

Bifocal optical coherence refractometry of turbid media

Sergey A. Alexandrov, Andrei V. Zvyagin, K. K. M. B. Dilusha Silva, and David D. Sampson

Optical+Biomedical Engineering Laboratory, Department of Electrical and Electronic Engineering, The University of Western Australia, Crawley, WA 6009, Australia

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We propose and demonstrate a novel technique, which we term bifocal optical coherence refractometry, for the rapid determination of the refractive index of a turbid medium. The technique is based on the simultaneous creation of two closely spaced confocal gates in a sample. The optical path-length difference between the gates is measured by means of low-coherence interferometry and used to determine the refractive index. We present experimental results for the refractive indices of milk solutions and of human skin *in vivo*. As the axial scan rate determines the acquisition time, which is potentially of the order of tens of milliseconds, the technique has potential for *in vivo* refractive-index measurements of turbid biological media under dynamic conditions. © 2003 Optical Society of America

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Detailed knowledge of the refractive index is essential in the understanding of light–tissue interactions in living tissue.¹ Such knowledge is important for *in vivo* optical diagnostics and laser treatments. Examples of the reported significance of the refractive index include the relationship of the refractive index of human stratum corneum to tissue hydration² and large refractive-index differences between normal and malignant breast tissue³ and between normal and calcified human aorta.⁴ Such differences are not necessarily observed in samples *in vitro*, and the results of measurements of samples *in vitro* and *in vivo* can differ significantly. For example, the refractive index of rat mesenteric tissue *in vitro* was found to be 1.52, compared with 1.38 *in vivo*.⁵

The majority of existing techniques for refractive-index determination, e.g., the method of critical angle,⁶ can be applied only *in vitro* to clear media or superficial layers. Recently a new technique that is applicable to turbid media has emerged that uses optical coherence tomography to track the shift in focal length that results from translating the focus of the objective along the optical axis within the medium.^{2,7} The refractive index of human skin, adipose tissue, and muscle has been measured *in vivo* by this technique² and by a modified version of it.⁸ However, measurements made with the technique are inherently slow, as they rely on an accurate, discrete axial displacement of focus.

In this Letter we propose a new technique for the direct, rapid measurement of refractive index in a turbid medium, which we term bifocal optical coherence refractometry. The technique is based on the simultaneous creation of two confocal gates and associated focal points within the sample. One uses low-coherence interferometry to determine the optical path length between the two points from which the refractive index is determined. The acquisition rate of the measurement is subject to the same scan rate as low-coherence interferometry, making the method well suited to the rapid (of the order of milliseconds) measurement of the refractive indices of turbid media, including biological tissue *in vivo*.

The proposed technique, illustrated schematically in Fig. 1, is based on a scanning Michelson interferome-

ter that employs a linearly polarized broadband source. For convenience, the optical circuit is illustrated (and implemented) by use of optical fiber. In the sample arm the beam is collimated, expanded, and split; a polarizing beam splitter (PBS₁) is used to minimize loss. One beam passes through a weak lens, L_f, and the other beam passes through a path-length compensator, C, to equalize the optical path lengths of the beams. The beams are recombined by a second polarizing beam splitter, PBS₂, and aligned to the axis of objective lens L_{obj}. The weak lens causes one beam to focus closer to the objective; thus two axially separated focal points are formed. Light that is backscattered from the sample is collected by the optical fiber, thus producing a confocal gate for each beam.

The optical path length between the two focal points Δl_{opt} is measured by axial scanning of the reference optical path length. The optical path length in a homogeneous sample of refractive index *n*, obtained by marginal ray-tracing analysis, is given by

$$\Delta l_{\text{opt}} = n \left\{ (f_{\text{obj}} - a) [NA^2(n^2 - 1) + n^2]^{1/2} - \frac{(f_{\text{obj}} - a - \Delta l) [NA^2 f_{\text{obj}}^4 (n^2 - 1) + (nf\Delta l)^2]^{1/2}}{f\Delta l} \right\}, \quad (1)$$

where NA is the numerical aperture of the objective lens, *f* and *f*_{obj} are the focal lengths of the weak lens and the objective lens, respectively, *a* is the distance in

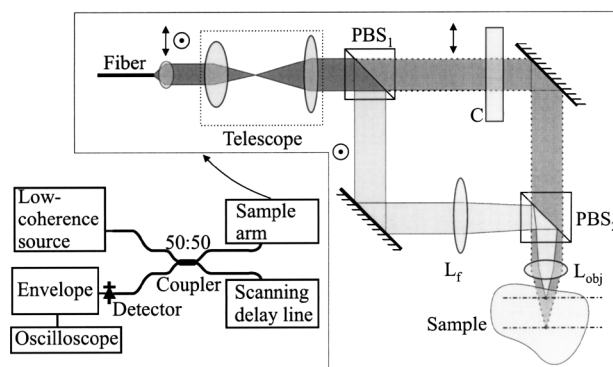


Fig. 1. Schematic diagram of the proposed technique.

air from the principal plane of the objective lens to the sample interface, and Δl is the distance between the two focal points in air. Equation (1) assumes that the group index and the refractive index are equal, implying negligible dispersion. Assuming that the parameters of the system are known, or were previously determined, the refractive index, averaged over the axial distance between the two focal points, can be calculated.

To test the technique we constructed an experimental setup based on a fiber-optic, low-coherence scanning interferometer. The interferometer was illuminated with polarized light from a superluminescent diode with a mean wavelength of 815 nm and a 3-dB bandwidth of 22 nm. The scanning optical delay line comprised a frequency-domain, folded-grating lens-and-tilted-mirror configuration operated off axis and in double pass.⁹ The grating had 830 lines/mm, and the lens was a 100-mm focal-length, infrared achromatic doublet. The mirror was 20 mm in diameter and was angle scanned by means of a galvanometer at 60 Hz. Polarization controllers were placed in both the reference and the sample arms, which enabled the power in each branch of the sample arm to be adjusted separately. A tilted plane-parallel glass plate was used as the path-length compensator. The angle of the plate to the optical axis was varied until the optical path-length difference between the two beams in the sample arm was equal to the optical path length between the two focal points. The focal lengths of the weak lens and the objective lens were 1000 and 10 mm, respectively. The $1/e^2$ beam diameter, measured by a scanning slit, was 7 mm. At the output of the interferometer, the signal was photodetected and its envelope was determined by use of a modified phase-locked loop circuit,¹⁰ displayed on an oscilloscope, and subsequently transferred to a personal computer.

We adopted a relatively simple calibration procedure. We determined the axial separation of the focal points in air by manually stepping a plane mirror in 10- μm intervals through the two focal points and recording the axial scan profile for each mirror location. The maximum values of the signals were determined for each measured position of the mirror. The resultant experimental points are plotted in Fig. 2. The scatter in the experimental points was due primarily to the imperfect axial alignment of the mirror and to the uncertainties in the axial position during manual translation. To determine the distance between the two focal points we least-squares fitted the points with a fourth-order polynomial (not shown in Fig. 2), and a peak-finding routine was used. The distance was thus measured to be $120 \pm 2 \mu\text{m}$. The theoretical curve shown in Fig. 2 was calculated from the axial response function of the system^{11,12} and the estimated fiber, lens, and beam parameters. Despite the limitations of the experimental setup, Fig. 2 shows that there is reasonable agreement between experiment and theory. We checked the calibration procedure for consistency by scanning the objective lens for a fixed mirror position, which gave the same results to within experimental error.

Experimental measurements were made of commercially available milk (nominal 2% fat content by volume) at various dilutions. Milk solutions were placed in a cuvette with a glass window of 150- μm nominal thickness. The results of a scan through a 20% milk solution are shown in Fig. 3. The trace is an average of 256 axial line scans acquired in approximately 2 s. A fourth-order polynomial was least-squares fitted to the experimental data. The fitted curves are shown as thicker curves in Fig. 3. We used a peak-finding routine to determine the separation between the fitted curves. Measurements were made for various dilutions from 5% to 75%. The resultant mean value of the refractive index was 1.34 ± 0.01 ($\pm\sigma$), which is consistent with the value reported in Ref. 13 of 1.344 ± 0.004 at a wavelength of 633 nm. To within experimental error, we did not find any dependence on dilution, which is consistent with the results of Ref. 13.

Differences in the functional forms of the two sets of peaks are evident in Fig. 3. The signal envelope is the convolution of the source autocorrelation function $S(\Delta l_i)$, with the axial sample reflectivity $R(l_s)$ modified by the axial response function of the sample-arm confocal optical system $h(l_s)$, is given by¹²

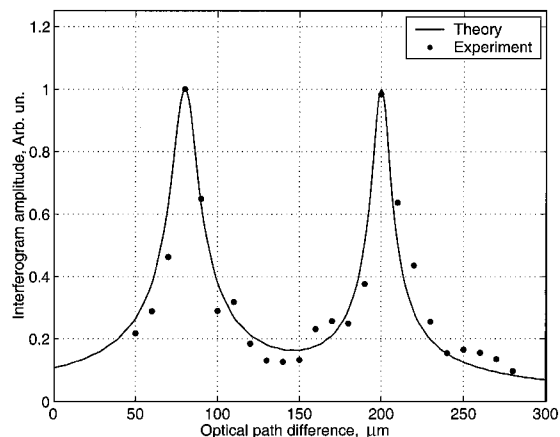


Fig. 2. Experimental response to a stepped mirror in air and corresponding theoretical axial intensity distribution.

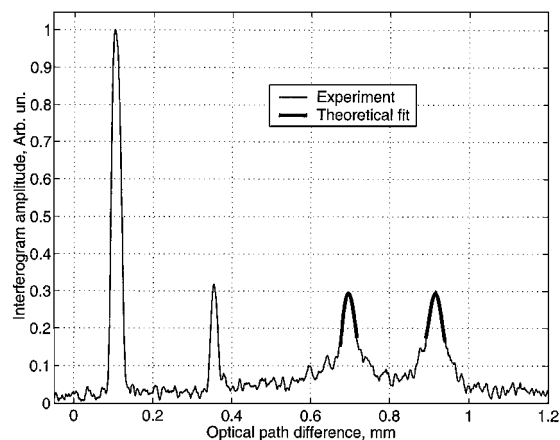


Fig. 3. Axial response for a 20% milk solution with theoretical curves fitted to the confocal peaks.

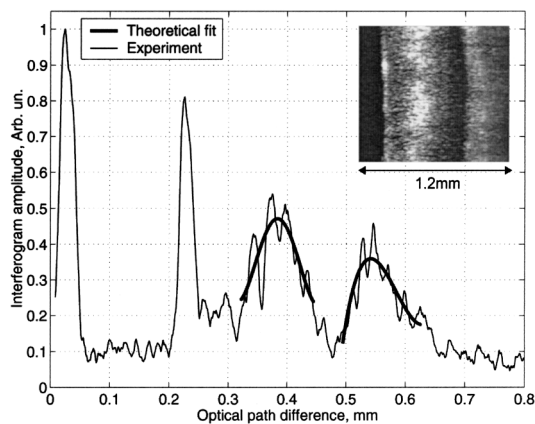


Fig. 4. Axial response for the stratum corneum of the thick skin on the dorsal surface of a human thumb *in vivo*. Inset: Optical coherence tomography image of the corresponding skin section.

$$I(l_r) \approx \int_0^\infty [R(l_s)h(l_s)]^{1/2} S(\Delta l_i) dl_s. \quad (2)$$

where $h(l_s) = h_1(l_s - l_{c1}) + h_2(l_s - l_{c2})$, h_1 and h_2 are the axial response functions of the two confocal gates, l_{c1} and l_{c2} are positions of the peak values of h_1 and h_2 , respectively, $\Delta l_i = l_r - l_s$ is the optical path-length difference, and l_r and l_s are the lengths of the reference and the sample arms, respectively. The glass surfaces of the cuvette window are planar scatterers and are located far from the focal points; hence the signal that arises from them is given by

$$I(l_r) \approx S(\Delta l_i). \quad (3)$$

The peaks that are due to the glass, located at the left side in Fig. 3, are described by Eq. (3) and are narrower and with steeper decay than peaks that arise from the milk sample, which are modified by the confocal response function as described by Eq. (2).

To measure the refractive index of human skin *in vivo* we exchanged the objective lens for one with a focal distance of 8 mm, for which the distance between the focal points in air was measured to be $75 \pm 2 \mu\text{m}$. The dorsal surface of the thumb of a human volunteer was placed against a glass coverslip, and the stratum corneum was scanned. The results are shown in Fig. 4. The trace in Fig. 4 is an average of 1024 axial line scans, representing an acquisition time of approximately 8.5 s. The inset in Fig. 4 is an image of the corresponding skin section acquired with a 980-nm optical coherence tomography system described elsewhere.⁹ This image confirms that the thickness of the stratum corneum was sufficient to encompass the two focal points. Based on the results shown in Fig. 4, we measured the refractive index to be 1.50 ± 0.02 . The error was assessed by multiple measurements at the same site. Our result is similar to the result reported in Ref. 2, i.e., 1.51 ± 0.02 , which was obtained *in vivo* at a wavelength of 1300 nm.

A unique advantage of our technique is the provision of a direct measurement without the requirement

for motion in the sample or the sample arm. The particular implementation demonstrated here is not unique, and configurations in which the distance between the focal points can be controlled are of particular interest. In our implementation the two foci were formed with orthogonal linear polarization states; hence any anisotropy of the medium would have affected the measurement. Polarization-insensitive configurations are also possible. The accuracy of the measurement is to first order independent of the refractive index of the medium between the objective lens and the two beam foci. In our experiments, the acquisition time was limited by the low optical power at the surface of the skin, which was approximately 0.1 mW for each beam. With power of the order of milliwatts, the refractive index could be measured from the average of a few collocated or adjacently located axial scans, limited primarily by speckle noise, making an acquisition time of some tens of milliseconds feasible.

In conclusion, we have proposed and demonstrated bifocal optical coherence refractometry for the rapid determination of the refractive index of a turbid medium. The results of experiments with milk solutions and with human skin *in vivo* are consistent with published data. High-speed acquisition would make the technique suitable for a range of applications and particularly attractive for *in vivo* refractive-index measurements of living biological media under dynamic conditions.

S. A. Alexandrov's e-mail address is sergey@ee.uwa.edu.au.

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