). According to the microphoto taken by a high-resolution CCD camera, the contact angle of the plugs is approximately $115^\circ - 118^\circ$ as shown in Fig. 4.24 (a). This ensures the confinement of light in the microchannel as discussed earlier in this chapter.

Fig 4.25 Tuning of grating period and plug length versus the flow rate of the immersion oil flow stream.

The length and period of the plugs can be tuned by varying the flow rate of the carrier flow as shown in Fig. 4.25. The average measurement values are based on 30 measurements. By maintaining the flow rate of the CaCl_2 solution at 0.05 $\mu\text{L/s}$, the flow rate of the carrier flow is increased from 0.035 to 0.05 $\mu\text{L/s}$. The CaCl_2 plug length is reduced at a rate of $-666.67 \mu\text{m s}/\mu\text{L}$. Consequently, the period of the optofluidic grating is increased at a rate of $-933.37 \mu\text{m min}/\mu\text{L}$. On the other hand, the length and period of the plugs can also be tuned by varying the flow rate of the dispersed flow as shown in Fig. 4.26. By maintaining the flow rate of the immersion oil solution at 0.05 $\mu\text{L/s}$, the flow rate of the CaCl_2 solution is increased from 0.05 to 0.065 $\mu\text{L/s}$. The CaCl_2 plug length is increased at a rate of $400 \mu\text{m s}/\mu\text{L}$. Consequently, the period of the optofluidic grating is increased at a rate of $600 \mu\text{m min}/\mu\text{L}$. The standard deviations of the plug length and the period are

Fig 4.26 Tuning of grating period and plug length versus the flow rate of the aqueous CaCl_2 flow stream.

determined to be within 5%. Since the plugs are highly uniform and monodisperse, the optical performance of the liquid grating is not significantly affected by these variations.

4.4.2 Tunable optical characteristics of optofluidic grating

Fig 4.27 Transmission spectra of the optofluidic grating with different grating periods. The output spectra have been smoothed using Savitzky-Golay method to show the attenuation peak and strength prominently.

The optical properties of the optofluidic grating are investigated against different grating period and grating length. When a stream of plugs with desired period is formed in the microchannel, the flow is halted and optical experiments are carried on. After the measurements, the flow is continued, leading to the tuning of the grating period for the next optical measurements. Fig. 4.27 shows the normalized transmission spectra of grating length of 6 mm with different grating periods of 70, 75 and 80 μm . The liquid grating is formed using immersion oil and CaCl_2 solution with refractive indices of 1.462 and 1.430, respectively. The index

Fig 4.28 Transmission spectra of the optofluidic grating with different grating lengths. The output spectra have been smoothed using Savitzky-Golay method to show the attenuation peak and strength prominently.

variation of the liquid grating is 0.032. With the increase in grating period, the attenuation peak is shifted from 1264.2 nm to longer wavelengths of 1274.6 and 1281.5 nm. In addition, the attenuation strength is reduced from 0.23 to 0.37 and 0.52. This is because the number of periods of the optofluidic grating with longer period is relatively smaller as compared to the one with shorter grating period.

Figure 4.28 shows the transmission spectra of the optofluidic grating with different grating lengths, i.e. 5, 6 and 7 mm. The grating period and the index variation are maintained at 75 μm and 0.032, respectively. Therefore, the attenuation peak is remained constant at 1274.6 nm but the attenuation strength is greatly affected. By increasing the grating length, the attenuation strength is

increased from 0.37 to 0.24 and 0.14. Subsequently, the bandwidth of the attenuation band is reduced from 10.2 to 9.0 and 7.6 nm. It can be concluded that the attenuation strength is improved when the grating length is increased, which leads to a sharper and narrower attenuation band.

The optofluidic grating has demonstrated the tuning ability in the central attenuation wavelength, the attenuation strength and the attenuation bandwidth. The experimental results show that the attenuation strength and bandwidth achieved are 0.14 and 7.6 nm, respectively, for a grating length of 7 mm and index variation of 0.032. The sharpness of the attenuation band can be further increased with a longer grating period but limited by the detectable intensity due to the absorption of liquids. Due to the presence of the interfacial curvature, the oil-water interface may not be an ideal grating interface. In such case, the optical performance of the liquid grating such as the resonant wavelength and the attenuation strength is slightly affected. However, these effects can be compensated by adjusting the period of the plugs and the RI variation. Alternatively, reducing the interfacial curvature could be achieved by surface treatment of PDMS chip and usage of surfactant [265].

The advantages of the optofluidic grating realized by droplet microfluidics are as follow. First, different combination of liquids can be employed to overcome material restrictions as commonly observed in solid gratings [257]. For example, in fiber grating interferometer, the modulation in the fiber core is limited to 0.0005 [266]. The modulation can be enhanced to 0.001 using doping techniques [266] or hydrogen loading [267]. Unlike solid grating, optofluidic grating can easily achieve high index modulation of 0.02 by selecting different liquids. Second, optofluidic grating provides flexible options of input light source and detectors. The period of the gratings and subsequently the attenuation bandwidth can be tuned easily. This

provides a wide tunable range in the central resonant wavelength and also the attenuation strength that is rather difficult for solid grating. Finally, optofluidic grating in the microchannel, which can be easily integrated onto a microfluidic chip and hence reduces the difficulties in fabrication processes as compared to solid gratings.

4.5 Summary

This chapter focuses on the development of the optofluidic microlens and the optofluidic grating. In the design of the optofluidic microlens, the theoretical analysis explores the focusing power of the optofluidic microlens based on the selected liquids and the formed lens profile. The microfluidic designs include the structural design of the expansion chamber and also the tunability of the microlens via the change of the flow rates of the liquids. In the design of the optofluidic grating, the theoretical analysis of droplet grating is performed and the structural design of the microchannel for grating period tuning is explored. Experimental results present the formation of optofluidic microlens and optofluidic grating in the LOC. The optofluidic components provide flexibility and tunability to suit different optical systems.

The advantages of the developed optofluidic microlens include (1) the capability to form different types of microlens via the tuning of flow rate ratio between the three laminar flows. The focusing power of the microlens is varied by different combinations of liquids (refractive index contrast), and (2) the interfaces between the flows are optically smooth, which reduce light scattering or optical losses. To further improve the optofluidic microlens, a new mechanism which provides vertical focusing can be employed and the 3D optofluidic microlens can be formed.

For the optofluidic grating, its advantages include (1) the capability to form different types of grating via the dynamic tunability of the two-phase flow. The grating period is varied by changing the flow rate ratio between the two-phase flow. The grating strength is varied by different combination of liquids (refractive index contrast), and (2) the developed optofluidic grating can overcome the material

limitation as in solid grating, such as higher refractive index contrast in optofluidic grating (0.02) as compared to the one in solid grating (0.001). In addition, the optofluidic grating provides a wide tunable range in the resonant wavelength and also the grating strength.

The innovation of the developed optofluidic components is summarized as follow,

- a) The optofluidic tunable microlens is theoretically analyzed using the hydrodynamic focusing principle and the expansion chamber is designed for ideal lens profile. The optofluidic microlens is experimentally demonstrated both as single and compound microlenses. It shows a great flexibility in design for integrated optofluidic devices.
- b) This is the first effort demonstrated to develop an optofluidic grating using droplet microfluidics. The design guideline is listed based on the liquid selection, plug size and shape profile. The optofluidic grating is experimentally demonstrated with flexible attenuation peak and attenuation strength.

CHAPTER 5

OPTOFLUIDIC FABRY-PÉROT REFRACTOMETERS

This chapter presents the designs of two optofluidic Fabry-Pérot (FP) resonant systems, i.e. optofluidic microlens FP resonator and complementary optofluidic FP resonant system. The optofluidic microlens FP resonator consists of a microcavity, which forms part of the microchannel to facilitate cell manipulation, and optofluidic microlenses, which are used for real-time efficient light coupling. On the other hand, the complementary optofluidic FP resonant system is employed for the effective refractive index measurement of single living cell using single-wavelength intensity-based measurement scheme.

First, the design of the optofluidic microlens FP resonator including the essential criteria to achieve high finesse resonator are presented. In the complementary optofluidic FP resonant system, the optical detection system is presented, including the theoretical study of the single-wavelength intensity-based cell refractive index measurement scheme, and the cell size signal modulation measurement scheme. Subsequently, the microfluidic manipulation system is discussed, which consists of a pressure driven integrated valve. Third, the fabrication processes for the optofluidic FP refractometers are illustrated. Finally, the experimental results are presented and discussed.

5.1 Optofluidic microlens FP refractometer

Fig 5.1 Schematic illustrations of (a) the optofluidic microlens FP refractometer, and (b) single cell optically trapped in the FP cavity.

The required precision or accuracy of a cell refractometer depends on the investigating subject. For example, a precision of 0.005 is sufficient to differentiate normal red blood cells and the one from anemia patients [268]; and a precision of

0.02 is sufficient to differentiate the G₂/M and G₀/G₁ cells in the cell cycle [269]. On the other hand, when monitoring the small refractive index change of a single cell such as that induced by antigen, a precision smaller than 0.001 is essential to detect the difference [270]. Based on this requirement, the previously developed on-chip grating interferometer with precision of 0.001 is not suitable. In addition, the sensitivity is relatively low for the system. To overcome these drawbacks, a high finesse FP refractometer with higher precision and higher sensitivity is proposed. The optofluidic microlens FP refractometer is illustrated in Fig. 5.1 (a). The optofluidic microlens FP refractometer consists of a pair of expansion chambers with two inlets for liquid microlens formation, a central microchannel as the FP cavity and a pair of optical fibers for optical input and output. Another pair of optical fibers is integrated for sample optical trapping. The liquid microlens is formed in the expansion chamber using three laminar flows. The curvatures of the microlenses can be easily tuned by controlling the ratio of the flow rates. In the FP cavity as shown in Fig. 5.1 (b), the sidewalls of the microchannel are coated with gold layer to increase the reflectance which is critical for high-finesse resonator. For cell measurement, a single cell is optically trapped in the FP cavity and differential measurement scheme is employed to measure the effective refractive index and the diameter of the single cell simultaneously as described in Chapter 3.

For a planar FP cavity with reflectance of R , the transmission function is given as

$$T = \frac{1}{1 + F \sin^2 \left[\left(\pi / \lambda \right) 2nL \right]} \quad (5.1)$$

where λ is the wavelength of the incident light, n is the refractive index of the medium in the cavity, L is the cavity length and F is the coefficient of finesse and is defined as

$$F = \frac{\pi\sqrt{R}}{1-R} \quad (5.2)$$

Resonator with high-finesse has sharper resonant peaks and lower transmission minima in the transmission spectrum. As the measurement of the refractive index is based on the positions of the resonant peaks, high-finesse resonator can increase the precision of the FP refractometer [271]. It is more precise to define the resonant wavelengths. To achieve a precision of 0.0005, the finesse of the microcavity has to be increased to be at least 10.

In the development of a high-finesse FP resonator, several criteria have to be addressed, i.e. the reflectance of the cavity, the nature of the input light and also the propagation loss.

5.1.1 Reflectance of FP cavity

As shown in Eq. 5.2, the reflectance of the FP cavity greatly affects the finesse of the FP resonator. The reflectance of an interface for normalized incident light is determined as

$$R = \left(\frac{n_a - n_b}{n_a + n_b} \right)^2 \quad (5.3)$$

where n_a and n_b are the refractive index of the index medium a and b , respectively.

From Eq. 5.3, it can be seen that higher refractive contrast ($n_a - n_b$) will lead to

higher reflectance. In this case, n_a is the chip material, i.e. PDMS ($n_a = 1.412$) and n_b is the medium in the cavity. The highest refractive index contrast is achieved when the medium in the cavity is air ($n_b = 1$), and then the reflectance achieved is 0.029. When the cavity is filled with higher refractive index solution as compared to air, the refractive index contrast and subsequently the reflectance are reduced. Therefore, in order to increase the reflectance of the PDMS-medium interfaces, a thin layer of gold has to be coated on the sidewalls of the microchannel. The reflectance of the interface can be improved by gold coating about 90 - 97% [272]. The reflectance greatly depends on the thickness of the coated gold layer, and the smoothness of the interface, which is the PDMS sidewall in this case. In this optofluidic FP resonator, the sidewalls of the central microchannel are coated with gold layer using the sputtering machine to increase their reflectance.

5.1.2 Propagation loss due to absorption and scattering

Losses due to absorption and scattering in medium of the cavity will degrade the finesse of the resonator. The round trip power attenuation factor is defined as

$$P_{as} = \exp(-2\alpha_s L) \quad (5.4)$$

where α_s is the absorption coefficient of the medium. The absorption of a medium depends on the wavelength of the light [273].

To reduce the losses due to absorption and scattering, three criteria can be considered, i.e. using (1) medium with lower absorption coefficient, (2) shorter propagation length, and (3) wavelength with lower absorption. In the optofluidic FP resonator, a pair of liquid microlenses is used. With the involvement of liquid in the

system, the wavelength used for measurement should avoid the region of 1550 nm because the absorption of aqueous solution at this wavelength region is high [274].

In addition, the transparency of PDMS has to be considered when selecting a suitable wavelength. Based on the transmission spectrum of PDMS in wavelength ranging from 400 nm to 2400 nm, PDMS has negligible or low absorption at wavelength of 400 to 800 nm and 1250 to 1350 nm [275]. Therefore, the operating wavelength of the optofluidic FP refractometer should be within these wavelength ranges.

5.1.3 Nature of the propagating light

In FP resonator, a pair of optical fibers with gold-coated facets is aligned and separated by a microchannel to form the resonant cavity. Ideally, the light should be effectively trapped in the cavity, i.e. collimated/parallel light beam in a perfectly aligned planar cavity as shown in Fig. 5.2 (a). With zero incident angle, all light rays undergo resonance (constructive interference) at the same condition, i.e.

$$m\lambda = 2nL \quad (5.5)$$

where n is the refractive index of the medium in the cavity and L is the cavity length. As a result, resonant peaks are observed in the transmission spectrum at wavelengths, λ , that match the resonant condition (Eq. 5.5) [276].

If the input light is not collimated but diverging such as the one emerged from the optical fiber (Fig. 5.2 (b)), the resonant condition is different than previous case. For the emerging light with diverging angle of θ_n , it consists of light rays with continuous incident angles from zero to θ_n . Therefore, the resonant condition for

Fig 5.2 Transmission spectrum of the FP resonator with (a) collimated propagating light, and (b) diverging propagating light.

each light rays can be expressed as

$$m\lambda_i = 2nL \cos \theta_i \quad \text{for } i=0,1,2,\dots,n \quad (5.6)$$

where $\theta_0 = 0$. As a result, for a particular order (m), broader range of wavelengths can match the resonant conditions and the resonant peak observed in the transmission spectrum has larger bandwidth and lower finesse. In addition, propagation loss exists with finite size of planar reflecting surfaces because a fraction of light can be leaked from the cavity [277].

In order to realize a high finesse resonator, the light emerged from the optical fiber has to be collimated. Light collimation for optical fiber has been demonstrated using commercially available packaged aspherical lens, hollow-core waveguide [278] or optical fiber slab [279], capillary tubing [280 -281], epoxy microlens [282] etc. The aspherical lens is normally suitable for free space applications and not for on-chip applications. Inserting hollow-core waveguide or optical fiber slab is costly and difficult in integration. Epoxy microlens is more suitable for single chip integration. However, it lacks of flexibility in fabricating the desired microlens as it is not easy to control the curvature and the height of the

Fig 5.3 Schematic illustrations of (a) the physics model of spherical FP resonator that consists of a pair of spherical curvature coated with metallic layer, and (b) optofluidic microlens FP resonator with a pair of liquid microlenses for light confinement.

microlens. In addition, with the fiber facets acted as resonator, fiber misalignment and poor facet quality degrade the finesse or the quality factor of the resonator.

To provide better light manipulation and confinement, spherical FP resonator, which is less sensitive to geometrical misalignment, is a better and more stable configuration as shown in Fig. 5.3 (a). The spherical curvatures provide two functions, i.e. diverging light is collimated by the spherical curvature and collimated light is confined to undergo resonance between the highly reflective spherical surfaces. The proposed optofluidic microlens FP resonator uses a pair of highly optical performing liquid microlenses to provide the function of light collimation with real-time tunability as shown in Fig. 5.3 (b). A gold-coated microchannel

between the liquid microlenses provides the function of light confinement and resonance and provides single cell manipulation. The microchannel is pre-aligned during fabrication to avoid losses due to geometrical misalignment. The optofluidic FP resonator separates light collimation from the resonance cavity which provides greater flexibility in the design of sample manipulation for measurement.

5.1.4 Design of the optofluidic microlenses for light collimation

To reduce propagation losses, a short focal distance is essential such that the distance between the fiber facet and the liquid microlens is shorter. Based on the lensmaker's equation, the focal distance (f) can be determined as

$$\frac{1}{f} = (n-1) \left[\frac{1}{R_1} - \frac{1}{R_2} + \frac{(n-1)t}{nR_1R_2} \right] \quad (5.7)$$

where n is the relative refractive index, t is the thickness of the lens and R_1 and R_2 are the radii of curvature of the first and second interface, respectively. To simplify the design of the liquid microlenses, symmetrical biconvex microlenses ($R_1 = -R_2 = R$) are formed and Eq. 5.7 is simplified as

$$\frac{1}{f} = (n-1) \left[\frac{2}{R} + \frac{(n-1)t}{nR^2} \right] \quad (5.8)$$

It can be clearly seen that higher relative refractive index value leads to shorter focal distance. For the core and cladding liquids, 4.16 mol L⁻¹ calcium chloride solution (CaCl₂) with refractive index of 1.462 and isopropanol solution (IPA) with refractive index of 1.382 are used, respectively. Therefore, a contrast of 0.08 in refractive index value is achieved.

To form the liquid microlenses, an expansion chamber with width and

Fig 5.4 Simulated propagating light is collimated by the liquid microlens.

length of 375 μm is used. A lateral expansion of 12.5 times is used in which the inlet and outlet has a width of 30 μm . This allows a sufficiently large tuning range in the radius of curvature of the liquid-liquid interfaces. The height of the microchannel is 125 μm for fiber insertion. The optical fibers to be used are single mode fibers with operating wavelength of 1350/1550 nm, core width of 9 μm , cladding width of 125 μm and numerical aperture (NA) of 0.13 (Wintek, SMF1550). When the light from the optical fiber is emerged into the PDMS ($n = 1.412$) chip, the diverging angle (θ) can be calculated as

$$\theta = \sin^{-1}(\text{NA}/n) = \sin^{-1}(0.13/1.412) = 5.28^\circ \quad . \quad (5.9)$$

With all the defined design parameters, the distance from the fiber facet to the expansion chamber is determined using ray tracing program (Zemax) to achieve collimation. The simulation result is shown in Fig. 5.4. A diverging beam with $\theta = 5.28^\circ$ is injected into the optical system. In the expansion chamber, a biconvex microlens with $R = 137.5 \mu\text{m}$ and $t = 175 \mu\text{m}$ is formed. Based on the simulated result, the required distance between the fiber facet and the expansion chamber (d_f) to collimate the diverging beam is 1 mm.

5.1.5 Design of single cell measurement

Fig 5.5 The schematic illustration of aperture to limit the beam waist of the light.

For single cell measurement, the optofluidic FP refractometer is integrated with another pair of optical fibers for optical trapping as shown in Fig. 5.5. The working principles are discussed in Chapter 3. When the cell is trapped in the cavity, differential buffer measurement scheme is employed to measure the cell refractive index and size simultaneously as discussed in Chapter 3. With the cell size in the range of 10 to 20 μm , an optical aperture has to be included to limit the beam waist of the collimated light. Based on the simulated result in Fig. 5.4, the beam waist of the collimated light is about 100 μm . When the cavity is employed to measure a single cell in the size of 20 μm , the refractometer is less sensitive because only part of the light undergoes a change in optical path length. Therefore, to improve the sensitivity of the refractometer, the light beam has to be limited to about 20 μm such that most of the light undergoes a change in the optical path length with the presence of the cell. The aperture is formed by two separate microchannels filled with visible light absorbing liquid, such as black ink.

5.2 Experimental results and discussions of optofluidic microlens FP resonator

5.2.1 Fabrication processes

The fabrication processes of the optofluidic FP refractometers are primarily based on the polymer soft lithography fabrication technique as discussed in Chapter 3. The mold is fabricated using SU8-50 on a silicon wafer. The patterned PDMS slab is bonded to the other PDMS slab using plasma bonding. Fibers are inserted into the chip and fixed together with the chip using UV-cured epoxy [283]. In this section, the gold coating process on the sidewalls of the microchannel and the characterization of the coated gold are discussed. In order to realize a high-finesse FP cavity, several issues need to be investigated, i.e. the parallelism between the sidewalls, the surface roughness of the sidewalls and the thickness of the gold coating layer.

The parallelism between the optical planes is very important to effectively confine light within the cavity and form a high finesse resonator [100]. For perfectly parallel optical planes as shown in Fig. 5.6 (a), an incident light which is normal to the optical plane is partly reflected and partly transmitted through the cavity as T_1 . The incident light is confined within the cavity to undergo multiple reflection for effective interference to occur in which the subsequent transmitted light $T_2, T_3 \dots T_n$ are all in parallel with T_1 . Therefore, light undergoes resonance based on the condition stated in Eq. 4.4 and sharp resonant peaks can be observed at the transmission spectrum of the resonant cavity.

However, the light confinement is disturbed when the two optical planes are not in parallel but slanted as shown in Fig. 5.6 (b). If the optical planes are slanted,

Fig 5.6 Schematic illustrations of the propagating light in the resonant cavity when the optical planes are (a) in parallel, and (b) slanted.

the incident light is not normal to the optical plane. At the optical plane, the incident light is partly reflected and partly transmitted as T_1 . The transmitted light T_1 is refracted and is not in the same direction as the incident light. The light cannot be efficiently confined within the cavity in which it is escaped from the cavity after several reflections. In addition, each refracted light for subsequent transmissions is not in parallel with one another. For example, the propagation direction for T_2 is different to the one for T_1 . Therefore, not all light can be detected and each refracted light has different optical path length. These lead to optical losses and a low-finesse resonant cavity is formed.

The parallelism of the microchannel sidewalls is investigated using SEM micrographs of cleaved test structures. Vertical PDMS sidewalls can be obtained using SU-8 master mold. SU-8 can provide uniform exposure conditions in the near-UV range and produce structures with highly vertical sidewalls and smooth features [284]. However, the soft lithography process does not guarantee parallel sidewalls. The unparallel microchannel can be formed as shown in Fig. 5.7 due to several possible causes such as the defects in the developed SU8 mold, the deformation of the PDMS microchannel during the peeling off process and the

Fig 5.7 SEM micrographs of (a) unparallel, and (b) parallel sidewalls of the PDMS microchannel.

bonding process etc. Unparallel sidewalls causes the finesse of the cavity to be relatively low and not much enhancement can be achieved even though liquid microlenses are formed. However, parallel PDMS sidewalls can be achieved by precise fabrication process. Fig. 5.7 (b) illustrates the PDMS sidewalls which are in parallel with the imperfection measured to be smaller than 0.1° .

The surface roughness of the microchannel sidewalls is critical for the optical performance of the resonant cavity. Ridges and valleys on the surface causes scattered wavelets. If the ridges and the valleys on the surface are relatively small compared to the wavelength of the incident light (smooth surface), the scattered wavelets have negligible difference in reflected angle or are still in-phase. Subsequently, specular reflection in which all rays are reflected in the same direction is observed as shown in Fig. 5.8 (a). On the other hand, when the ridges and the valleys on the surface are comparable to the wavelength of the incident light (rough surface), the rays are reflected in a large variation of reflected angles as shown in Fig. 5.8 (b). This is known as diffuse reflection and subsequently rays in the cavity will not be collimated and effectively trapped within the resonant cavity.

Fig 5.8 Schematic illustrations of the reflection of the incident light on a (a) smooth surface, and (b) rough surface.

Fig 5.9 SEM micrographs of the microchannel structure in (a) SU-8 mold, (b) PDMS chip, and (c) PDMS sidewall at high magnification.

The surface roughness of the microchannels is observed using SEM technique. The SEM microphotos of the microchannel structure in SU-8 mold and PDMS chip are shown in Fig. 5.9. Instead of its relatively high mechanical strength and biocompatibility, SU-8 as an epoxy type negative photoresist is suitable for soft lithography due to its possibility in fabricating thick vertical sidewalls with relatively low surface roughness as compared to deep reactive ion etching in silicon wafer which causes vertical striation and rougher surface. Fig. 5.9 (c) shows the surface of the sidewall in high resolution. It can be concluded that no visible roughness at the 100 nm level is found. The roughness of the sidewall is lower than 100 nm which

becomes lower with higher microchannel. Since the surface roughness is much smaller as compared to the wavelength of the incident light (1275 nm), the reflections from the sidewalls are specular dominated and it can be concluded that surface roughness of the sidewalls has negligible effect on the resonant cavity.

The reflectance of the sidewalls is increased by coating a nanometer thickness of gold layer using the sputtering machine. The microchannel is coated with gold using the sputtering machine. Based on the specification of the sputtering machine, the coating rate of the gold layer at the bottom of the microchannel is approximately 10 nm min^{-1} . However, the coating time for the PDMS sidewall is relatively long due to the difficulty in coating the sidewall. Based on the experimental characterization, the coating rate for the PDMS sidewall is 5.33 nm min^{-1} .

Figure 5.10 illustrates the optical spectra of the FP cavity with different gold thickness. As the thickness of the coated gold layer is increased from 23 to 40 nm, the minimum transmission is reduced from 0.64 to 0.02 at the resonant wavelength of 1278.3 nm. However, further increment in the thickness of the coated gold layer causes lesser light being able to transmit into the output fiber. No distinct and visible peaks are observed in the output spectrum as the signal is mostly embedded in the noise. As a result, the optimized thickness of the coated gold layer is 40 nm with a minimum transmission of 0.02 and a finesse of 3.48. The low finesse value of the FP cavity is due to the large propagation loss of the diverging light emerging from the optical fiber. Since the separation between the fibers in the optofluidic FP resonator is 3 mm, without the formation of the liquid microlenses, a great loss is incurred before light reaches the resonant cavity and only a small portion of light undergoes resonance in the cavity.

Fig 5.10 Transmission spectra of the FP cavity with different thicknesses from 23 nm, 31 nm to 40 nm of the coated gold layers at the sidewalls of the central microchannel Fig 5.7 SEM micrographs of (a) unparallel, and (b) parallel sidewalls of the PDMS microchannel.

Therefore, the finesse of the FP cavity is relatively low. The finesse of the FP cavity can be increased using the liquid microlenses.

5.2.2 Experimental setup

Fig 5.11 Schematic illustration of the experimental setup of the optofluidic microlens FP resonator.

Figure 5.11 illustrates schematically the experimental setup of the optofluidic microlens FP resonator. In the characterization of the optofluidic FP resonator, a superluminescence light emitting diode (SLED) with a central wavelength of 1275 nm and a bandwidth of 70 nm is used (Denselight, DL-CS2079A). The transmission spectrum is detected using the optical spectrum analyzer (Advantest, Q8384) with a resolution of 0.01 nm. All liquids are injected into the chip using syringe pumps. In order to visualize the light path, fluorescein dyes are introduced into the liquid flows. The SLED is replaced with the Argon ion laser with an output wavelength of 488 nm for the fluorescent excitation. The device is imaged under the microscope attached with a CCD camera. A high-power laser with wavelength 1550 nm and maximum power of 45 mW (Ultrale,

CL16e) is used to realize the fiber optical trap. The experiments are carried out for two main objectives. Firstly, the characterization of the microlens FP resonant cavity is investigated. These results are presented in section 5.2.3. Secondly, the optical measurement of the chemical buffer solutions and the (Madin-Darby canine kidney) MDCK cell are carried out. These results are presented in section 5.2.4.

5.2.3 Characterization of the microlens FP resonator

The formation of liquid microlens using three laminar flow streams in the expansion chamber provides the flexibility in tuning the radius of curvature of the interfaces and also the shape of the microlens as discussed in Chapter 4. However, biconvex microlens has greater focusing power or shorter focus distance as compared with other types of microlens. Therefore, light focusing or collimating using biconvex microlens can reduce the spatial distance of the optical system and the size of the PDMS chip. In addition, the formation of biconvex microlens is easier and more robust in operation because the flow rates of both cladding flow streams are the same by sharing a single inlet and single syringe pump. For the formation of the liquid microlenses, 4.16 mol L⁻¹ calcium chloride solution (CaCl₂) with refractive index of 1.462 and isopropanol solution (IPA) with refractive index of 1.382 are used as the core and cladding flow streams, respectively.

Figure 5.12 shows the formation of the liquid microlenses when the flow rate of the core flow stream is maintained at 35 μ L/min. The diffusion at the liquid interfaces due to the concentration difference between the two liquid layers can be minimized using faster flow rate because the diffusion distance of Ca²⁺ and Cl⁻ ions is reduced [262]. With the chosen liquids, the diffusive broadening of the interfaces

Fig 5.12 Microphoto of the optofluidic microlenses captured using a CCD camera with a $10\times$ objective lens. The sidewalls of the central microchannel are coated with gold layer.

is eliminated and the microlenses formed have a better match with the ideal lens. In addition, the liquid microlenses have optically smooth curvatures which ensure high quality light manipulation and collimation.

The light propagation path is illustrated in Fig.5.13. Diverging beam is collimated by the first liquid microlens and coupled back into the optical output fiber by the second liquid microlens. The collimation efficiency with respect to the radius of the curvature of the microlenses is shown in Fig. 5.14. Without collimation, only a small portion of light (parallel light at the central beam) undergoes resonance within the FP cavity and diverging light is observed outside the FP cavity in the microchannel as shown in Fig. 5.14 (a). However, when the liquid microlens of $r = 150\ \mu\text{m}$ is formed, it can be seen that more light is collimated and undergoes resonance as shown in Fig. 5.14 (b). Less diverging light is observed outside the FP cavity in the microchannel. The light emerged from the optical fiber is fully collimated when a liquid microlens of $r = 138\ \mu\text{m}$ is formed as shown in Fig. 5.14 (c). It can be clearly seen that most light is collimated and undergoes resonance in the FP cavity and nearly negligible diverging light is

Fig 5.13 Optical micrograph of the diverging light from the optical fiber being collimated by the first microlens. The collimated beam undergoes resonance in the FP cavity in the central microchannel, which then is coupled back into the optical output fiber by the second microlens.

Fig 5.14 Optical micrographs of the light beam when (a) no microlens, (b) microlens with $r = 150 \mu\text{m}$, and (c) microlens with $r = 138 \mu\text{m}$ is formed. (d) Zoom view of the collimated beam in the cavity that is captured using a $40 \times$ objective lens.

Fig 5.15 Transmission spectra of the optofluidic FP resonator without microlens and with microlens ($r = 138 \mu\text{m}$). The finesse of the cavity is greatly improved with the formation of microlenses.

observed outside the FP cavity in the microchannel. The light beam is clearly seen to be in parallel when the light beam in the FP cavity is observed using a $40\times$ objective lens as shown in Fig. 5.14 (d).

Figure 5.15 shows the quality of the FP resonant cavity with and without liquid microlenses. The microchannel of the resonant cavity has a width of $15 \mu\text{m}$ and filled with DI water. The finesse of the FP cavity is greatly improved from 3.48 to 18.79 when liquid microlenses with radius of curvature of $138 \mu\text{m}$ are formed. At this condition, most of the light is collimated, undergoes resonance and efficiently couples back to the detection fiber. When the radius of the curvature of the liquid microlenses is further reduced, the light from the optical fiber converges. Converging light is inefficient in undergoing resonance. Therefore, the FP cavity becomes lossier and the finesse is reduced as shown in Fig. 5.16. From the result, microlenses with the radii of the curvature in the range of $130 \mu\text{m}$ to $145 \mu\text{m}$ are

Fig 5.16 Finesse of the resonant cavity versus the radius of curvature of the microlenses. The region of operation is highlighted in which the finesse is higher than 15.

within the operational range to ensure the finesse of the FP cavity is higher than 15. The finesse of the FP cavity is greatly improved as compared to the previous reported FP resonators in microfluidic systems, which are 5 – 7 [100, 102].

In order to show the importance of achieving a higher finesse FP cavity, the precision of the measurement is investigated using different finesse of the FP cavity with the resolution of the OSA set to 0.02 nm. In the experiment, 30 output spectra are captured in each cases and a peak is located. The standard deviation and standard error are calculated based on the 30 output spectra. A low finesse cavity of 3.48 has a standard deviation of 2.28×10^{-2} and a standard error of 4.0×10^{-3} as shown in Table 5.1. When a high finesse cavity is used, the standard deviation and standard error is reduced to 2.7×10^{-3} and 8.0×10^{-4} , respectively. The standard deviation achieved is improved as compared with previous devices [100, 102]. Based on the experimental results, the precision of the measurement is increased by employing a high finesse resonant cavity.

TABLE 5.1. Precision of peak localization

Finesse	3.48	18.79
Standard deviation	2.28×10^{-2}	2.7×10^{-3}
Standard error	4.6×10^{-3}	8×10^{-4}

5.2.4 Single cell volume refractometer

Fig 5.17 Transmission spectra of the FP resonator filled with buffer solutions of different refractive indices.

The developed optofluidic FP resonator is characterized and employed as a volume refractometer. For characterization, the microchannel which forms the FP cavity is filled with different liquids to measure the responsivity of the system (the shift of the resonant peak) to the change of the refractive index. In the measurement, certified refractive index liquids (series AA, Cargille Laboratories) with refractive index ranging from 1.493 to 1.528 are used. The refractive index liquids have low

Fig 5.18 Shift of the resonant peak as a function of the refractive index of the buffer solution.

temperature dependence ($0.0004\text{ }^{\circ}\text{C}^{-1}$) so as to avoid measurement error due to the surrounding temperature.

Figure 5.17 shows the output transmission spectra when the refractive index liquids are injected into the microchannel. It can be observed that the resonant peak is shifted to longer wavelength as the refractive index of the liquid increases. The relation between the resonant peak and the refractive index of the sample liquid in the microchannel is shown in Fig. 5.18. The developed FP resonator has a detection sensitivity of 960 nm/RIU and a detection range of 0.043 RIU. The highest resolution for the optical spectrum analyzer is 0.01 nm. Therefore, the refractometer has a resolution (smallest detectable change) in refractive index measurement of 1×10^{-5} RIU. Since the refractive index measurement is limited within one free spectral range, the detection range is dependent on the width of the FP cavity. With larger width, the free spectral range of the transmission spectrum is reduced and subsequently the detection range is decreased. The sensitivity of the volume

TABLE 5.2. Measurement results of different buffer solutions in room temperature (20°C)

	FP volume refractometer	Handheld refractometer
Calcium chloride solution ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)		
0.5 mol L ⁻¹	1.34662 ± 0.00002	1.3466
1.0 mol L ⁻¹	1.35441 ± 0.00003	1.3544
1.5 mol L ⁻¹	1.36223 ± 0.00002	1.3622
2.0 mol L ⁻¹	1.34988 ± 0.00002	1.3699
Fetal bovine serum solution (volume percentage)		
10%	1.33708 ± 0.00003	1.3371
20%	1.33870 ± 0.00002	1.3387
30%	1.34134 ± 0.00003	1.3413
40%	1.34321 ± 0.00002	1.3432

refractometer is not affect by the width of the cavity but by the wavelength of the detecting light. Higher wavelength of the detecting light has to be used to achieve higher sensitivity. In addition, the free spectral range can be increased as well using higher wavelength of the detecting light. However, the selection of the wavelength of the detecting light is also depending on the absorption of the liquids used or measured as discussed.

The FP volume refractometer is used to measure different concentrations of calcium chloride solutions and fetal bovine serum solutions. The measurement results are listed in Table 5.2 and compared with the one measured using the handheld refractometer with resolution of 1×10^{-4} RIU.

The design of the optofluidic FP refractometer is modified to suit for cell measurement. Since the normal cell diameter is ranging from several microns to < 20 microns, the microchannel width is set at 20 microns and the beam waist of the resonator has to be reduced. Using single mode fiber, the beam waist is about 13 μm . However, the beam waist in the FP cavity is enlarged to 100 μm because the

Fig 5.19 Optical micrographs of the beam width in the expansion chamber (a) without, or (b) with the aperture.

Fig 5.20 Transmission spectra of the aperture-integrated optofluidic FP resonator without microlenses and with microlenses.

beam emerged from the fiber facet is diverging. An aperture is integrated in the resonant system between the input fiber and the first expansion chamber. The 15- μm aperture is formed with a gap between two microchannels which are filled with light absorbing black ink. The light emerged from the input fiber can only passing through the aperture while those reach the microchannels filled with black ink is

Fig 5.21 Microphoto of (a) the FP resonator integrated with liquid microlenses for light collimation and optical fibers for optical trapping, and (b) a single MDCK cell being trapped in the cavity.

completely absorbed. The microphotos of the propagating light in the expansion chamber with the formation of the microlens are shown in Fig. 5.19 (a) and (b). It is shown that the beam width of the collimated light is reduced from $100\ \mu\text{m}$ to $15\ \mu\text{m}$ with the integration of the on-chip aperture.

The output spectra without and with aperture are shown in Fig. 5.20. The FP cavity is filled with DI water. It can be seen that the optimal finesse of the FP resonator achieved with the integration of the aperture is measured as 13.31. On the other hand, the signal to noise ratio is reduced by 20 times, which may affect the precision of the system but the resonance peaks are still prominent for measurement.

The aperture-integrated FP resonator is characterized using certified RI liquids (series AA, Cargille Laboratories) with RI ranging from 1.493 to 1.528. The developed FP resonator has a detection sensitivity of $950\ \text{nm/RIU}$ and a detection range of 0.043 RIU. After the characterization, the system is then employed for Madin-Darby canine Kidney (MDCK) cell measurement. The MDCK cell is injected into the central microchannel filled with $1\times$ PBS solution and is optically

Table 5.3 Measurement results of MDCK cell effective refractive index and diameter.

	1 st cell		2 nd cell	
	Refractive index	Size (μm)	Refractive index	Size
Measurement 1	1.3823	17.5	1.3837	16.3
Measurement 2	1.3831	16.7	1.3834	16.6
Measurement 3	1.3825	17.2	1.3831	16.7
Measurement 4	1.3836	16.5	1.3828	17.0
Arithmetic mean	1.3829	17.0	1.3833	16.7
Standard deviation	0.0006	0.5	0.0004	0.3

trapped using a pair of optical fibers within the FP cavity as shown in Fig. 5.21 (a). Same as the on-chip grating interferometer, the optical force is adjusted to avoid cell distortion on the shape so that the cell is maintained in spherical shape as shown in Fig. 5.21 (b). With the presence of optical trapping, the flow is halted to avoid its effect on the cell. The optical trap is applied on the cell less than 5 sec to minimize the phototoxicity and the output spectrum is obtained after the peaks are stabilized. Then, a higher concentration of PBS solution is injected into the central microchannel to replace the original buffer solution. The refractive indices of the buffers before/after mixing are determined as $n_1 = 1.3553$ and $n_2 = 1.3611$, respectively. The calculated MDCK cell refractive index and diameter are listed in Table 5.3. Two MDCK cells are measured four times using the same chip. The first measured MDCK cell has an effective refractive index of 1.3829 ± 0.0006 and size of $17.0 \pm 0.5 \mu\text{m}$. Similarly for the second measured cell, the effective refractive index is 1.3833 ± 0.0004 and the size is $16.7 \pm 0.3 \mu\text{m}$. Based on the measured results, the standard deviation of measured cell refractive index is 5×10^{-4} . With

such precision, the developed refractometer can be used to monitor the small refractive index change within a single cell.

Cell viability test is performed using Trypan blue. Based on the viability test, after the refractive index measurement, 90 % of the cells are remained viable (sample size: 30). Similar to the on-chip grating interferometer, the cell viability is not affected significantly by the measurement procedures.

Several issues and challenges of the developed optofluidic microlens FP refractometer for single cell measurement are discussed as follow,

- a) Current design does not include single cell separation mechanism. Similar to previous technique, the same approaches can be employed to improve the efficiency for single cell samples in the microchannel, i.e. by shaking the trypsinized cells in the culture flask, carrying out the experiments soon after cell resuspension, and by diluting the cell density. In the experiment, single cell is manually selected for RI and size measurements.
- b) Cell stiction on microchannel occurs as the cells are not isolated from the walls. To avoid cell stiction, surface modification can be done to improve cell-repelling from the channel wall.
- c) For real-time monitoring the viability of the cells in the microchannel, the cells can be pre-stained with Calcein AM, which is transported into live cell and emitting green fluorescence. This can provide an insight on the viability of the cell during refractive index measurement.
- d) For each measurement, the time period with the cell in the cavity was ranging from 40 to 50 s due to low flow rate buffer exchange. Sequential

measurement is possible by integrating a full microfluidic control network. But unlike on-chip grating interferometer, cell isolation is not practical in this system.

The developed optofluidic microlens FP resonator provides several advantages as compared to previous techniques. First, the finesse of the FP resonator is improved by 5.4 times via the liquid microlenses. Second, the sensitivity is increased from 2.63 nm in on-chip grating interferometer to 950 nm in optofluidic microlens FP resonator. The detection range is remained the same for both systems. Third, the liquid microlenses are optically smooth to reduce light scattering or propagation loss. The diffusion broadening effect of the interfaces is reduced using higher flow rates. Fourth, the cavity length is not limited by the resonator as found in the Bragg reflector type resonator. Smaller cavity length is preferred to have a larger detection range. For the detection of 17- μm MDCK cell, the cavity length is chosen as 20 μm . Fourth, as the FP cavity is not formed by the fibers, the alignment of the fibers has less effect on the finesse of the resonator. In addition, the fiber grooves are pre-aligned in the design and the fabrication of the microfluidic chip. The alignment of the fibers with the liquid microlenses and the FP cavity is relatively easy.

5.3 Complementary optofluidic FP refractometer

Fig 5.22 Schematic illustrations of (a) the complementary microfluidic FP resonant system, (b) the optical measurement region, and (c) the pressure valve in the off- and on-states.

The two previous reported refractometers are based on the shift of the resonant peaks in the wavelength domain. Besides wavelength-based measurement scheme, the measurement of refractive index can also be achieved using single-wavelength intensity measurement scheme in a resonant microcavity. The intensity

is varied when a particle or cell is passing through the microcavity. By analyzing the intensity variation, the refractive index of the sample can be determined. Based on this working principle, a complementary microfluidic FP resonant system is developed.

The schematic illustration for the complementary microfluidic FP resonant system integrated onto a microfluidic chip is shown in Fig. 5.22 (a). The microfluidic chip consists of an inlet and two outlets, i.e. sample outlet and waste outlet. Cells suspended in buffer solution are injected into the microfluidic chip. Measurements are taken in region **b**, which consists of three pairs of optical fibers as shown in Fig. 5.22 (b). The Fiber-Pair I are bare fibers used to measure the intensity attenuation due to absorption/scattering. Fiber-Pair A and B are fibers with gold coated facets to form FP resonator A and B. Both FP resonators are used to measure the intensity variation due to the change in the resonant mode. The size of the cell can be determined from the three signals in time domain with an assumption that the cell is spherical which is normally valid. Subsequently, with the measured size of the cell, the refractive index of it can be determined from the intensity variation of the complementary FP resonators. The details of the measurement scheme are discussed in the next section. After the measurement region, the microchannel is divided into two branches, one to the sample outlet and the other one to the waste outlet, respectively. The microchannel connected to the sample outlet is designed with a series of restricting structures, which has a width of 40 μm . The microchannel connected to the waste outlet is integrated with a pressure valve as shown in Fig. 5.22 (c). In the off-state of the valve, the microchannel has a width of 40 μm . Due to the presence of the restricting structures, the flow of the sample solution is limited to the waste outlet. When a cell is detected in region **b**, the

Fig. 5.23 Refractive index measurement using wavelength-based measurement scheme. The shift of resonant peaks when the resonant cavity is filled with solutions of different refractive indices.

pressure valve is changed to the on-state in which the microchannel is closed by the pressure valve. In the close state, the flow of the sample solution is changed to the sample outlet. The integrated system has high potential in determine low concentration of sample cell in solution and perform preconcentration as required for further biochemical/biophysical analyses.

The common measurement scheme employed in optical resonators to measure the volume refractive index change is based on the shift of the resonant peaks in the wavelength domain [285]. When the refractive index of the solution filled in the cavity is gradually increased, the resonant peaks are shifted to longer wavelength as shown in Fig. 5.23. The shift of the resonant peaks is due to the change of the optical path length within the cavity as discussed in Chapter 3. The use of wavelength domain can ignore the fluctuation in optical intensity. However, there are several limitations in the wavelength-based measurement scheme.

Fig. 5.24 Transmission spectra of the optical resonators with different

First, the main drawback of the wavelength-based measurement scheme is the lack of the cell's absorption or scattering measurement [286]. Based on the wavelength-based measurement scheme, the only measurable parameters are the cell refractive index and size. However, there specificity is limited for different cells with the same size and the same refractive index. One can differentiate cells with the same size and the same refractive index with the presence of absorption/scattering measurement.

Second, a high finesse resonant cavity is essential to achieve high precision refractometer in wavelength-based measurement scheme. With higher finesse, the resonant peaks are sharper and have lower bandwidth, which has higher precision in determining the wavelength of the resonant peaks. To achieve a high finesse resonant cavity, one has to increase the reflectances of the cavity sidewalls as shown in Fig. 5.24. The cavity sidewalls can be coated with a thin layer of gold or using Bragg reflectors to increase the reflectances. However, gold coating has

limited reflectivity and Bragg reflectors impose fabrication and integration difficulties. In addition, fiber-based FP resonator cannot effectively confine light within the cavity due to the diverging nature of the emerging light which induces optical loss and subsequently reduce the finesse of the resonator.

Third, cell trapping mechanism is essential to measure the wavelength spectrum. Single cell has to be stabilized and trapped in the cavity before the transmission spectrum is measured. Any motion or vibration during measurement will lead to errors and affect the accuracy. The integration of the trapping mechanism will further impose fabrication difficulties and affect the robustness of the chip [287].

Fourth, shorter cavity length is preferred because it can increase the detection range and also the sensitivity of the system in wavelength based measurement scheme [288]. But short cavity length leads to larger free spectral range and larger bandwidth of resonant peaks. As a result, the input light source should have larger bandwidth in several tens of nanometers. On the other hand, the spectrum span recorded has to be widened and subsequently the reading time for one full spectrum is increased. Any motion or vibration during measurement leads to errors and affect the accuracy.

To overcome these drawbacks, a complementary microfluidic FP resonant system is designed to measure the absorption/scattering, the size and the refractive index of single cell using the single-wavelength intensity measurement scheme. This measurement scheme does not require high finesse resonant cavity or cell trapping mechanism, which greatly simplifies the fabrication process and improves the robustness of the system.

5.3.1 Single-wavelength intensity based measurement scheme

Fig. 5.25 (a) Single FP resonant cavity integrated with microchannel for sample injection, and (b) the intensity variation in respect to the different cell refractive indices.

Figure 5.25 (a) shows a single FP resonator with gold coated facets. When the facets have a reflectance R , the transmission function of the FP cavity is expressed as

$$T = \frac{(1-R)^2}{1+R^2-2R\cos(\delta)} \quad (5.10)$$

where δ is the phase difference between each succeeding reflections and can be expressed as

$$\delta = \left(\frac{2\pi}{\lambda} \right) 2nL \cos \theta \quad (5.11)$$

where n is the refractive index of the medium in the cavity, L is the cavity length, θ and λ are the incident angle and the wavelength of the incident light, respectively.

In wavelength-based measurement scheme, the refractive index value is determined based on the position of the resonant peak. The resonant peak in the transmission spectrum is shifted when the cavity is filled with solutions of different

refractive indices. By increasing the refractive index value of the cell, the resonant peak is shifted to longer wavelength as shown in Fig. 5.23. On the other hand, in the single-wavelength intensity based measurement scheme, the intensity levels for different refractive index values are recorded at single wavelength, such as 514 nm. The variation of the transmission intensity with the cell of different refractive indices is simulated and shown in Fig. 5.25 (b). The transmission spectrum is simulated using buffer with refractive index value of 1.333, cavity length of 25 μm , cell size of 15 μm and the cell refractive index values ranging from 1.333 to 1.343.

There are two critical issues of using a single FP resonator. First, the measurement range is limited due to the symmetrical of the transmission intensity. The resonator only has a measurement range of 0.05 refractive index unit (RIU) using the single-wavelength intensity based measurement scheme, which is half of the one in wavelength based measurement scheme. For example, one cannot determine whether the cell refractive index value is 1.340 or 1.344 when the transmission intensity is 0.5. Second, the resonator is only sensitive in the highlighted region for refractive index value ranging from 1.336 to 1.347. It is insensitive in regions with low gradient. For example, it is unable to differentiate cells with refractive index value of 1.333 and 1.334 because they have the same intensity level.

To overcome these two issues, a complementary FP resonant system is proposed as shown in Fig. 5.26 (a), in which the two FP resonators are fine tuned to have different sensitive measurement regions as shown in Fig. 5.26 (b). The FP resonator A has low sensitive regions ranging from 1.333 to 1.336 and 1.347 to 1.351. These regions are complemented by the FP resonator B, which has high sensitivity within these regions. In addition, the measurement range is increased by

Fig. 5.26 (a) Complementary FP resonant system, and (b) the intensity variation in respective to the different cell refractive indices.

two times to 0.1 RIU using the two FP resonators because every refractive index value in the measurement range has two distinct intensity values.

5.3.1.1 Effect of the reflectance of the fiber facet

FP resonator with higher reflectance leads to higher finesse cavity, in which the finesse is determined as

$$F = \frac{\pi}{2 \sin^{-1} \left[(1-R)/2\sqrt{R} \right]} \quad (5.12)$$

The resonant peaks are sharper and the bandwidth is narrower. This is one of the essential criteria for resonator using wavelength based measurement scheme. However, for resonator using single-wavelength intensity based measurement scheme, high finesse cavity is not a criterion. Fig. 5.27 shows the simulated intensity variation for different refractive index values for single FP resonator with different reflectances. For cavity with $R = 0.1$, the intensity gradually increased to the maximum transmission and subsequently decreased by

Fig. 5.27 Intensity variation in respect to different buffer solutions measured at 514 nm for FP resonators with different reflectances.

increasing the refractive index value. The intensity contrast is relatively low. By increasing the reflectance of the cavity, the slopes become steeper and the intensity contrast is higher. For cavity with $R = 0.9$, a higher intensity contrast is observed for the range from 1.341 to 1.343, but the cavity is insensitive for the other refractive index values, ranging from 1.333 to 1.341 and 1.343 to 1.351, which limits the measurement range of the cavity. Therefore, it can be deduced that when R is increasing, the intensity contrast between the maximum and the minimum (sensitivity) is increased, but the bandwidth of the sensitive measurement region is reduced. As a result, there is a tradeoff between intensity contrast and measurement range. To have a comparable sensitivity (0.9) and bandwidth (0.008 RIU), $R = 0.4 - 0.5$ is preferred. Without the need of high reflecting surfaces or high finesse microcavity, the fabrication process is greatly reduced.

5.3.1.2 Effect of the cell size

Fig. 5.28 Intensity variation in respect to cell with different sizes and refractive indices simulated at 514 nm.

For the measurement of single cell using the complementary FP resonant system, theoretical analysis is performed to investigate the measurement range and the sensitivity of the FP resonant system by varying the cell size from 5 μm to 20 μm and the cell refractive index from 1.333 to 1.357. The cavity has a length of 25 μm and the surrounding solution has a refractive index of 1.333. The intensity variations with respect to the refractive index values for particles with different sizes are shown in Fig. 5.28. It can be seen that the measurement range is increased with smaller particle (measurement range is reduced by 2-fold with particle size increased by 2-fold). This is because the change of optical path for the same refractive index value is smaller for smaller particle. Although measurement range is increased, the sensitivity of the system is reduced with smaller particle. Therefore, it is a trade-off between the measurement range and the sensitivity of the FP system for the measurement of single cell. In addition,

the measurement range and sensitivity is independent with the cavity length. For wavelength based measurement scheme, short cavity length is preferred because larger shift is observed which requires lower resolution in measurement. However, short cavity length imposes difficulties in fabrication. Since single-wavelength intensity based measurement scheme does not require short cavity length, the fabrication process is greatly simplified.

For system characterization, spherical particles with uniform refractive index value are used. In the experiment, polystyrene particles with size of 15.41 μm are used for characterization and Madin-Darby canine kidney cell is used for cell measurement. Therefore, the cavity length of 25 μm is designed.

5.3.1.3 Effect of the absorption/scattering of the cell

Intensity attenuation is normally observed with the presence of particle/cell in the cavity. The degree of attenuation is depending on the absorption and scattering levels of the particle/cell [98 - 99]. Due to the presence of intensity attenuation, another pair of fibers is integrated in addition to the complementary microfluidic FP resonant system. These fibers are not gold coated and is used to measure the intensity attenuation of different particles/cells.

Fig. 5.29 shows the transmission spectra of FP resonator without/with the presence of the particle or cell. By monitoring the intensity level at single wavelength of 514 nm, a total intensity variation of observed by the FP resonator is

$$\Delta I_T = I_1 / I_3 \quad (5.13)$$

Fig. 5.29 Transmission spectra of the resonant cavity without/with the presence of the particle.

where I_1 and I_3 are the intensity level with and without the cell in the cavity, respectively. However, the intensity attenuation due to absorption/scattering is

$$A_{AS} = I_2 / I_3 \quad (5.14)$$

where I_2 is the intensity level due to the absorption/scattering of the cell. The intensity variation due to optical path difference can be determined as

$$\Delta I_{OPD} = I_1 / I_2 \quad (5.15)$$

In order to decouple these two components (A_{AS} and ΔI_{OPD}) from the FP measurement (ΔI_T), the pair of bare fibers is used to measure A_{AS} , and then ΔI_{OPD} can be determined as

$$\Delta I_{OPD} = \Delta I_T / A_{AS} \quad (5.16)$$

Therefore, the absorption/scattering of the cell and the optical path difference induced by the presence of the cell in the FP cavity can be measured by the three pairs of fibers. However, the refractive index value of the cell can only be known if the size of the cell is known. In the next section, the measurement of the cell size using the same optical system, i.e. the three pairs of fibers, is presented.

5.3.2 Measurement of cell size

Fig. 5.30 Intensity signals observed with respect to time when single cell flows through (a) the measurement region, and (b) the FP resonator B.

In order to decouple the information on the refractive index of the cell from the measured optical path difference, the size of the cell is determined by signal modulation as shown in Fig. 5.30. The time period between signal I and signal A is t_1 , and the time period between signal A and signal B is t_2 . The separation distance between Fiber-Pair 1 and FP resonator A is 800 μm. FP resonator A and FP resonator B have a separation distance of 400 μm. The flow velocity of the cell in the microchannel can be determined as

$$v_{\text{cell}} = \frac{1}{2} \left(\frac{800}{t_1} + \frac{400}{t_2} \right) \quad (5.17)$$

With the determined flow velocity, the size of cell can be determined from the individual signal. When the cell starts to pass through the light beam, a change in transmission intensity is observed at time t_{bef} as shown in Fig. 5.30 (b). When the cell leaves the light beam, the transmission intensity returns to the original level at time t_{aft} . The size of cell, by averaging the values obtained from the three pairs of fibers, can be determined as

$$d_{\text{cell}} = -d_{\text{beam}} + \frac{1}{3} \sum_{n=3} v_{\text{cell}} (t_{\text{aft}} - t_{\text{bef}}) \quad (5.18)$$

where d_{beam} is the width of the light beam.

5.3.3 Pressure driven valve for flow switching

Fig. 5.31 Schematic illustration of the microfluidic system for flow switching, which consists of pressure driven valve and restricting structure.

The design of the microfluidic system is shown in Fig. 5.31. After the measurement region, the microchannel is divided into two branches, one to the sample outlet and the other one to the waste outlet, respectively. The microchannel connected to the sample outlet is designed with a series of restricting structures, which has a width of $40\ \mu\text{m}$ and a length of $1\ \text{mm}$. The microchannel connected to the waste outlet is integrated with a pressure valve as shown in Fig. 5.31 [289]. In the off-state, the microchannel has a width of $40\ \mu\text{m}$. Due to the presence of the restricting structures, the flow of the sample solution is limited to the waste outlet. When a cell is detected, the pressure valve is changed to the close state in which the microchannel is closed by the pressure valve. In the on-state, the flow of the sample solution is changed to the sample outlet.

The flow in the microchannel with the operation of the pressure valve is simulated using Comsol Multiphysics as shown in Fig. 5.32. When the pressure

Fig. 5.32 Simulation results of the flow velocity in the microchannel when the pressure valve is in (a) the off-state, and (b) the on-state.

valve is in the off-state, the flow is dominated in the microchannel to the waste outlet. However, when the pressure valve is in the on-state, the flow in microchannel to the waste outlet becomes stagnant. The flow is switched to the microchannel to the sample outlet. The pressure valve is normally in the off-state such that the sample liquid flows to the waste outlet. When the cell is detected in the complementary microfluidic FP resonant system, the pressure valve is switched to the on-state and the sample fluid flows to the sample outlet. Cells are accumulated in the sample outlet for further biochemical/biophysical analyses.

5.4 Experimental results and discussions of complementary optofluidic FP resonant system

5.4.1 Fabrication processes

Fig 5.33 Microphotos of (a) integrated fibers with desired separation distance and at optimal alignment, (b) the pressure valve in the off-state, and (c) the pressure valve in the on-state.

The fabrication processes of the optofluidic FP refractometers are primarily based on the polymer soft lithography fabrication technique as discussed in Chapter 3. The mold is fabricated using SU8-50 on a silicon wafer. The patterned PDMS slab is bonded to the other PDMS slab using plasma bonding. Figure 5.33 shows the fabricated complimentary optofluidic FP resonant system. Integrated fibers are

fixed using UV cure epoxy with desired separation distance and at optimal alignment as shown in Fig. 5.33 (a). The fabricated pressure valve in the off-state is shown in Fig. 5.33 (b). Liquid is pressurized on the two side chambers to bend the sidewalls of the microchannel of the waste outlet and the close state is obtained without any liquid flows to the waste outlet as shown in Fig. 5.33 (c).

5.4.2 Experimental setups

Fig 5.34 Schematic illustration of the experimental setup of the complementary optofluidic FP resonant system.

The experiment setup of the complementary optofluidic FP resonant system is shown in Fig. 5.34. The microfluidic system provides the injection of sample solution into the microfluidic chip and the control of the pressure driven valve. On

the other hand, the fibers are connected to the external optical system for optical measurement. The sample solution is injected into the microchannel using a syringe pump (New Era, NE1000). For the pressure driven valve, the microchannels are initially injected with DI water. The inlets are then connected to the pressure controllers to control the pressure in the microchannels using computer control for the operation of the valve. The optical system consists of two sets of setup. First, a He-Ne laser with central wavelength of 633 nm and a bandwidth of 30 nm is connected as the input for spectrum analysis. The output fiber is connected to the spectrometer (Ocean Optics, HR4000). This setup is used for wavelength-based measurement scheme in which the transmission spectra of the FP resonators are detected. Second, the input source is changed to an argon ion laser with output wavelength of 514 nm (Modu-Laser, Stella-PRO Select) for intensity measurement. The input light is split into the three input fibers. The output fibers are connected to the photomultiplier tube (PMT) detectors with peak sensitivity at the wavelength of 500 nm (Hamamatsu, H5784-02) and the output signals from the PMT detectors are detected using USB data acquisition (DAQ) hardware at 10 kHz (National Instruments, USB-6008). This setup is used for single-wavelength intensity based measurement scheme in which the output intensity of each output fibers is measured. The experiments are carried out for two main objectives. Firstly, the characterization of the complementary microfluidic FP resonant system using 15.41- μm polystyrene beads is experimented. These results are presented in section 5.4.3. Secondly, the optical measurement of the MDCK cells is presented in section 5.4.4.

5.4.3 Characterization of complementary optofluidic FP resonant system

Fig 5.35 Transmission spectra of the complementary microfluidic FP resonant system with He-Ne laser as the input source.

The two pairs of gold-coated fibers are inserted into the chip and auto aligned by the patterned grooves. A He-Ne laser with central wavelength of 633 nm and a bandwidth of 30 nm is connected as the input. The output spectra for the two FP resonators are shown in Fig. 5.35. The finesse of the FP resonators measured are 3.12 and 2.31, respectively. The finesse is lower than the simulated FP with reflectance of 0.5. This is because optical losses due to the emerged diverging light are not included in the simulation. However, the interference patterns are clearly observed in the output spectra and can be used for this measurement scheme as high finesse FP resonator is not essential.

The complementary microfluidic FP resonator system has to be fine-tuned to obtain two FP resonators with different sensitive regions. The light source is

changed to a single-wavelength argon ion laser and the PMTs are connected to measure the intensity for each output fibers. The microchannel is filled with DI water ($RI = 1.333$) that is used as the reference buffer for the characterization of the resonators. The cavity distance between the fibers are fine-tuned using micromanipulators to obtain a minimum intensity level and $\frac{1}{4}$ with respect to the maximum intensity level for FP resonator A and B, respectively. All fibers are fixed in position using UV-cured epoxy.

The transmission intensity of each resonator is measured for buffers with refractive index in the range from 1.334 to 1.340. $CaCl_2$ solutions with different concentrations are prepared and the refractive indices are measured using a handheld refractometer. The intensity variations (the ratio of measured intensity to the initial intensity) due to optical path difference (refractive index value $\times$ cavity length) are shown in Fig. 5.36. It can be seen that the two FP resonators have different sensitive regions and both signals can be used to determine the refractive index change or the optical path difference.

The characterized complementary optofluidic FP resonant system is used to measure polystyrene beads ($15.41 \pm 0.15 \mu m$ with $RI = 1.585$ to 1.595). The polystyrene beads have a nominal diameter of $15.41 \mu m$ with a standard deviation of $0.15 \mu m$ (Invitrogen, F8843). The measurement of the polystyrene beads can be used to verify the accuracy of the FP resonant system because the bead is spherical in size and has uniform refractive index distribution. The size of 100 beads is measured using SEM micrograph as shown in Fig. 5.37. The measured nominal diameter is 15.41 with a standard deviation of 0.14 , which is consistent with the specification provided by the manufacturer. Polystyrene beads are diluted in DI water and injected into the microchannel. Fig. 5.38 shows a single bead in the FP

Fig 5.36 Intensity variation of the complementary FP resonant system by injecting different buffer solutions using argon ion laser as the input source.

Fig 5.37 SEM micrograph of the polystyrene beads.

resonator when it is passing through the detection region. The intensity measurement results are shown in Fig. 5.39. The time period for the bead to flow from the first pair to the second pair of fibers is 1.6312 s. Subsequently the time from the second pair to the third pair of fibers is 0.8093 s. With the predefined distance of 800 and 400 μm between the fibers, the velocity of the bead is 492 μm/s.

Fig 5.38 Microphoto of the polystyrene bead flowing through the detection region.

Fig 5.39 Measured intensity when the polystyrene bead was flowing through the detection region.

Since the time period for the polystyrene bead to pass through the light beam is 40.8 ms, $d_{\text{cell}} + d_{\text{beam}} = 20.07 \mu\text{m}$. The diameter of the bead is measured using optical microscope with $20\times$ objective lens and has a value of $15.51 \mu\text{m}$. Therefore, the beam width is determined as $4.56 \mu\text{m}$.

Based on the intensity change detected by Fiber-Pair I as shown in Fig. 5.39, the attenuation/scattering of the polystyrene bead is $A_{\text{AS}} = 0.7364$. The intensity

Fig 5.40 Comparison with the measured intensity variations with the characteristic curves of both resonators in the refractive index range from 1.585 to 1.595.

Fig 5.41 Size and refractive index distributions of 30 polystyrene beads.

variations for FP Resonator A and B due to the shift of the FP resonant peaks/ the change of the optical path in the cavity are $\Delta I_{\text{OPD}} = 1.9028$ and 2.2272 , respectively. The intensity variations (ΔI_{OPD}) of the FP resonant system for a $15.51\text{-}\mu\text{m}$ particle in the range of 1.585 to 1.595 RIU are shown in Fig. 5.40. Based on the two measured intensity variations, the polystyrene bead has refractive index value of

1.592. The experiment is repeated for 30 polystyrene beads. The size and refractive index distributions of 30 measured polystyrene beads are shown in Fig. 5.41. The measured 30 beads have a mean diameter of 15.41 ± 0.12 and a mean refractive index of 1.590 ± 0.002 .

5.4.4 Measurement of single MDCK cell

Fig 5.42 Microphotos of the flowing MDCK cell in the microchannel. A point is highlighted in red to prominently show the rotating direction of the flowing cell.

The developed complementary microfluidic FP resonant system is used for MDCK cell measurement. The MDCK cell is suspended in the PBS solution (RI = 1.356). The flowing pattern of the cell in the microchannel is critical for the refractive index and size measurements because the measurement is uni-dimensional. It may lead to measurement error if the cell is spinning or pulsating in the microchannel. The flow path of the MDCK cell in the microchannel is monitored by high-speed camera (Photron, FASTCAM SA3). The captured microphotos are shown in Fig. 5.42. Based on the captured microphotos, the cell is rolling in the flow direction with a constant velocity. In addition, the MDCK cell is spherical in shape. Therefore, the measurements are not affected significantly by the

Fig 5.43 Measured intensity when the MDCK cell was flowing through the detection region.

flowing pattern of the cell.

The measurement results are shown in Fig. 5.43. The size of the MDCK cell is measured to be $16.17\ \mu\text{m}$. The attenuation/scattering of MDCK cell is $A_{AS} = 0.9753$. The intensity variations for FP Resonator A and B due to the shift of the FP resonant peaks/ the change of the optical path in the cavity are $\Delta I_{OPD} = 0.3213$ and 0.5147 , respectively. The intensity variations of the FP resonant system for a $16.17\text{-}\mu\text{m}$ cell in the range of 1.372 to 1.387 RIU are shown in Fig. 5.44. Based on the two measured intensity variations, the MDCK cell has refractive index value of 1.382 .

The size and refractive index distributions of 30 measured MDCK cells are shown in Fig. 5.45. The measured 30 MDCK cells have a mean refractive index of 1.382 ± 0.001 . For viability test, the cells are injected into the LOC system and collected at the outlets. The collected cells are then stained with Trypan blue. Based on the viability test, 96 % of the cells are remained viable (sample size: 50). This shows

Fig 5.44 Comparison with the measured intensity variations with the characteristic curves of both resonators in the refractive index range from 1.372 to 1.378 for a 16.17- μm cell.

Fig 5.45 Size and refractive index distributions of 30 MDCK cells.

that the complementary optofluidic FP refractometer has no significant effect on the cell viability.

The integrated valve is operated using pressure controller. The pressure driven valve is in off-state such that the flow is directed to the waste outlet as shown

Fig 5.46 Microphotos of (a) the pressure driven valve in the off-state, and (b) the flow trajectory of a single bead to the waste outlet.

Fig 5.47 Microphotos of (a) the pressure driven valve in the on-state, and (b) the flow trajectory of a single bead to the sample outlet.

in Fig. 5.46. Polystyrene beads with a diameter of $15.41\ \mu\text{m}$ suspended in deionized water is injected into the microfluidic system. When the valve is in off-state, the polystyrene beads are flowing directly to the waste outlet. When the valve is in on-state, the polystyrene beads are directed to the sample outlet as shown in Fig. 5.47. Based on the demonstrated results, the pressure-driven valve can be employed as a cell sorting mechanism when it is operated with a computerized auto-selection system based on the size and refractive index measurement in the upstream of the microchannel.

Several issues and challenges of the developed complementary optofluidic FP refractometer are discussed as follow,

- a) In the current design, single cell separation mechanism is not integrated. However, single cell separation can be easily designed using a T-junction structure as discussed in Chapter 3 or a hydrodynamic focusing structure by limiting the cell medium flow to the size of a single cell. In the experiment, similar approaches same as the one discussed previously are employed to increase single cell measurement efficiency. The measurement results are confirmed by microscopic approach. Results for single cell measurement are presented and other results caused by cell cluster are omitted.
- b) Cell stiction is not a significant problem in this system because the cells are injected and flowing in the microchannel at a velocity of approximately 500 $\mu\text{m/s}$.
- c) Each cell can be measured within 5 s and this greatly improves the throughput of the cell refractometer as compared with the previous two systems. The limitation of the measurement speed is mainly due to the limited acquisition rate of the DAQ. With a fixed acquisition rate, a slower flow rate leads to better resolution in measurement with more recorded data points.
- d) Sequential measurement is applicable. However, the detection part and the flow switching part have to be synchronized to add the functionality of cell sorting. Other functionalities can also be integrated easily using the LOC platform.

5.5 Summary

This chapter focuses on the theoretical analysis, optical designs, microfluidic designs, chip fabrication and experiments of the optofluidic FP refractometers. The optofluidic microlens FP resonator is designed, fabricated and demonstrated. A pair of liquid microlenses is integrated onto the FP cavity. It is a simple way to realize a real-time tunable FP resonator such that light is manipulated easily using liquid microlenses and confined in the FP cavity. The collimated light propagation enhances the finesse and reduces the loss of the FP resonator. The resonant cavity is formed in the central microchannel with the sidewalls coated with gold layers. The gold layers increase the reflectance of the sidewalls and subsequently the finesse of the FP cavity. By tuning the radii of the curvature of the liquid microlenses, the finesse of the FP cavity can be enhanced by 5.4 times, i. e. 18.79. The optofluidic microlens FP resonator has a measuring range of 0.043 RIU and a detection sensitivity of 960 nm per RIU for buffer solution measurement. The developed optofluidic FP refractometer is employed to measure the refractive index and diameter of MDCK cell. The refractometer demonstrates a precision of 0.0005 RIU in refractive index measurement and 0.4 μm in size measurement.

The innovation of the developed optofluidic microlens FP resonator is summarized as follow,

- a) This is the first effort demonstrated to integrate a pair of optofluidic microlenses in the fiber-based FP resonator. The microlenses provide real-time tunability to collimate the light path and increase the finesse of the FP resonator by 5.4-fold as compared to the one without the

microlenses [102]. The finesse of the FP resonator can be further improved using 3-dimensional optofluidic microlenses.

- b) Unlike conventional fiber-based FP resonator, in which the FP cavity is formed by the gold coated fiber facets, the developed FP resonators firstly presented the usage of the sidewalls of the PDMS microchannel to form the cavity. The sidewalls of the microchannel are gold-coated with the developed fabrication process.

The complementary optofluidic FP resonant system is also designed, fabricated and demonstrated. The measurement of single cell refractive index and size is performed using a single-wavelength intensity-based measurement scheme. In addition, another pair of fibers is integrated to measure the transmission of the cell to obtain the absorption/scattering information. In contrast with a single FP resonator using frequency-based measurement scheme, which requires high-finesse resonator and high resolution spectrometer to increase its precision, the complementary optofluidic FP resonant system using single-wavelength intensity based measurement scheme relies on the signal-to-noise ratio of the detection system for its precision. It has several advantages as compared to the single FP resonator using common frequency-based measurement scheme. First, it does not require a high finesse resonant cavity, which greatly reduces fabrication and integration difficulties. Second, cell trapping mechanism is not required which greatly simplifies the design of the integrated system and improves the robustness of the integrated chip. Third, the light source requires only single wavelength which greatly improves the data acquisition rate and avoids measurement errors due to motion and vibration. The complementary optofluidic FP resonant system is used

for real-time cell measurement. Based on the experimental results, MDCK cells are measured to have refractive index of 1.382 ± 0.001 .

The innovation of the developed complementary optofluidic FP resonant system is summarized as follow,

- a) This is the first effort demonstrated to employ single-wavelength intensity-based measurement scheme in cell refractometer. The working and operating principles are theoretically analyzed to summarize a set of design criteria for the system.
- b) A LOC system with the measurements of multiple biophysical properties is developed. The system does not only measure the cell size and refractive index, but also the cell absorption/scattering, which is another important optical parameter that researchers can investigate on and relate it to the biological properties of the cell.
- c) The developed LOC system is a real-time monitoring system, which does not require any cell trapping mechanism and also demonstrating shorter processing time as compared to previously demonstrated frequency-based cell refractometer.

CHAPTER 6

CONCLUSIONS

6.1 Conclusions

Three different Lab-on-a-chip (LOC) system which employ optical resonant cavities for cell refractive index measurement have been innovated, i.e. on-chip grating interferometer, optofluidic microlens Fabry-Pérot (FP) refractometer, and complementary optofluidic FP resonant system. Various aspects, including theoretical studies, system designs, fabrication technology, system characterization and cell measurements have been presented. The contents cover mainly the theoretical analyses for cell refractive index measurement based on the optical signal variation in the resonant cavities, and the experiments of the three successfully designed and integrated LOC systems. The major conclusions drawn are listed here.

The on-chip grating interferometer is studied theoretically, fabricated and experimented. The working principles in employing the grating interferometer with buffer modulation scheme for cell refractive index measurement are discussed thoroughly. A simplified measurement model has been developed based on the FP cavity formulation. In addition, the system is designed with microfluidic network and integrated with fiber optical trapping for robust and precise cell manipulation in the microchannel.

- (i) This is the first effort demonstrated to design a cell refractometer using the Fiber Bragg grating interferometer combined with differential measurement scheme. The accuracy of refractive index measurement is improved. The precision of the measurement is improved to 0.001 as compared to the one with FP resonator [102]. The precision of the measurement can be further improved by increasing the finesse of the grating interferometer. This can be done by reducing the light propagation loss in the microchannel using microlenses or improving the Q-factor of the fiber grating.
- (ii) A single pair of optical fibers is employed for cell trapping and cell refractive index measurement, which avoid the alignment problem in measurement and reduce the complexity in integration and packaging. In previous demonstrated FP resonator, the pair of fibers cannot be used for optical trapping as they are gold-coated. An external micro-capillary tube is used to fix cell in the cavity, which complicates the packaging process and may leads to cell distortion [102]. In this system, optical trapping is used as non-contact trapping technique. The optical power can be easily tuned not only to efficiently trap the cell within the micro-cavity, but also to avoid cell distortion.
- (iii) A droplet microfluidic system is designed, fabricated and demonstrated to realize differential measurement scheme with enhance mixing. It has the capability to isolate suspended cells into a series of plugs for single cell measurement with the designed microfluidic networks. Further biological analytic or handling processes can be easily integrated into the microfluidic networks.

The optofluidic microlens FP resonator, which consists of a pair of innovative liquid microlenses, is studied theoretically, fabricated and experimented. The expansion chamber, which the liquid microlens is formed, is designed by the guidance of the flow simulation in the chamber to avoid the presence of secondary flow and optimize the lens profile formed by the liquid interfaces. The system is integrated with optical trapping and designed with on-chip to perform experiments on cell refractive index measurement.

- (i) This is the first effort demonstrated to integrate a pair of optofluidic microlenses in the fiber-based FP resonator. The microlenses provide real-time tunability to collimate the light path and increase the finesse of the FP resonator by 5.4-fold as compared to the one without the microlenses [102]. The finesse of the FP resonator can be further improved using 3-dimensional optofluidic microlenses.
- (ii) Unlike conventional fiber-based FP resonator, in which the FP cavity is formed by the gold coated fiber facets, the developed FP resonators firstly presented the usage of the sidewalls of the PDMS microchannel to form the cavity. The sidewalls of the microchannel are gold-coated with the developed fabrication process.

The complementary optofluidic FP resonant system, which employs single-wavelength intensity-based measurement scheme for cell refractive index measurement, is studied theoretically, fabricated and experimented. Based on the signal modulation in time domain, the size of the sample can be determined concurrently. The system provides a real-time cell refractive index measurement system that does not require cell trapping. In addition, a pressure-driven valve is integrated into the system for cell sorting.

- (i) This is the first effort demonstrated to employ single-wavelength intensity-based measurement scheme in cell refractometer. The working and operating principles are theoretically analyzed to summarize a set of design criteria for the system.
- (ii) A LOC system with the measurements of multiple biophysical properties is developed. The system does not only measure the cell size and refractive index, but also the cell absorption/scattering, which is another important optical parameter that researchers can investigate on and relate it to the biological properties of the cell.
- (iii) The developed LOC system is a real-time monitoring system, which does not require any cell trapping mechanism and also demonstrating shorter processing time as compared to previously demonstrated frequency-based cell refractometer.

Table 6.1 summarized the key metrics of interest of the developed LOC systems. The on-chip grating interferometer and the optofluidic microlens FP refractometer are operating in the frequency (or wavelength) domain. Therefore, the finesse of the microcavities is critical. The optofluidic microlens FP refractometer has excelled in term of precision in both measurements of cell refractive index and size (2-fold improvement). The finesse is improved by 5-fold as compared to the on-chip grating interferometer, which consequently increase the resolution in determining the peak shift during measurement. In addition, the sensitivity of the optofluidic microlens FP refractometer is also relatively higher than the one of the on-chip grating interferometer, which greatly reduces the difficulties in detecting refractive index change. The limited sensitivity of on-chip grating interferometer

Table 6.1 Comparisons of the developed LOC systems

	On-chip grating interferometer	Optofluidic microlens FP refractometer	Complementar y optofluidic FP refractometer
Standard deviation in RI measurement	0.001	0.0005	0.001*
Standard deviation in size measurement	0.9 μm	0.4 μm	0.9 μm^*
RI range (for 20- μm sample)	0.032	0.032	0.011
Sensitivity per RIU	2.63 nm	950 nm	-
Finesse	5.13	18.79	2.31 & 3.12
Merits	Cell isolation	Improved finesse	Absorption/scat tering measurement
	Optical trap	Real-time tunable	Real-time measurement
Drawbacks	Cavity length limitation	Complex optical trap	Limited RI range
	Low sensitivity	Complicated gold coating fabrication	Only one measurement

* The standard deviations for on-chip grating interferometer and optofluidic microlens FP refractometer are calculated based on several measurements of a MDCK cell. But the one for complementary optofluidic FP refractometer is calculated based on a sample size of 30 MDCK cells because it is difficult to have multiple measurements in this system.

is due to the long cavity length (Bragg grating in fiber), which limiting the performance of the system. However, the on-chip grating interferometer provides the function of cell encapsulation and isolation, which moves from conventional microfluidics to digital microfluidics. Each cell plug is treated as an isolated sample

for easy manipulation and avoiding cross-contamination. The optical trap system is also relatively simpler as compared to the one of the optofluidic FP refractometer. The on-chip grating interferometer can be used if the refractive index difference between the targeted cells and the whole sample is large, which does not required high precision, due to its relatively ease packaging process and its ability in compartmentalization. In contrast to the first two LOC systems, the complementary optofluidic FP refractometer is operating in the time domain. The main advantage of this LOC system is its real-time measurement process, which does not require any trapping mechanism. In addition to refractive index and size measurements, the adsorption/scattering information is also measured consequently. It does not require high finesse microcavities, which greatly simplify the fabrication and packaging process. The demonstrated precision is comparable to the on-chip grating interferometer and it may be improved using high resolution photodetectors. However, the drawback of the complementary optofluidic FP refractometer is its relatively narrow refractive index range of 0.011 RIU for 20- μm sample.

In conjunction to the development of on-chip refractometers, two optofluidic components have been innovated, i.e. optofluidic microlens and optofluidic grating. Various aspects, including theoretical studies, microfluidic designs, fabrication technology and optical characterizations have been presented. The major conclusions drawn are as follows:

- (i) The optofluidic microlens is studied theoretically, fabricated and experimented. The structural design of the expansion chamber in which the microlens is formed and the tuning of the interfaces with the flow rates of the three laminar flow streams are discussed thoroughly. The formation of

microlens with different lens profiles and radii of curvature are experimentally presented.

- (ii) The optofluidic grating is studied theoretically, fabricated and experimented. The structural design of the microchannel and the formation mechanism of the plugs are discussed thoroughly. The optical characteristics of the optofluidic grating are investigated with different grating period and grating length.

LOC technologies enable the incorporation of several functionalities which facilitate the measurement of cell refractive index with greater stability, efficiency and ease of measurement in a more controllable environment. With the design of the microfluidic network, single cell can be easily manipulated to direct the cell into the measurement area, to trap the cell during measurement and to sort the cell after measurement. In addition, buffer modulation method is not easily feasible unless in the microchannel environment with the cell trapping functionalities. In addition, it introduces other biological or chemical functionalities to broaden its applications and the miniaturization with portability makes LOC devices much more attractive.

To further improve the development of LOC systems for single cell refractive index measurement, several future works with relevant recommendations are described in the following section.

6.2 Recommendations

Recommendations for future research are summarized as follows

- a) In the development of the on-chip grating interferometer, it is significant to increase the finesse or reduce the bandwidth of the resonant peaks so that the precision of the measurement can be improved. Two possible solutions can be studied. First, the optical loss can be reduced in the microcavity using microlenses for collimation. The emerging light from the fiber is diverging, which lead to optical loss and subsequently reduced finesse. By collimating the emerging light, the optical loss is reduced and subsequently the finesse of the cavity can be improved. Second, the optimization of the grating performance can be performed by increasing the refractive index modulation on the fiber core or using suitable apodization profile.
- b) In the development of the optofluidic microlens FP refractometer, the optofluidic microlens focuses light along the plane of the microchannels, which is a 2-dimensional microlens. In order to further increase the finesse of the resonant cavity, one can design an optofluidic 3-dimensional microlens. The 3-dimensional optofluidic microlens can focus light along the plane of microchannels and also perpendicular to the plane of the microchannels. This can be achieved by employing the centrifugal effect on the density-mismatched liquids when the liquids flow through a curved microchannel.
- c) With the demonstrated optofluidic grating, it can be extended to form an interferometric system for cell measurement. Michelson interferometer can be designed with a single grating and a reflecting mirror. On the other hand, Mach Zehnder interferometer can be designed with a pair of grating.

- d) In the development of the complementary optofluidic FP resonant system, a 3-dimensional hydrofocusing mechanism can be employed to improve the measurement efficiency. By integrating the 3-dimensional hydrofocusing mechanism, the biological samples such as cells can be aligned horizontally and vertically such that it passes through the core of the fibers for detection.
- e) With the developed LOC systems in this thesis for the cell refractive index and size measurement, it is recommended to extend the LOC systems into real life biological applications. For example, the system can be used to identify protozoa in treated water. In this case, with a limited range of possible biosamples in treated water, the LOC systems can be employed to differentiate protozoa based on refractive index and size. Another example is iron-deficiency anemia or Thalassemia detections in which the hemoglobin concentration of red blood cell determines its refractive index. The LOC systems can also be extended to other biological applications in which the parameters of refractive index and size can be used with sufficient precision to differentiate the targeted samples from a sample group.
- f) In this thesis, the LOC systems developed are mainly focused on the measurement of cell refractive index. It is highly recommended to incorporate further optical/chemical analyses such as biomedical functionalities into the chip such as drug screening or bio-reagent concentration effect etc. This will expand the usefulness of the systems for different applications.

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