Refractive index measurement of single living cells using on-chip Fabry-Pérot cavity

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This letter reports the measurement of single living cells’ refractive index (RI) using an on-chip fiber-based Fabry-Pérot cavity by a differential method. In experiment a single cell is captured into the cavity, then the spectral shift in response to the buffer change and the cell presence/absence can be used to determine the cell’s RI and size. Experiment on kidney cancer cells measures an effective RI of 1.399 at 0.1% accuracy. Compared with other approaches, the differential method eliminates uncertain factors and thus ensures high accuracy. The microchip facilitates automatic detection and makes it promising for label-free drug screening. © 2006 American Institute of Physics.

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Recent years have seen a rapid increase of research interests in determining the cell physical parameters such as size, shape, and refractive index (RI) of living cells as demanded by biological studies and cell-based drug screening. 1 The RI measurement also provides an approach to label-free cell detection in addition to the various available technologies such as cavity ring-down spectroscopy, autofluorescence, confocal Raman and optical scattering, etc. 2–5 The RI has long been used to determine the concentration of solvents in a uniform solution. 6 For nonuniform medium such as living cells, it becomes more complicated since each cell is composed of many bimolecular ingredients such as lipids, sugars, proteins, nucleic acids, and metabolites. 7 As most of the cells are inhomogeneous, different parts of the cell may have different RIs. 8 However, the entire cell can be well represented by an effective refractive index. It has been found that among all the ingredients the protein is one of the main contributors due to its large amount and high refractive index (approximately 1.50–1.58). 7 Therefore, the effective RI is a key indicator to reflect the cell states (e.g., protein level) and to discriminate the cells (e.g., a normal cell from a cancerous cell of the same type). 7,8 Currently several methods have already been demonstrated for measuring the effective RI of cells based on optical scattering, optical coherence tomography, computer aided phase microscopy, digital holographic microscopy, counterpropagating optical trapping, etc. 5,6,8–15 However, the scattering method and the tomography are restricted to the tissue samples and cannot be used from single cell study. 5,11 The phase microscopy and the holographic microscopy require bulky microscope setups and complicated algorithms, which are expensive and difficult to miniaturize. The optical trapping method makes use of two counterpropagating laser beams to trap a cell, whose optical deformability 34 and equilibrium position 15 are dependent on the cell’s RI. 16 However, it requires complex optical system 14,15 and has low accuracy (approximately 3%) in dealing with real cells. 15 To tackle these problems, this letter proposes a simple but precise method and also implements it using a microfluidic chip.

The principle of the differential method is shown schematically in Fig. 1, which consists of two aligned single mode fibers and a cell holder submerged in the buffer solution. The holder is to temporarily fix the cell during the measurement. The end facets of the two fibers are high-reflection coated so as to form the Fabry-Pérot (FP) cavity. The fibers also serve as the optical input and output. The wavelength λ of the mth FP mode in the output spectrum is given by

\[ \frac{1}{2} m \lambda = nD + (n_v - n) l_c \xi, \]

where \( n \) is the RI of the buffer solution that surrounds the cell, \( D \) is the free-space distance of the two fiber ends, and \( n_v \) and \( l_c \) stand for the effective RI and effective size of the cell. The variable \( \xi \) indicates whether the cell is in the FP cavity: \( \xi = 1 \) for the presence of the cell and \( \xi = 0 \) for the absence of the cell.

FIG. 1. Working principle of the microfluidic Fabry-Pérot cavity for cell detection.
In the measurement, two buffer solutions with slightly different RIs ($n_1$ and $n_2$) are used alternatively. As a result, four spectra can be obtained in response to the buffer change and the absence/presence of cells, i.e., $\lambda_{n_1}$ for no cell and buffer 1, $\lambda_{n_2}$ for the presence of the cell in buffer 1, $\lambda_{c_2}$ for the presence of the cell in buffer 2. But differentiating the wavelength values, it yields

$$l_c = D \left(1 - \frac{\Delta \lambda_c}{\Delta \lambda_n}\right),$$  

and

$$n_c = n_1 \left[1 + \frac{\Delta \lambda_1 (\Delta \lambda_n - \Delta \lambda_c)}{\lambda_{n_1} (\Delta \lambda_n - \Delta \lambda_c)}\right],$$

where $\Delta \lambda_c = \lambda_{c_2} - \lambda_{c_1}$, $\Delta \lambda_n = \lambda_{n_2} - \lambda_{n_1}$, and $\Delta \lambda_1 = \lambda_{c_1} - \lambda_{n_1}$. If $n_2 > n_1$, it has $\Delta \lambda_n > \Delta \lambda_c$. From Eq. (2) it can be observed that the cell size can be simply determined by the spectral shift even without the knowledge of the actual RIs of the two buffers, while from Eq. (3), the cell RI needs only the accurate RI value of buffer 1. These features are unique compared with the other RI measurement methods. They ensure high accuracy of measurement even in the presence of uncertain factors such as the variation of instantaneous RI due to the mixing of two buffers.

The design and photograph of the optofluidic chip for the implementation of the differential method are illustrated in Figs. 2(a) and 2(b). In addition to the FP cavity part as discussed above, the chip has a specially designed microfluidic structure for the purpose of flow generation and control. The two buffer inlets are connected to two syringe pumps so as to provide two types of buffers, buffers 1 and 2. At any time, only one of the two types of buffer is pumped. By switching the on/off of the two pumps, it can inject two buffers into the main channel alternatively. This is one of the key steps to realize the differential detection method. In the cell inlet, the living cells are injected one by one into the main channel for detection. For the convenience of measurement, buffer 1 is used in the cell inlet. The cell holder is actually a micropipette controlled by another syringe pump. The microchip in Fig. 2(b) is formed by scribing the microfluidic structures on poly methylmethacrylate (PMMA) substrate using an excimer laser (wavelength of 248 nm). After integration, the input and output fibers form a gap of 35.5 µm (measured using an optical microscope). The end facets of the input and output fibers (Corning SMF-28) are coated with a layer of gold (approximately 40 nm thick) to have a reflectivity of 80%. The spreading of the light beam in the cavity induces an addition loss of 31% in every roundtrip. As estimation, the cavity has a finesse of 5.2 and a $Q$ factor of 330. Figure 3 shows the capture of a single cell by the cell holder inside the FP cavity.

![FIG. 2. Optofluidic microchip to implement the differential method using Fabry-Pérot cavity. (a) Design and (b) photograph of an integrated chip.](image)

Before measuring the real cells, the FP cavity is calibrated by putting purely buffer 1 and then buffer 2 without any cell. Buffer 1 is a phosphate buffered saline (PBS) solution having a nominal RI of 1.350. Buffer 2 is prepared by adding a small amount of ethylene glycol (RI of 1.431) to the PBS so as to obtain a slightly higher RI. The measured spectra are shown in Fig. 4(a). It can be seen that the multiple FP modes are shifted to longer wavelength in response to the change of buffer 1 to buffer 2. The mode at $m=63$ is initially at a wavelength of $\lambda_{n_1} = 1510.50$ nm for buffer 1, and is shifted to $\lambda_{n_2} = 1527.25$ nm for buffer 2. Therefore, the RI of buffer 2 can be calculated to be 1.365, though its accurate value is not absolutely necessary for determining the cell size and RI according to Eqs. (2) and (3). To calibrate the repeatability and accuracy of the fluidic FP cavity, the measurement has been conducted for four times as listed in Table 1. The standard deviation of measurement is 0.1%. As the buffer does not change, the standard deviation actually indicates the measurement accuracy.

After the calibration, the measurement is conducted on the Madin Darby canine kidney (MDCK) cell, which is a type of cancer cell with a nominal size of 16–20 µm and an

<table>
<thead>
<tr>
<th>Buffer 2 (PBS+ethylene glycol)</th>
<th>Refractive index</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Average</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCK cell</td>
<td>Refractive index</td>
<td>1.398</td>
<td>1.395</td>
<td>1.400</td>
<td>1.401</td>
<td>1.399</td>
<td>±0.2%</td>
</tr>
<tr>
<td>Size (µm)</td>
<td></td>
<td>18.2</td>
<td>17.0</td>
<td>18.5</td>
<td>17.3</td>
<td>17.8</td>
<td>±4.0%</td>
</tr>
</tbody>
</table>
effective refractive index of 1.385–1.404. As discussed above, a single cell is captured by hydroforce and held still by the holder, then the buffer is alternated. The spectra for the two buffers are compared in Fig. 4(b). The presence of the cell in buffer 1 shifts the monitored nth mode to $\lambda_{nc}^1=1538.25$ nm. When buffer 2 is used, the mode is further shifted to $\lambda_{nc}^2=1546.41$ nm. Therefore, it has $\Delta \lambda_{nc}=8.16$ nm, $\Delta \lambda_p=16.75$ nm, and $\Delta \lambda_s=27.75$ nm. According to Eqs. (2) and (3), it has $l_c=18.2 \, \mu$m and $n_c=1.398$. The measurement is also repeated for four times by releasing and recapturing the same cell; the data are listed in Table I. The result shows that the cell RI has a standard deviation of ±0.2%. However, the cell size has large fluctuation (±4.0%).

The method and chip presented in this letter exhibit clear advantages over the conventional ones. First, the size and RI of the cell can be measured simultaneously, while in some other methods the measured value is actually the optical path length (i.e., the product of the RI and size) and needs other measurements to decouple the RI and size.6,8 Secondly, the determination of cell size does not need any information of the buffers’ RI, which simplifies the measurement and also tolerates the uncertainties and noise factors. This is clearly advantageous over the optical trapping method that depends on the RIs of both the cell and the buffer.14,15 Lastly, the value of effective RI is relatively stable regardless of the capture position and orientation of the cell, presenting a significant improvement in comparison to the other FP cavity methods that detect the transverse light distribution and are thus strongly affected by the cell orientation.7,8

In conclusion, this letter presents an optofluidic microchip to determine both the size and refractive index of single living cells with the advantages of high accuracy, simple method, single-chip integration, and continuous detection. It utilizes high-reflection coated single modes fibers to form a Fabry-Pérot cavity in the microfluidic environment and changes the buffer solution using microfluidic technology. The refractive index and size of the cell can be measured simultaneously by differentiating the spectral shift. The chip is fabricated by laser machining on PMMA substrate. The experiment on MDCK cells measures an effective refractive of 1.398 and an average cell size of 17.8 $\mu$m. The accuracy can be better than 0.1%. Such chip allows label-free detection of single living cells in microfluidic environment, and would find many applications in cell biological research, disease diagnoses, cell-based drug screening, etc.