

# Live cell refractometry using microfluidic devices

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Using Hilbert phase microscopy for extracting quantitative phase images, we measured the average refractive index associated with live cells in culture. To decouple the contributions to the phase signal from the cell refractive index and thickness, we confined the cells in microchannels. The results are confirmed by comparison with measurements of spherical cells in suspension. © 2006 Optical Society of America  
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Interaction of optical fields with tissues is largely determined by the three-dimensional refractive index distribution associated with the biological structure.<sup>1–4</sup> Scattering models such as Mie theory,<sup>5</sup> the first Born approximation,<sup>6</sup> and the radiative transport equation<sup>7</sup> use the refractive index contrast between the scattering particles and their surroundings to make predictions about the interaction of the optical field with biological media. Highly scattering tissue has been characterized in terms of an average refractive index by optical coherence tomography<sup>8</sup> and, more recently, total internal reflection.<sup>9</sup> Live cells, on the other hand, are essentially transparent under visible light and thus can be described as weak scatterers, with subtle refractive index variations in space. Recently, Faber *et al.* demonstrated that the oxygen saturation modulates the hemoglobin refractive index and thus the scattering properties of red blood cells.<sup>10</sup> Light scattered by cells carries information about the biological architecture and thus can be used for noninvasive investigation and diagnosis.<sup>1–3</sup> Therefore, precise knowledge of the cell refractive index provides both an optical assay for live cell studies and a valuable input for scattering models.

The information about the cell structure and, hence, the refractive index is encoded in the wavefront modification of the illuminating field. Quantifying the optical path-length shift associated with the cell can potentially provide noninvasive information about structural changes associated with cell cycling or disease progression. However, this optical phase change produced by the cells is due to the combined effect of both refractive index and thickness variations across the sample. To decouple the contributions of these two variables, quantitative phase and confocal microscopy have been used in tandem.<sup>11</sup> However, this approach is experimentally demanding and may have limited applicability. Recently, Rappaz *et al.* have used pairs of digital holography measurements of cells at two different values of solution refractive index, which also helped decoupling of the cell morphology and refractive index information.<sup>12</sup>

Nevertheless, this approach requires exposing the same cells to two different surrounding media, which adds constraints to the cell preparation procedure and prevents high-throughput investigation.

In this Letter, we present a new modality for measuring the average refractive index of live cells by confining them in microfluidic devices. The method uses Hilbert phase microscopy (HPM), which was developed in our laboratory for measuring quantitative phase images of cells with a high acquisition rate and low noise.<sup>13,14</sup>

The HPM setup and phase retrieval procedure are described in detail elsewhere.<sup>14</sup> The phase distribution associated with a microscope image is encoded by using a plane-wave reference field that creates spatial modulation, and a quantitative phase image is then obtained via a spatial Hilbert transform. In our experiments, a He–Ne laser ( $\lambda = 632.8$  nm) acts as the illumination source for an inverted microscope equipped with a 40 $\times$  objective. The CCD used (C7770, Hamamatsu Photonics) has an acquisition rate of 291 frames/s at the full resolution of 640  $\times$  480 pixels, and the fringes are sampled by 6 pixels per period.

To extract the refractive index information independently from the cell thickness, we placed live HeLa cells (a human epithelial carcinoma cell line) in microchannels of fixed dimension that confine the cell in the vertical direction. Single input and single output microchannels of rectangular cross sections were prepared by molding elastomer on microstructures fabricated on a silicon wafer (for details, see Ref. 15). First, the refractive index of the culture medium (CM) was determined by acquiring successive phase images of microchannels filled with both CM and water. The CM is Dulbecco's Modified Eagle Medium, containing 10% fetal calf serum. Figure 1(a) shows an HPM quantitative phase image of the microchannel filled with this fluid. The phase profile along the direction indicated by the arrows in Fig. 1(a) is shown in Fig. 1(b). By use of the refractive index of the mi-

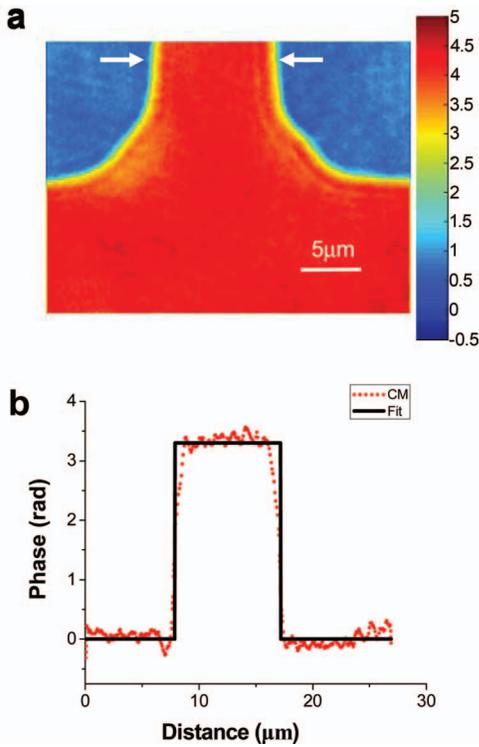


Fig. 1. a, Quantitative phase image of a microchannel filled with CM. The color bar indicates phase in radians. b, Phase profile across the microchannel, along the direction indicated by the arrows in a.

crochannel material (Silicon Elastomer, Sylgard 184),  $n_{\text{channel}}=1.430$ , and that of water,  $n_{\text{water}}=1.333$ , for the incident wavelength at 20 °C, the CM refractive index,  $n_{\text{CM}}$ , was obtained as

$$n_{\text{CM}} = n_{\text{channel}} + \left( \frac{\Delta\phi_{\text{channel-CM}}}{\Delta\phi_{\text{channel-water}}} \right) (n_{\text{channel}} - n_{\text{water}}), \quad (1)$$

where  $\Delta\phi_{\text{channel-CM}}$  and  $\Delta\phi_{\text{channel-water}}$  are, respectively, the phase shifts of the microchannel with respect to CM and water. Using Eq. (1), we measured  $n_{\text{CM}}=1.337$  with a standard deviation of 0.0015.

HeLa cells suspended in CM were then introduced into the microchannels, which deformed the cells and confined them in the vertical direction. Figure 2(a) shows an example of a quantitative phase image of such a sample. Because the microchannel thickness exhibits some variability due to the fabrication process, we performed a relative measurement that eliminated the need for *a priori* knowledge of the microchannel height. Thus, we quantified the phase shift of both the cell and CM with respect to the microchannel, as shown in Fig. 2(b). Using this information, the refractive index  $n_{\text{cell}}$  of a cell confined in microchannel is obtained as

$$n_{\text{cell}} = n_{\text{CM}} + \left( \frac{\Delta\phi_{\text{channel-cell}}}{\Delta\phi_{\text{channel-CM}}} \right) (n_{\text{channel}} - n_{\text{CM}}). \quad (2)$$

This procedure was applied for measuring the refractive index of 17 cells, and the results are summa-

rized in Fig. 2(c). This histogram shows that the measured refractive index values are characterized by a small standard deviation,  $n_{\text{cell}}=1.384\pm 0.0018$ . This value for the average refractive index is comparable with other results published in the literature.<sup>16–18</sup> To investigate the potential cell size effect on our measurement procedure, we plotted the refractive index of each individual cell as a function of the projected cell diameter [Fig. 2(d)]. This graph demonstrates that the cell size and refractive index are not correlated in our measurement.

To validate this technique further, we performed a second, independent measurement of cell average refractive index. In this latter approach, we used the fact that cells in suspension assume a spherical shape, which enables extraction of the refractive index from the quantitative phase image. HeLa cells were trypsinized and resuspended in CM. HPM images of cells were collected before the cells had time to attach to the substrate, as shown in Fig. 3(a). The cross-section phase profile and the fit to the expression predicted by assuming a spherical cell shape is shown in Fig. 3(b). By use of the value of  $n_{\text{CM}}$  previously measured, the cell refractive index can be obtained from the following expression:

$$n_{\text{cell}} = n_{\text{CM}} + \frac{\lambda \Delta\phi(x,y)}{2\pi h(x,y)}. \quad (3)$$

In Eq. (3),  $\Delta\phi$  and  $h$  are the local phase shift and the thickness of the cell, respectively. The refractive index across the cell diameter is also shown in Fig. 3(b). The constant behavior of this profile indicates that the spherical model is a good approximation for cells in suspension. The histogram of refractive indices obtained for 28 cells is shown in Fig. 3(c). Remarkably, the value obtained,  $n_{\text{cell}}=1.385\pm 0.0049$ , agrees very well with the result obtained from the microchannel experiments. The independence of cell size and refractive index is shown in Fig. 3(d). Thus, the spheri-

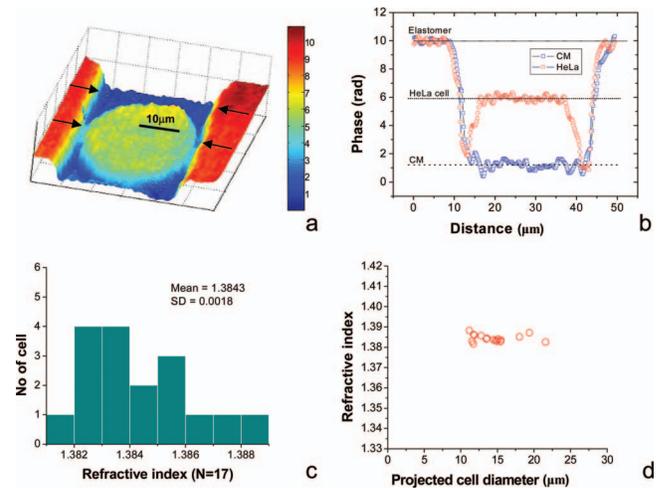


Fig. 2. a, Quantitative phase image of a HeLa cell in a microchannel (color bar indicates phase in radians). b, Phase profile along the arrows indicated in a. c, Histogram of the measured cell refractive index. The mean value and standard deviation are indicated. d, Cell refractive index versus the projected cell diameter.

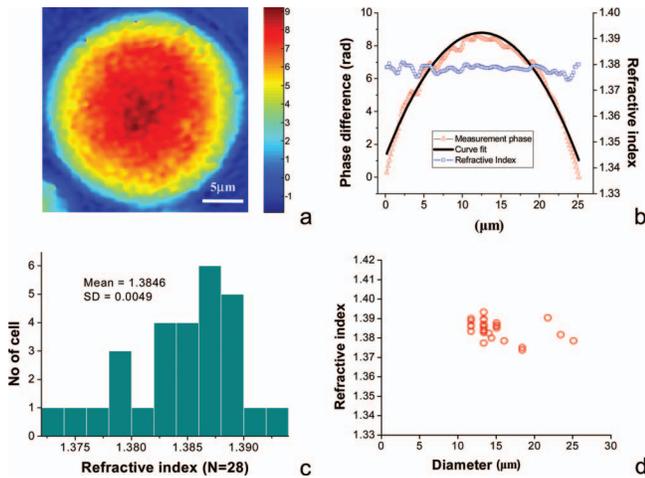


Fig. 3. a, Quantitative phase image of a spherical HeLa cell in suspension (color bar indicates phase in radians). b, Phase profile along the diameter of the cell in a. The fit with the spherical model and the resulting refractive index profile are also indicated. c, Cell refractive index histogram. d, Refractive index versus the cell diameter.

cal model confirms the results obtained using the microchannel geometry.

The technique presented here can be automated by combining the imaging geometry with flow in the microfluidic channels, allowing for high-throughput cyto-refractometry. We anticipate that the direct method of assessing the cell refractive index in microfluidic devices will become a valuable tool for quantifying both cell physiology (e.g., cell metabolism) and pathology (e.g., progression/prognosis of cancer and other diseases).

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