MULTIPHASE FLOW MANIPULATION FOR CONTINUOUS REFRACTIVE INDEX ANALYSIS OF SINGLE LIVING CELL

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ABSTRACT

This paper reports an integrated microfluidic system for single cell’s refractive index measurement. In this system, a serial multiphase flow manipulation is proposed. Comparison to previous methods developed to measure the cell’s refractive index in a microfluidic chip, it ensures that each cell is trapped inside a buffer plug and individually passes through the detection area. This method also avoids cell’s wastage and measurement failure due to several cells gathered in the detection area. Results show that measured MDCK cells have an average effective refractive index of 1.383 ± 0.001.

Keywords: Multiphase flow, cell analysis, fiber Bragg grating, refractometry

1. INTRODUCTION

In biomedical field, the cell’s refractive index (RI) is is found to be associated with certain diseases such as Thalassemia in haematology and cancer diagnosis in oncology. Different optical methods [1-2] have been employed to measure a single cell’s RI in a microfluidic chip, where a continuous microfluidic flow is used. However, such microfluidic flow creates difficulties in single cell separation, isolation and trapping. Therefore, to tackle all these problems, a low-capillary multiphase flow [3] is employed for single cell isolation. In this case, prior to the formation of multiphase flow, single cell separation is ensured by hydrodynamic focusing. Furthermore, as buffer modulation method is used for the measurement of cell’s RI, smaller buffer plug is mixed with the cell plug in the measurement area.

Figure 1. (a) Multiphase flow system for cell manipulation. (b) Mixing in measurement area.
2. WORKING PRINCIPLE

Initially, the injected cells are separated after passing through the hydrofocusing region so that each cell individually reaches the multiphase flow formation region, as shown in Fig. 1a. Then, each cell is trapped within a buffer plug (standard PBS solution) separated by olive oil. Both flows have very slow flow rates in order to obtain a low capillary number (Ca < 0.1), so that the plug formation is based only on the channel’s geometry and the flow rate ratio. In the detection region, the width of the channel is reduced to extend the length of the plug by 2.5 times. Such arrangement will facilitate the mixing and measuring processes, as shown in Fig. 1b.

The measurement of cell’s effective RI is based on the fiber Bragg grating resonant cavity, in which the wavelength shift in the output spectrum is associated with the optical path length within the cavity, i.e. RI and associated physical length. By trapping a single cell within the cavity and changing the surrounding buffer, the cell’s effective RI \( n_c \) and effective thickness \( l_c \) can be determined by:

\[
l_c = (\Delta \lambda_1 - \Delta \lambda_2) /[k(n_2 - n_1)] \tag{1}
\]

and

\[
n_c = (\Delta \lambda_1 / k l_c) + n_1 = (\Delta \lambda_2 / k l_c) + n_2 \tag{2}
\]

where \( n_1 \) and \( n_2 \) are the RI of buffer 1 and buffer 2, respectively, \( \Delta \lambda_1 \) and \( \Delta \lambda_2 \) are the wavelength shifts compared between the absence/presence of cell in the cavity with buffer 1 and buffer 2, respectively and \( k \) is the peak shift constant.

3. DEVICE DESCRIPTION

The chip is fabricated by PDMS soft lithography. The structures are molded from the SU8 master and then the PDMS slab is bonded on a coverslip by plasma bonding. A pair of FBG fibers with Bragg wavelength of 1258 nm is aligned and forms a cavity for cell trapping by optical traps (high-power laser) as well as for cell measurement (SLED light source) based on the detected output spectra measured by Optical Spectrum Analyzer.

Figure 2. Fabricated PDMS chip.

Figure 3. (a) Cell plug formation: (i) \( t = 0 \) s, (ii) \( t = 1 \) s. (b) Flow mixing: (i) mixing with dye, (ii) mixing of buffer plug with cell plug for cell measurement.
4. RESULTS AND DISCUSSION

The chip is calibrated with NaCl solution of different concentration to obtain the peak shift constant, \( k \), and it is determined to be \( 1.56 \times 10^3 \). After that, Madin-Darby Canine Kidney (MDCK) cell is used for the measurement. Fig. 3a shows the formation of cell plug after the cell is separated by hydrofocusing. A single MDCK cell is successfully trapped within a buffer plug \((n_1)\) with a length of 120 µm. To monitor the mixing process, a dye plug is mixed with a buffer plug as shown in Fig. 3b(i).

Recirculating flow generated within the buffer plug has enhanced the mixing process [3]. Fig. 3b(ii) illustrates the MDCK cell trapped within the fiber cavity for RI measurement while a PBS (higher concentration) plug is mixed with the cell plug \((n_2)\) to perform buffer modulation. The output spectra without/with cell before mixing are shown in Fig. 4. The corresponding peak is shifted by 0.0072 nm \((\Delta \lambda_1)\). Similarly, the wavelength shift can be obtained after mixing, i.e. 0.0059 nm \((\Delta \lambda_2)\). Subsequently, the MDCK cell’s effective RI and effective thickness can be determined as shown in Table 1. It shows that MDCK cell has an average effective RI of 1.383 and an average effective thickness of 16.5 µm.

Table 1. Measurement results of cell’s effective RI and effective thickness

<table>
<thead>
<tr>
<th></th>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
<th>Cell 4</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n_1 )</td>
<td>1.355</td>
<td>1.355</td>
<td>1.356</td>
<td>1.355</td>
<td>-</td>
</tr>
<tr>
<td>( n_2 )</td>
<td>1.360</td>
<td>1.359</td>
<td>1.359</td>
<td>1.357</td>
<td>-</td>
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<tr>
<td>( \Delta \lambda_1 ) (nm)</td>
<td>0.072</td>
<td>0.072</td>
<td>0.072</td>
<td>0.067</td>
<td>-</td>
</tr>
<tr>
<td>( \Delta \lambda_2 ) (nm)</td>
<td>0.059</td>
<td>0.062</td>
<td>0.064</td>
<td>0.062</td>
<td>-</td>
</tr>
<tr>
<td>Cell’s effective RI</td>
<td>1.383</td>
<td>1.384</td>
<td>1.383</td>
<td>1.382</td>
<td>1.383</td>
</tr>
<tr>
<td>Cell’s effective thickness (µm)</td>
<td>16.7</td>
<td>16.0</td>
<td>17.1</td>
<td>16.0</td>
<td>16.5</td>
</tr>
</tbody>
</table>

5. CONCLUSIONS

In conclusion, an integrated single cell’s RI measurement system has been developed. Serial multiphase flow control is employed to enhance the robustness of the system and reduce cell’s wastage in the chip. The measurement results show that MDCK cell has an average effective RI of 1.383 ± 0.001. Furthermore, measured cell can be exported for further diagnosis while single cell in plug can be ensured.

REFERENCES