Determination of single living cell’s dry/water mass using optofluidic chip

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This paper presents an interferometric method for measuring the dry/wet mass of a single living cell using an optofluidic chip. It consists of the fiber Mach-Zehnder interferometer and the fiber-optical trap, both of which are integrated onto a single chip. In experiment, a single living cell is captured by the fiber-optical trap, and then the cell’s refractive index and diameter are simultaneously determined by the spectral shift in response to the buffer modulation. Hence, both cell dry and water masses can be calculated with a precision higher than 5%. © 2007 American Institute of Physics. [DOI: 10.1063/1.2789287]

Recently, there has been a rapidly growing interest in developing optofluidic technology through the fusion of microfluidics and optics, which are becoming popular tools to perform single cell experiment. In this letter, we present the design and testing of an optofluidic chip for cell detection. Both the refractive index (RI) and diameter of a single living cell can be determined simultaneously, from which the cell volume, dry mass, water mass, and sum mass can be calculated immediately.

The cell dry mass means the weight of the total substance other than water inside the living cell; namely, the dry mass and water mass comprise the sum mass of the cell. Measurements of the single cell’s physical parameters such as volume, dry mass, and water mass are demanded in biology studies and cell-based drug screens. For example, they can be used for studying the cell membrane osmotic water permeability.1,2

However, single cell mass measurement is very challenging, especially for a single living cell. Currently, only few methods have been demonstrated for measuring the dry mass of a single cell based on transmission electron microscopy3 (TEM) or optical interferometry.4–6 The TEM method, is too complicated and is not suitable for a living cell. Cell interferometry is the most general method for measuring the cell dry mass, which is calculated from the integral of the optical path difference of a living cell. Both of these two methods need bulky instruments and only give the cell dry mass without the water mass or volume information. Nevertheless, it is much more desirable to simultaneously determine the cell dry mass, water mass, and volume, from which the solid concentration, water concentration, and cell total mass are also obtained.

In this letter, we demonstrate trapping a cell via a fiber-optical trap and its integration with a fiber MZ interferometer onto an optofluidic chip for determining the size and refractive index of a single living cell simultaneously. Compared with the previous method, this design improves the repeatability and accuracy. Moreover, the cell is nearly a perfect sphere while being held by a fiber-optical trap at low intensity.7,8 Therefore, the cell’s volume can be calculated from the measured diameter value. As there exists a linear relationship between the dry masses density and refractive index of the cell, the cell dry and water masses can be calculated from the value of volume and refractive index of the cell.6

The optical system of the measurement setup is illustrated in Fig. 1. The MZ interferometer consists of four single-mode fiber splitters with a ratio of 97:3. This arrangement of the splitters allows both the detection beam and the optical trapping beam to be multiplexed into the fiber of the detection arm. It also ensures the transmission power of each MZ interferometer arm to be nearly equal. The cell is temporarily fixed by optical trapping during the measurement, and the detection beam passes through the cell and its surrounding buffer, which is shown in Fig. 2. The output intensity at wavelength λ of the MZ interferometer is given by

![FIG. 1. Schematic of the measurement setup.](image-url)
where \( I_r \) and \( I_s \) are the field amplitudes in the reference arm and sample arm, respectively, and \( n \) is the RI of the buffer solution that surrounds the cell. By assuming that the cell has the homogenous RI, \( n_c \) and \( l_c \) stand for the effective RI and effective diameter of the cell, respectively, and \( \phi_0 \) represents the initial phase difference. \( \xi \) is a variable to indicate whether the cell is in the optical path: \( \xi = 1 \) for the presence of the cell and \( \xi = 0 \) for the absence of the cell. By tracking a certain peak of the output spectra with maximum intensity; i.e., the \( m^{th} \) peak at wavelength \( \lambda \) can be expressed as

\[
I = I_r + I_s + 2 \sqrt{I_r I_s} \cos \left( \phi_0 + \frac{nD + (n_c - n)n_c \xi}{\lambda} \frac{2\pi}{2\pi} \right),
\]

where \( I_r \) and \( I_s \) are the field amplitudes in the reference arm and sample arm, respectively, and \( \phi_0 \) is the initial phase difference. \( \xi \) is a variable to indicate whether the cell is in the optical path: \( \xi = 1 \) for the presence of the cell and \( \xi = 0 \) for the absence of the cell. By tracking a certain peak of the output spectra with maximum intensity; i.e., the \( m^{th} \) peak at wavelength \( \lambda \) can be expressed as

\[
\left( m - \frac{\phi_0}{2\pi} \right) \lambda = nD + (n_c - n)n_c \xi.
\]

In order to decouple the unknown \( n_c \) and \( l_c \), a differential method is utilized via buffer modulation. 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 the cells, i.e., \( \lambda_{n1} \) for no cell and buffer 1, \( \lambda_{nc} \) for no cell and buffer 2, \( \lambda_{c1} \) for the presence of the cell in buffer 1, and \( \lambda_{c2} \) for the presence of the cell in buffer 2. By differentiating the wavelength values, it yields

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

where \( \Delta \lambda_c = \lambda_{c2} - \lambda_{c1} \), \( \Delta \lambda_n = \lambda_{n2} - \lambda_{n1} \), and \( \Delta \lambda_1 = \lambda_1 - \lambda_{n1} \). From Eq. (2), it can be observed that the cell diameter can be simply determined by the spectral shift and the cell RI needs only the accurate RI value of buffer 1 plus the spectral shift. Thanks to the differential method, these unique features ensure high accuracy of measurement even in the presence of the uncertain factors such as the variation of the instantaneous RI due to the mixing of two buffers.
The usual formula, for solutions, for the mass density of the substance can be expressed as

\[ \rho = \frac{\Delta n}{\chi}, \]

where \( \Delta n \) is the increment of the RI produced by the substance and \( \chi \) is a constant known as the specific refractive increment. As a reasonable approximation, this relationship is applicable to a cell composed mainly of protein. So, from the values of effective RI, the mean dry mass density \( \rho \) of the cell can be given as

\[ \rho = \frac{n_i - n_0}{\chi}, \]

where \( n_i \) is the RI of the normal phosphate buffered saline (PBS) medium of 1.350, \( \chi \) is assumed to be an approximation value of 0.19 ml/g since the value of \( \chi \) has been proven to be nearly independent of intracellular composition or concentration. Hence, the dry mass, water mass, and sum mass of the cell can be expressed, respectively, as

\[ M_{\text{dry}} = \rho V, \quad M_{\text{water}} = \rho_0 V, \quad M_{\text{cell}} = M_{\text{dry}} + M_{\text{water}}, \]

where \( V = (4\pi/3)(l^3/8) \) is the cell volume and \( \rho_0 = 1 \, \text{g/ml} \) is the density of water.

The design of the optofluidic chip is shown in Fig. 3. The two buffer inlets are connected to two programmed syringe pumps. By swapping the on/off of the two pumps periodically, it can inject two buffers into the main channel alternately. In the cell inlet, the diluted living cells in the PBS medium are injected one by one into the main channel for detection. The trapping light is provided by a fiber laser source (wavelength of 1064 nm, power of 45 mW). The cell is captured when it passes by the trapping region formed by two counterpropagating laser beams. After detection, the cell is released by turning off the trapping laser before repeating the capture and detection. The chip is fabricated by scribing the microfluidic structures on the polymethylmethacrylate substrate using an excimer laser. All the channel widths are about 50 \( \mu \text{m} \). After integration, the two fibers are precisely aligned and form a gap, which allows the cell to be captured. The precise distance of the gap is initially at a wavelength of \( \lambda_{n_1} = 1285.51 \, \text{nm} \) for buffer 1, and is shifted to \( \lambda_{n_2} = 1294.08 \, \text{nm} \) for buffer 2. Therefore, the RI of buffer 2 is calculated to be 1.359 \pm 0.001.

After calibration, the measurement is conducted on the Madin Darby canine kidney cell. The cell is injected in the main channel and captured by the fiber-optical trap. The cell can keep in the middle position of the channel by adjusting each trapping light’s power. Then, the buffer is alternated. The spectra for the two buffers are compared in Fig. 5(b). The presence of the cell shifts the monitored peak to \( \lambda_{c_1} = 1308.40 \, \text{nm} \) in buffer 1, and the peak is further shifted to \( \lambda_{c_2} = 1308.39 \, \text{nm} \) in buffer 2. According to the Eqs. (3)–(6), it has \( \ell = 15.2 \, \mu \text{m}, \quad n_c = 1.395, \quad V = 1.84 \times 10^{-15} \, \text{m}^3, \quad M_{\text{dry}} = 4.60 \times 10^{-10} \, \text{g}, \quad M_{\text{water}} = 1.84 \times 10^{-9} \, \text{g}, \quad \text{and} \quad M_{\text{cell}} = 2.3 \times 10^{-9} \, \text{g}. \) To estimate the precision, the measurement is also repeated three times by releasing and trapping the same cell. The data are listed in Table I. The result shows that both deviations of the cell dry and water masses are lower than 5\%. Compared with the experimental error, the uncertainty due to the unknown intracellular composition can be neglected for the calculated result of cell dry/water mass. For the cell’s RI, the accuracy of this method is estimated to be higher than \( \pm 0.005 \) in RI by comparing with a standard method.

The method presented in this letter exhibits several advantages over the conventional methods. It is applicable for the detection of the single living cell in a suspending state unlike cell interferometry in which the cell must be attached to the substrate. Secondly, compared with the hydrodynamic method for cell capture, the fiber-optical trapping improves the repeatability and stability. Moreover, as the trapping light is from the fibers, the cell is self-aligned to the fiber cores for detection.

<table>
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<th>Test</th>
<th>( \lambda_{c_1} )</th>
<th>( \lambda_{c_2} )</th>
<th>( M_{\text{dry}} )</th>
<th>( M_{\text{water}} )</th>
<th>( M_{\text{cell}} )</th>
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<tr>
<td>Test 1</td>
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<td>1.396</td>
<td>1.84 \times 10^{-10}</td>
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<td>1.400</td>
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<td>2.26 \times 10^{-9}</td>
<td>2.26 \times 10^{-9}</td>
</tr>
<tr>
<td>Test 3</td>
<td>1.396</td>
<td>1.397</td>
<td>1.84 \times 10^{-10}</td>
<td>2.31 \times 10^{-9}</td>
<td>2.31 \times 10^{-9}</td>
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