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Determining refractive index of single living cell using an integrated microchip

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Abstract

We report a novel method for measuring the effective refractive index (RI) of single living cell using a small integrated chip, which might be an efficient approach for diseases diagnosis. This microchip is able to determine the refractive index of single living cell in real time without any extra cell treatments such as fluorescence labelling, chemical modification and so forth, meanwhile, providing low cost, small size, easy operation and high accuracy. The measurement system integrates an external cavity laser, a microlens, and some microfluidic channels onto a monolithic chip. In the experiments, two standard polystyrene beads with nominal RI are utilized to calibrate the measurement system and five different types of cancerous cells are subsequently measured in the chip. The experimental results show that the refractive indices of the cancerous cells tested ranges from 1.392 to 1.401, which is larger than typical value of normal cell of 1.35–1.37. This integrated chip potentially has a serial of applications on biodefense, disease diagnosis, biomedical and biochemical analysis.

Keywords: Refractive index; External cavity laser; Single living cell; Microfluidics; Microlens

1. Introduction

In recent years, rapid in situ analysis of biological cells and detection of ultra-small-volume samples even single cell are in great demand [1] since it covers a range of applications from environmental monitoring and biodefense to disease diagnosis and point-of-care (POC). The refractive index (RI) plays a vital role in many places of biophysics, biochemistry and biomedicine. It is of crucial importance in monitoring the characteristics of living cells. For example, chemical or ingredient changes inside the cells can be detected by refractive index measurement. One of many potential applications is disease diagnosis like cancers. Decades ago, it was well known that cancer was a genetic disease and cancer was the result of cumulative alterations in DNA [2]. Most researchers have long focused on gene mutations and developed several theories of how cells turn malignant. However, recent evidence shows that the number of chromosomes inside cells is a critical mark in the early stage of cancer. One living cell is a complex structure, which contains numerous organelles with different refractive index. For example, the refractive index of cytoplasm is normally 1.35–1.38 [3–5], and the index of nucleus is around 1.39 [6]. However, the protein concentration within the cell components mainly determines the effective refractive index of cells because of the large amount and high refractive index of 1.50–1.58 [3,6]. Compared to normal cells, cancer cells have more protein staying in the relatively larger nucleus in order to adapt to the rapid cell division [7,8], which means cancer cells have a relatively larger refractive index than normal cell due to the larger amount of protein. Therefore, some cancers probably can be diagnosed by the effective refractive index of the cell as an indicator before it becomes invasive in the beginning of the disease.

Currently, there are several techniques have already been developed to measure the refractive index of liquid and homogenous materials. The most common method is interferometry including Rayleigh refractometer, Mach–Zehnder, Michelson and Fabry–Perot interferometer and more [9–11]. By means of them, the RI of the medium can be measured by detecting the

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critical angle of total reflection, backscatter or interference patterns. However, all of these conventional methods have their inevitable disadvantages. Firstly, the interferometric methods are mainly used to determine the refractive index of homogenous medium such as liquids, particles and so on. It is difficult to handle the inhomogeneous matters like biological cells. Secondly, the interferometry is extremely prone to be influenced by slight disturbance even the breath. It is so tricky to obtain a steady pattern in the environment with moving or shaking. The movements of living cell and surrounding buffer will easily and completely ruin the measurements. Thirdly, due to the low efficiency of scattering from small size particles, a laser with high power is normally equipped in those traditional methods to enhance the signal-to-noise ratio. Last but clearly not least, those methods perform in the large and expensive machine and require the specifically trained operators, which exclude the applications in personal or field cases.

The present work aims to determine the effective refractive index of single living cell in real time with an integrated chip, which might be helpful for early stage disease diagnosis. A microfluidic system is built to manipulate the cell flow and deliver the single cell into an analysis region, where a laser beam keeps scanning the cell stream flowing through the external cavity of laser. Compared to the cavity with and without cell loading, the cells to be analyzed will alter the emission frequency and output power from the external cavity laser when they are passing through the beam. By recording the changes of wavelength and power, the refractive index of single living cell can be subsequently determined. In the experiments, two standard polystyrene beads with nominal refractive index are employed to calibrate the system. The result shows the high accuracy of the system with the error of 0.3%. After that, five types of different cells are measured followed the calibration; the outcomes indicate that cancerous cells tested have higher refractive indices. In this sense, the cancer diseases could be diagnosed using this novel method by screening the refractive index of single living cell.

2. Design and fabrication

To measure the refractive index of single living cell, an integrated biophotonic chip is designed and demonstrated in Fig. 1. A laser diode with the dimension of 215 μm × 300 μm × 100 μm is embedded on the chip to serve as the light source, whose central wavelength λ_0 is 1.550 μm. A gold-coated mirror oppositely positioned constructs the external cavity with one surface of the diode. Besides, one microlens set is designed and fabricated to improve the beam quality so that the laser can be only focused on one cell, which avoids the interference evoked by other cells. By applying an electrical field on the electrodes, the samples to be analyzed with phosphate buffer saline (PBS) buffer are steered by electrokinetic force and delivered into the analysis region. A hydrofocusing mechanism is also utilized to narrow the flow down to single cell level so that the biological cells are lined up and checked one by one in the analysis region [12]. In addition, the identified cells are able to be sorted into different reservoirs for further testing by

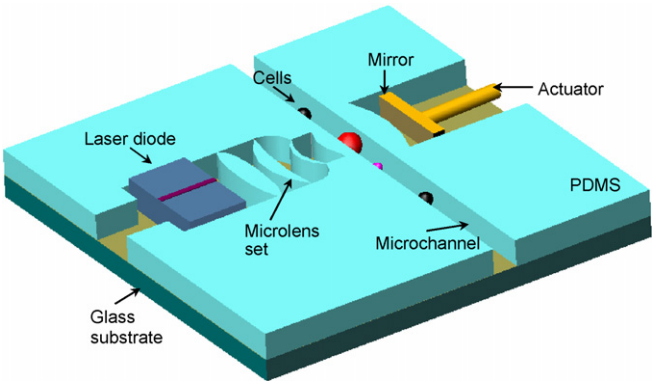

Fig. 1. Schematic diagram of the integrated detection chip.

a switching apparatus. The cells are delivered into the external cavity of laser along a microfluidic channel with the width of 100 μm. On account of the different indices between the living cell and the buffer, the cell moving through the analysis region changes the effective length of external cavity and subsequently affects the emission characteristics of laser diode. The signals of spectrum and power are detected and analyzed by a spectrometer and a power meter, respectively.

Soft lithography technology was used to fabricate the microchip [13,14]. The process flow is shown in Fig. 2. The master is made of SU-8 on a silicon substrate by the photolithography. The transparent photomask is employed to bring the cost down and speed up the process. Once the SU-8 mold is achieved, the 10:1 polydimethylsiloxame (PDMS, SYLGARD 184 Silicone Elastomer Kit) will be poured on the master to form a replica by curing for 1 h at 95 °C. After that, the

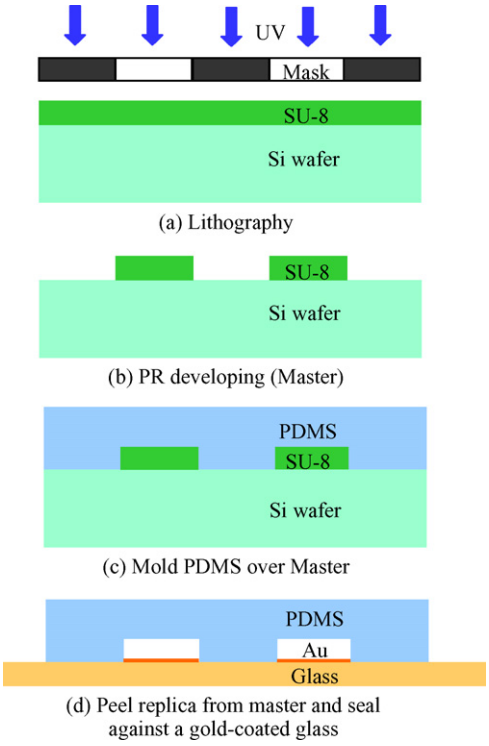

Fig. 2. The fabrication process of the integrated microchip.