Differential single living cell refractometry using grating resonant cavity with optical trap

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This letter reports the measurement of single living cell’s refractive index \( \text{RI} \) using the optical grating resonant cavity with buffer modulation on a biochip. The cell’s RI is a significant cell biophysical property which has potential in cancer and disease diagnoses. The single cell is trapped optically within the cavity, and the transmission spectra shift due to the change of surrounding buffer, without/with cell, are used to determine the cell’s effective RI and effective thickness. The measurement shows that Madin-Darby canine kidney cell has an effective RI of 1.383±0.001. Moreover, the optical trapping technique eliminates uncertainty and avoids cell damage. © 2007 American Institute of Physics. [DOI: 10.1063/1.2823610]

Recently, biophysical properties of single living cell, such as refractive index (RI), have gained attention from scientists to investigate the measurement methods and the interpretation of their meanings. The cell’s RI is used as a fundamental quantity, which is related to other biophysical properties (e.g., size and cell mass), the studies on cell permeability and hematolysis.\(^1\) For example, hemoglobin is the main constituent of red blood cell. Therefore, the abnormal ratio of hemoglobin in a red blood cell, such as iron deficiency anemia or thalassemia, will cause a deviation in RI. In addition, the RI of the cell is also related to oncology in which the cancerous cell has higher RI as compared to the normal cell due to higher protein content.\(^2\)

Several methods have been developed to measure cell’s RI, such as microscopic methods,\(^3,4\) air-bubble reference method,\(^5\) two-beam optical trap,\(^6\) and interference.\(^7\) However, microscopy methods require bulky imaging system and complex algorithm analysis. The air-bubble reference method is applied by squeezing confining the cells between slides. The two-beam optical trap technique, on the other hand, is found complicated with low accuracy. In a recently demonstrated Fabry-Pérot (FP) interferometer,\(^7\) the cell’s RI is measured by holding it inside the cavity using a micropipette which may lead to the deformation of cell and consequently experimental error. Moreover, the use of the micropipette makes it difficult to be integrated onto a single chip. Therefore, in this letter, a simple, precise, and noncontact method is proposed to overcome such shortcomings.

A single living cell refractometer is developed and shown in Fig. 1(a). Three inlets are introduced in this biochip for cell loading, oil injection to form multiphase flow, and buffer modulation. A pair of fiber Bragg grating (FBG) single-mode fibers is used for the RI measurement and the noncontact trapping of the cell. As shown in Fig. 1(b), the FBG fibers are separated and aligned to form a resonant cavity with cavity length of \( D \). Laser light is coupled into both fibers to trap a single cell optically between the fibers. For RI measurement, a broadband light source is used to observe the transmission spectrum of the resonant cavity.

In principle, the cell’s effective RI \( (n_c) \) and effective thickness \( (l_c) \) can be determined simultaneously by monitoring the output spectrum shift using differential interferometry method\(^3\) where the surrounding buffer in the cavity

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changes during measurement. Based on FP cavity, the transmittance of the uniform FBG resonant cavity can be expressed as

\[
T = \frac{(1 - R)^2}{1 + R^2 - 2R \cos[2(\beta_0 D + 2\beta_1 L_1 + \varphi)]},
\]

where \( R \) and \( \varphi \) are the reflectance and phase shift of FBG, respectively, \( L_1 \) is the distance between the fiber edge and FBG, \( \beta_0 \) and \( \beta_1 \) are the respective propagation constants. To verify the effectiveness of Eq. (1), the calculated transmittance spectrum is compared with the one simulated by the standard T-matrix method as shown in Fig. 2. The simulation results show that both methods match well with each other within the band-stop region and give identical peaks. As a result, the relationship between the peak shift and the change of the optical path length can be correctly determined by FP cavity. When the RI of the buffer in the cavity is changed slightly, the resonant peaks are shifted by

\[
\frac{\Delta \lambda}{\lambda} = k \frac{\Delta n D}{2n_{\text{eff}} L_{\text{eff}}} = \frac{\Delta \text{OPL}}{\text{OPL}_{\text{eff}}},
\]

where \( \lambda \) is the resonant wavelength, \( \Delta n \) is the change of the RI, \( n_{\text{eff}} \) and \( L_{\text{eff}} \) are the effective RI and the effective length of the resonant cavity, respectively. As the change of the effective optical path length (OPL\(_{\text{eff}}\)) due to the slight change in the RI is negligible, a linear relation between \( \Delta \lambda \) and \( \Delta \text{OPL} \) can be simplified as

\[
\Delta \lambda = k \Delta \text{OPL},
\]

where \( k \) is a dimensionless constant.
With the defined $k$, the differential method is employed to determine $n_c$ and $l_c$. Two buffers with different RIs are injected into the cavity alternatively. Four transmission spectra can be obtained with two spectra for each buffer, i.e., with the absence/presence of the single cell in the cavity. The peak shift can be expressed as

$$\Delta \lambda_m = k(n_c - n_m)l_c,$$  \hspace{1cm} (4)

where $n_m$ is the RI of buffer $m$ (1 or 2) and $\Delta \lambda_m$ is the wavelength shift in buffer $m$. Subsequently, $n_c$ and $l_c$ can be determined as

$$n_c = (n_2 \Delta \lambda_1 - n_1 \Delta \lambda_2)/(\Delta \lambda_1 - \Delta \lambda_2)$$  \hspace{1cm} (5)

and

$$l_c = (\Delta \lambda_1 - \Delta \lambda_2)/[k(n_2 - n_1)].$$  \hspace{1cm} (6)

The biochip was fabricated on a polydimethylsiloxane slab by soft lithography. Then, the doped-photosensitive fibers were inscribed with 2 mm uniform FBG with Bragg wavelength of 1258 nm and then aligned to form a 20 µm cavity. For the RI measurement, a superluminescent light-emitting diode with a central wavelength of 1275 nm and a bandwidth of 70 nm is coupled into the input fiber as the light source (Denselight, DL-CS2079A). In addition, a 45 mW laser source at the wavelength of 1550 nm (Ultrale, CL16e) is coupled into both fibers to realize the fiber optical trap.

The characterization of the coefficient $k$ is carried out before the measurements. Initially, the de-ionized water ($n=1.3337$) is used as the reference buffer in the channel. Then, saline solutions with different concentrations, which are used by a handheld refractometer with a sensitivity of 0.0001 (Reichert, AR200), are used to obtain the peak shifts with respect to the RIs of the solutions. Based on the measured results, as shown in Fig. 3, the value of $k$ for the system is determined to be $1.56 \times 10^{-4}$. For one free spectral range, the refractometer is sufficient to measure the range of change of optical path length ($\Delta OPL$) up to 640 nm.

After the characterization, the system is employed for Madin-Darby canine kidney (MDCK) cell measurement. The MDCK cell is first encapsulated in a phosphate-buffered saline (PBS) plug (buffer 1), as shown in Fig. 4. The cell is then optically trapped within the fiber cavity and almost perfectly aligned along the fibers’ core which greatly improves the stability of the system. The optical trap is applied on the cell less than 5 s to minimize the phototoxicity and the output spectrum is obtained. Then, a higher concentration of PBS solution is mixed into the plug to change the RI (buffer 2), as shown in Fig. 4(c). The RI of the buffers before/after mixing are $n_1=1.355$ and $n_2=1.360$, respectively.

Figure 5 shows the measurement results of the MDCK cell. Based on Figs. 5(a) and 5(b), the peak shifts are determined as $\Delta \lambda_1=0.071$ nm and $\Delta \lambda_2=0.058$ nm. The calculated MDCK cell’s effective RI and effective thickness can then be determined, as listed in Table I. The first measured MDCK cell has an nc of 1.383±0.001 and lc of 16.7±1.0 µm. Similarly, for the second measured cell, the nc is 1.383±0.001 and the lc is 16.1±0.8 µm.

From the results, the standard deviation of measured cell’s effective RI is 0.001 which is improved in precision as compared with the FP method, i.e., 0.002. For the cell’s effective thickness, the standard deviation is larger because the cell’s effective thickness 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%. There are several advantages of using this biochip as a cell refractometer. First, this biochip offers high precision (up to 0.001 in RI) with reproducibility in measurement because of the fiber optical trap. Second, the fabrication process of the biochip is simple and easy for integration of the fiber optical system. Third, this biochip has a potential in label-free cell detection and sorting. Complicated labeling techniques are not required and different cells may be sorted out by observing the RI variations.

In conclusion, a single living cell refractometer is developed with the cell effectively trapped in the resonant cavity between a pair of FBG fibers. The effective RI of the cell is measured by the buffer modulation method, in which the resonant wavelength shifts are observed with the absence/presence of cell in two different buffers. The measured MDCK cell has an average effective RI of 1.383±0.001. This device has a potential in label-free cell detection and sorting especially in oncology and hematology.

<p>| TABLE I. Measurement results of the cell’s effective refractive index and effective thickness. |
|--------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>First measurement</th>
<th>Second measurement</th>
<th>Third measurement</th>
<th>Fourth measurement</th>
<th>Arithmetic mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective refractive index ($n_c$)</td>
<td>1.382</td>
<td>1.383</td>
<td>1.382</td>
<td>1.384</td>
<td>1.383</td>
</tr>
<tr>
<td>Effective thickness ($l_c$) (µm)</td>
<td>16.7</td>
<td>16.7</td>
<td>17.9</td>
<td>15.4</td>
<td>16.7</td>
</tr>
<tr>
<td>Effective refractive index ($n_c$)</td>
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<td>1.384</td>
<td>1.382</td>
<td>1.382</td>
<td>1.383</td>
</tr>
<tr>
<td>Effective thickness ($l_c$) (µm)</td>
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<td>15.4</td>
<td>16.7</td>
<td>16.7</td>
<td>16.0</td>
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