Refractive index measurements of multiple layers using numerical refocusing in FF-OCT

Gihyeon Min,1 Woo June Choi,2 Ju Wan Kim,3 and Byeong Ha Lee1,*

1School of Information and Communications, Gwangju Institute of Science and Technology, 261 Cheomdan-gwagiro, Buk-gu, Gwangju, 500-712, South Korea
2Department of Bioengineering, University of Washington, 3720 15th Avenue NE, Seattle, Washington 98195, USA
3Department of Medical System Engineering, Gwangju Institute of Science and Technology, 261 Cheomdan-gwagiro, Buk-gu, Gwangju, 500-712, South Korea
*leebh@gist.ac.kr

Abstract: We propose and demonstrate the novel method of refractive index (RI) measurement for each layer of multilayered samples, which is based on numerical refocusing in full field optical coherence tomography (FF-OCT). The en-face FF-OCT image on an inner layer boundary of a multilayered sample is unintentionally blurred or defocused due to the RI of the sample itself, but can be numerically refocused. The refocusing is performed by numerically shifting the image sensor plane of the system, in general. However, by calculating the corresponding sample shift and then compared it with the actual sample shifting distance, we could extract the average RI of the layer between any two layer boundaries within the multilayered sample. In addition, the thickness of that particular layer could be derived at the same time. For the idea proof, several samples were prepared by stacking, for each sample, two transparent plates with a gap in between. While changing the material of the plate and filling the gap with oil, the RIs of the plate and the oil were measured. For oils of various RIs, from 1.2977 to 1.3857, the measured RIs were well matched with the reported ones within 0.205%. Moreover, even with a stack of various and multiple plates in front of the same oil layer, the oil RI and the physical thickness of the oil layer were extracted with average errors of only 0.065% and 0.990%, respectively.

©2013 Optical Society of America

OCIS codes: (290.3030) Index measurements; (120.3180) Interferometry; (110.4500) Optical coherence tomography; (090.1995) Digital holography.

References and links

1. W. V. Sorin and D. F. Gray, “Simultaneous thickness and group index measurement using optical low-coherence reflectometry,” IEEE Photon. Technol. Lett. 4(1), 105–107 (1992).
2. M. Haruna, M. Ohmi, T. Mitsuyama, H. Tajiri, H. Maruyama, and M. Hashimoto, “Simultaneous measurement of the phase and group indices and the thickness of transparent plates by low-coherence interferometry,” Opt. Lett. 23(12), 966–968 (1998).
3. D. F. Murphy and D. A. Flavin, “Dispersion-insensitive measurement of thickness and group refractive index by low-coherence interferometry,” Appl. Opt. 39(25), 4607–4615 (2000).
4. H. Maruyama, T. Mitsuyama, M. Ohmi, and M. Haruna, “Simultaneous Measurement of Refractive Index and Thickness by Low Coherence Interferometry Considering Chromatic Dispersion of Index,” Opt. Rev. 7(5), 468–472 (2000).
5. J. Na, H. Y. Choi, E. S. Choi, C. S. Lee, and B. H. Lee, “Self-referenced spectral interferometry for simultaneous measurements of thickness and refractive index,” Appl. Opt. 48(13), 2461–2467 (2009).
6. G. J. Tearney, M. E. Brezinski, J. F. Southern, B. E. Bouna, M. R. Hee, and J. G. Fujimoto, “Determination of the refractive index of highly scattering human tissue by optical coherence tomography,” Opt. Lett. 20(21), 2258–2260 (1995).
7. S. Kim, J. Na, M. J. Kim, and B. H. Lee, “Simultaneous measurement of refractive index and thickness by combining low-coherence interferometry and confocal optics,” Opt. Express 16(8), 5516–5526 (2008).
8. K. Lee, S. Y. Ryu, Y. K. Kwak, S. Kim, and Y. W. Lee, “Separation algorithm for a 2D refractive index distribution and thickness profile of a phase object by laser diode-based multiwavelength interferometry,” Rev. Sci. Instrum. 80(5), 053114 (2009).
1. **Introduction**

Measurements of refractive index (RI) and its distribution, lateral or/and longitudinal, are essential for understanding the optical properties of materials such as thin films, glass plates, multilayered devices and biological tissues. Various methods for RI measurements have been proposed including low coherence interferometry [1–5], focus tracking method [6], combination of low-coherence interferometry and confocal optics [7] and multi-wavelength interferometry [8]. In addition, laser feedback technique [9], full field optical coherence tomography (FF-OCT) [10], fiber point diffraction longitudinal shearing interferometry [11], time-of-flight measurements of terahertz pulse [12] and combining polarized reflectance and vision image [13] have been proposed also. However, these methods were possible only to measure the RI of a single layered sample. For the RI measurements of each layer in a multilayered sample low-coherence confocal interference microscope [14], combination of confocal microscope and low-coherence interference [15], and wavelength-scanning interferometer (WSI) with a confocal microscope [16] have been reported. Zvyagin et al. [17] obtained the RI tomography of a turbid medium by using the bifocal optical coherence refractometry (BOCR) method. However, these methods were quite time consuming and cumbersome [14,15], and some systems were too much complicated for implementing [16,17].

Recently defocusing correction methods [18,19] were proposed for the RI measurement, which used the image defocus phenomenon happening in a FF-OCT system. In general, the
focal plane (FP) of an internal en-face image of FF-OCT is separated from the image plane (IP) of the system due to the RI mismatch of the sample from its surrounding medium [18–22]. By mechanically adjusting the path length of the reference and the sample arms, the image can be focused; and from which the RI of the sample can be extracted. However, this method provides just axially-averaged RI and is time consuming. Moreover, mechanically adjusting the position of the FP, with a fixed IP, is a somewhat difficult task. Zhou et al. [20] has proposed the RI measurement for each layer of a multilayered sample using the separation of the FP from the IP. The positions of both planes were measured with multiphoton microscopy and OCT, respectively. Since it needed both images of different modalities at the same time, the system was much complicated. Furthermore, complicated comparison process was required to find out the same layers between two images. Srinivasan et al. [21] also, presented local RIs for cortical layers with spectral/Fourier domain OCT microscope; however, 4D mechanical scanning was required for getting a 4D data set.

In this paper, we propose the numerical correction method that can give the axial RI profile of a multilayered sample. Similar to our previous work [22], the proposing method utilizes the typical digital refocusing technique based on the Fresnel diffraction theory, in which the image sensor location is numerically moved so as to be placed at the conjugate focal plane of the sample while checking its focusing status with the amplitude analysis method (AMP) [22,23]. Expanding this method, we are able to simultaneously measure the RI and the thickness of a specific layer within a multilayered sample without using additional equipment or extra scanning. With the numerical image sensor shift distance, the corresponding object position is calculated, from which we can extract the average RI and the physical thickness of the layer between any two layer boundaries within the sample. The principle of the numerical refocusing process for FF-OCT is briefly reviewed and the core concept of extracting the RI of each layer in a multilayered sample is presented in detail. Furthermore, extracting the thickness of each layer with the measured RI of that layer is explained. To confirm the concept, we present the axial RI profile measurement of a multilayered sample, made by stacking two glass plates with a gap of air or oil. Finally, we experimentally demonstrate the extraction of RI and thickness of an interesting specific layer within a multilayered sample without measuring the RIs and thicknesses of any other layers.

2. Principles

In an FF-OCT system, in general, the en-face image of the layer boundary located at a deep region of a sample becomes easily blurred. The RI mismatch between the sample and its surrounding medium, such as air or water, separates the FP of the imaging optics from the IP of the coherent gating optics of the FF-OCT system [18–22].

As an example, let us think about the FF-OCT imaging of a sample having double layers. It is assumed that each layer has RI of \( n_1 \) and \( n_2 \), respectively, and the sample is immersed in a medium of RI \( n_0 \). Initially, the FP and the IP of the FF-OCT system are aligned to coincide at the front surface of the sample as shown in Fig. 1(a). When the sample is moved for the imaging of its inside, however, the separation of FP from IP occurs. As shown in Fig. 1(b), when the sample is mechanically moved to the right by a distance \( d_s \) to see the boundary between both layers, IP1 is moved right by \( d_{ip} \) but FP1 is moved left by \( d_{fp} \). Therefore, the obtained image goes out of focus. Assuming that the optical dispersion of the sample material is not severe, the separation between the IP1 and the FP1 is approximately given as [22]:

\[
\ell_1 = d_{ip} + d_{fp} = \left( n_1 - n_0 \right) d_s.
\]

with \( d_{ip} = \left( 1 - \frac{n_1}{n_0} \right) d_s \) and \( d_{fp} = \left( 1 + \frac{n_1}{n_2} \right) d_s \).
Fig. 1. Image coordinates in the FF-OCT system and the ray tracing for the cases of $n_0 = n_1 = n_2$ (dashed line), $n_0 \neq n_1 \neq n_2$ (black and red solid line), and $n_0 \neq n_1 = n_2$ (green solid line). The image coordinates of (a) the initial sample position, (b) when the sample is moved by $d_s$, and (c) when the sample moved further to $d_s + d_{s2}$. L1 and L2: lenses, FP1, FP1’ and FP2: focal planes, IP1 and IP2: image planes, CP1 and CP2: CCD positions for imaging of IP1 and IP2, respectively, CJP1 and CJP2: conjugate planes of CP1 and CP2 in the $n_0$ medium, respectively. MIB: middle interface boundary.

The en-face interference image of an FF-OCT system is captured at the CCD plane, where a two dimensional detector array (charge coupled device (CCD) in our case) is physically placed. At this CCD plane, we obtain a complex field $\Psi(x, y)$ of the sample using the well-known phase shifting interference method [22, 24]. Because IP1 is shifted to the right by $d_{ip}$ with the sample movement of $d_s$, to obtain its focused image the CCD should be moved right to its conjugate plane denoted by CP1. The ray trace for the focused imaging of the boundary between layers is depicted with solid red ray lines in Fig. 1(b). However, since the CCD plane is physically fixed, the image captured by the CCD goes out of focus. The out-of-focus image can be corrected by numerically moving the CCD plane to the right, to CP1. With the complex field $\Psi(x, y)$ obtained at the fixed CCD plane, however, the complex field at CP1 is numerically calculated as [22, 25]:

$$\Phi(X, Y, Z) = \mathcal{F}^{-1} \left[ \exp \left( -j\pi\lambda Z \left( \xi^2 + \eta^2 \right) \right) \mathcal{F} \left[ \Psi(x, y) \right] \right], \quad (2)$$
where $\xi$ and $\eta$ are the spatial frequencies, $i$ is the index of the sample movement, and $Z_i$ is the numerically adjusted distance of the CCD plane for the $i$-th sample movement. As shown in Fig. 1(b), $Z_i$ is the distance from the CCD plane to CP1, at which the blurred image of the boundary between layers is numerically refocused [22]. The optimal distance $Z_i$ is acquired by the focus evaluation method of amplitude analysis (AMP) [22, 23], which is based on the assumption that the integration of $\Phi(X,Y,Z_i)$ (AMP value) over the X-Y plane reaches the minimum when $\Phi(X,Y,Z_i)$ is in a focused state [23].

The position of the conjugate plane of CP1 in the background medium of RI $n_0$, denoted as CJP1, can be calculated with the parameters of the imaging optics or can be obtained experimentally. In the $n_0$ background medium, the location of CJP1 $s_{o2}$ is related with the location of CP1 $s_{i2}$ [26, 27] as

$$s_{i2} \left( d-f_2 - \frac{(s_{i2}/n_0)(f_i/n_0)}{(s_{i2}/n_0)-(f_i/n_0)} \right) = f_2d - \frac{(f_i/n_0)f_2(s_{i2}/n_0)}{(s_{i2}/n_0)-(f_i/n_0)}.$$  \hspace{1cm} (3)

Where, the optic system is assumed as being composed of two lenses, of focal lengths $f_1$ and $f_2$, separated by a distance $d$. When we define $\ell_i$ as the distance from the initial top surface plane of the sample object to CP1, we have the second set of conjugated planes with the initial set as $s_{i2} = s_{i2} + Z_i$ and $s_{o2} = s_{o2} - \ell_i$. By substituting these relations into Eq. (3) and performing some mathematical manipulations we have $Z_i$ as a function of $\ell_i$:

$$Z_i = -s_{oi} + \frac{f_2d - f_1f_2(s_{oi} - \ell_i)}{(s_{oi} - \ell_i - f_1)n_0}.$$  \hspace{1cm} (4)

It says that if the top surface of the sample moves in the $n_0$ background medium by a distance $\ell_i$, we have its focused image at the CP1, which is moved to the right by $Z_i$ from the CCD plane. Reversely, if we get $Z_i$ with the numerical movement of the CCD plane, we can also calculate the distance $\ell_i$ with the given parameters of the optics. However, in general, it is not easy to obtain all parameters especially such as $s_{oi}$ and $s_{oi}$. Thus, we need to think of obtaining empirical equation of Eq. (4). At first, the CCD is randomly moved by a distance $Z_i$. Then, the sample is mechanically shifted till it gives the minimum AMP value of the top surface of the sample, which gives the distance $\ell_i$. After obtaining $\ell_i$ values for several $Z_i$’s, the empirical equation of Eq. (4) is obtained by fitting the data set. We note that since Eq. (4) can be applied to any distance of $\ell_i$, it is generalized with $Z_i$ and $\ell_i$ instead of $Z_i$ and $\ell_i$.

From Eq. (4) and Fig. 1(b) we know that the first sample movement $d_{oi}$, for imaging the MIB (middle interface boundary) of the sample, gives a defocused image at the CCD plane. With the numerical movement of the CCD plane by $Z_i$ we have its focused image at CP1. However, in the medium of RI $n_0$, the conjugate plane of CP1 is not at MIB but at the front of the interface separated by $(\ell_i - d_{oi})$. From this discrepancy, the average RI of the first layer of the sample can be extracted; in our case, this is the region of RI $n_i$. In Fig. 1(b), we can think that $\ell_i$ is the distance between the sample positions, in the background medium of $n_0$, that are focused at two CCD planes separated by $Z_i$. The same thing happens in the medium of RI $n_i$, but each sample position is moved to the left (depicted with the solid ray line) due to the higher $n_i$ over $n_0$. It is not difficult to show that the distance $\ell_i$ in the medium of $n_i$ is related with the distance $\ell_i$ in the medium of $n_0$ as [22]:

#197050 - $15.00 USD  
#197050 - $15.00 USD  
(C) 2013 OSA  
2 December 2013 | Vol. 21, No. 24 | DOI:10.1364/OE.21.029955 | OPTICS EXPRESS 29959
Substituting $\ell_1$ of Eq. (1) into Eq. (5.1) gives

$$\ell_1' = \left(1 - \frac{n_0^2}{n_1^2}\right)d_{s1}. \tag{5.2}$$

Therefore, the RI of the first layer of the sample is simply calculated with the sample movement distance $d_{s1}$, numerically optimized distance $\ell_1'$, and the known background $n_0$ as:

$$n_1 = \left(\frac{d_{s1}}{d_{s1} - \ell_1'}\right)^{1/2}n_0. \tag{6}$$

For the imaging of the second interface boundary, the bottom surface of the sample, the sample is moved to the right by an additional distance $d_{s2}$. With this sample movement, due to the RI $n_2$ of the second layer, FP2 and IP2 become separated by a distance $\ell_2$ as shown in Fig. 1(c). The distance $\ell_2$ is due to the double refractions happened at the boundaries between $n_0$ and $n_1$ and between $n_1$ and $n_2$. At the first boundary, between $n_0$ and $n_1$, the beam becomes focused at FP1’ with the total sample moving distance of $(d_{s1} + d_{s2})$, as depicted with the solid green ray lines in Fig. 1(c). Assuming that the first layer is thick enough, due to the sample movement of $(d_{s1} + d_{s2})$, the FP is moved from the initial top surface plane of Fig. 1(a) to the FP1’ of Fig. 1(c) [22]:

$$z_{fp1} = \frac{n_1}{n_0}(d_{s1} + d_{s2}). \tag{7.1}$$

However, due to the finite thickness of the first layer, the beam is refracted again at the second interface boundary, between $n_1$ and $n_2$, and focused at FP2, as depicted with the black solid ray lines in Fig. 1(c). Similarly, when we assume a thick enough second layer, the distance from the second interface to the FP2 is given as [22]:

$$z_{fp2} = \frac{n_2}{n_1}\left(z_{fp1} - t_1\right), \tag{7.2}$$

where $t_1$ is the physical thickness of the first layer of the sample.

Meanwhile, for the imaging of the MIB with the coherent gating, the optical path lengths should be the same with and without the 1st layer, $n_0t_1 = n_0d_{s1}$. Thus, in addition to the RI $n_1$ already given in Eq. (6), the thickness of the first layer is determined with the first sample movement distance as

$$t_1 = \frac{n_0}{n_1}d_{s1}. \tag{8.1}$$

By the same token, the thickness of the second layer is obtained from the distance of the second sample movement made for the bottom boundary imaging as

$$t_2 = \frac{n_0}{n_2}d_{s2}. \tag{8.2}$$

Further, the distance $\ell_2$ between IP2 and FP2 is

$$\ell_2 = z_{fp2} - t_2. \tag{9}$$
By substituting Eqs. (7) and (8) into Eq. (9), we have:

\[
\ell_2 = \left( \frac{n_2}{n_0} - \frac{n_2 n_0}{n_1^2} \right) d_s + \left( \frac{n_2}{n_0} - \frac{n_0}{n_2} \right) d_{s2},
\]

(10)

As was mentioned, the distance \( \ell'_2 \) ' in the background medium \( n_0 \) is obtained from the distance \( \ell_2 \) in the medium of RI \( n_2 \) as in Eq. (5.1)

\[
\ell'_2 = \frac{n_0}{n_2} \ell_2.
\]

(11)

Of course, \( \ell'_2 \) ' is obtained from Eq. (4) or already made empirical equation with the numerical correction distance \( Z_2 \) (\( i = 2 \)) for the CP2. Interestingly, from Eqs. (5.1), (1), and (10), we have the difference between \( \ell'_2 \) ' and \( \ell'_1 \) ' as a function of only the second sample moving distance \( d_{s2} \):

\[
\ell'_2 - \ell'_1 = \frac{n_0}{n_2} \ell_2 - \frac{n_0}{n_1} \ell_1 = \left( \frac{n_0^2}{n_2^2} \right) \frac{d_{s2}}{d_{s2} - (\ell'_2 - \ell'_1)}
\]

(12)

From this, the RI \( n_2 \) of the second layer is simply obtained as

\[
n_2 = \left( \frac{d_{s2}}{d_{s2} - (\ell'_2 - \ell'_1)} \right)^{1/2} n_0.
\]

(13)

As a result, we have the RI of the \( i^{th} \) layer of the sample having many internal layers as

\[
n_i = \left( \frac{d_i}{d_i - (\ell'_i - \ell'_{i-1})} \right)^{1/2} n_0,
\]

(14.1)

with \( i = 1, 2, 3, \ldots \) and \( \ell'_0 = 0 \). Of course, with this \( n_i \) the thickness \( t_i \) of the \( i^{th} \) layer of the sample is simply obtained as the extension of Eq. (8) as

\[
t_i = \frac{n_0}{n_i} d_{ai}.
\]

(14.2)

In a word, the RI and thickness of a particular hidden layer of a multilayered sample are simultaneously extracted only from the CCD correction distances, \( Z_i \) and \( Z_{i+1} \), used for numerical refocusing of both the top and bottom interface boundaries of that particular layer. Figure 2 summaries the process for obtaining the RI profile along the depth of the sample.
3. Experiment and results

For the concept proof, an FF-OCT system was implemented based on Michelson interferometry [22]. As the broadband light source, a superluminescent diode (SLD) having a center wavelength of 830 nm and a spectral bandwidth of 65 nm was used. At both sample and reference arms, NA 0.3 water immersion microscopic objectives were used. To obtain the empirical equation of Eq. (4), the function relating $\frac{\ell_i'}{\lambda}$ with $Z_i$, the 1951 USAF resolution test target was used as a sample. The setup was pre-aligned to ensure focusing of the test pattern on the resolution target. After that, the position of the CCD was mechanically shifted by a certain distance. The position of the resolution target was then adjusted till the best focused image of the target pattern was appeared at the CCD. The same measurements were performed for several CCD positions; these measurements gave the data set of Fig. 3. The solid line is a fitted curve; the data points were well fitted with an exponential curve.
Empirical equation for Eq. (4). The sample position was moved by \( \ell \) for having a focused target pattern image at the CCD located at a given distance \( Z \). The solid line is a fitted exponential curve.

\[
\ell = A \exp(-Z_i/\alpha) + b
\]

\( A = 7.99218 \)
\( \alpha = 1.14832 \)
\( b = -8.80156 \)

Schematic of the sample A having double layers, the top substrate and the middle air layer.

FF-OCT images and numerically corrected images in the double-layered sample A. (a) FF-OCT image of the top resolution target through its glass substrate. (b) the enlarged image of the red box region in (a). (c) and (d) FF-OCT image of the bottom resolution target and its enlarged image. (e) the numerically corrected image of (a) obtained at \( Z_1 = 58.55 \) mm. (f) the enlarged image of the red box region in (e). (g) and (h) the numerically corrected image of (d) obtained at \( Z_2 = 45 \) mm and its enlarged image. (i) and (j) AMP values used for obtaining (e) and (h), respectively.
Table 1. Tomographic RIs and thicknesses measured for several double layered samples

| Sample # | First layer | Second layer | Measured RI and thickness (mm) | Reference RI and thickness (mm) | Standard deviation | Error (%) |
|----------|-------------|--------------|--------------------------------|--------------------------------|--------------------|-----------|
| A        | Resolution target | air | 1.5064 | 1.5078 | 3.879 × 10⁻³ | 0.093 |
|          |             | | 1.5851 | 1.585 | 4.480 × 10⁻³ | 0.004 |
|          | B           | Fused silica | 1.4519 | 1.4528 | 1.915 × 10⁻³ | 0.062 |
|          |             | Oil B | 1.3755 | 1.3759 | 4.897 × 10⁻³ | 0.029 |
|          | C           | Fused silica | 1.4519 | 1.4528 | 1.606 × 10⁻³ | 0.062 |
|          |             | Oil C | 1.3588 | 1.3563 | 4.440 × 10⁻³ | 0.184 |
| D        | Fused silica | Oil D | 1.4518 | 1.4528 | 8.319 × 10⁻⁴ | 0.069 |
|          |             | | 1.3365 | 1.3368 | 4.775 × 10⁻³ | 0.022 |
| E        | Fused silica | Oil E | 1.4523 | 1.4528 | 4.775 × 10⁻³ | 0.034 |
|          |             | | 1.3152 | 1.3172 | 1.895 × 10⁻³ | 0.152 |
| F        | Fused silica | Oil F | 1.4507 | 1.4528 | 2.501 × 10⁻³ | 0.145 |
|          |             | | 1.2963 | 1.2977 | 1.475 × 10⁻³ | 0.108 |
| G        | BK7          | Oil G | 1.5085 | 1.5102 | 2.557 × 10⁻³ | 0.113 |
|          |             | | 1.3874 | 1.3857 | 4.361 × 10⁻³ | 0.123 |
| H        | BK7          | Oil H | 1.5096 | 1.5102 | 1.594 × 10⁻³ | 0.040 |
|          |             | | 1.3633 | 1.3661 | 3.176 × 10⁻³ | 0.205 |
| I        | BK7          | Oil I | 1.5097 | 1.5102 | 1.546 × 10⁻³ | 0.033 |
|          |             | | 1.3444 | 1.3466 | 4.077 × 10⁻³ | 0.163 |
| J        | BK7          | Oil J | 1.5099 | 1.5102 | 1.379 × 10⁻³ | 0.020 |
|          |             | | 1.3256 | 1.3279 | 3.913 × 10⁻³ | 0.173 |
| K        | BK7          | Oil K | 1.5104 | 1.5102 | 1.263 × 10⁻³ | 0.013 |
|          |             | | 1.3063 | 1.3075 | 4.187 × 10⁻³ | 0.092 |
| Average  | First layer | - | - | - | 2.849 × 10⁻³ | 0.062 |
|          | Second layer | - | - | - | 3.721 × 10⁻³ | 0.128 |

As a double-layered sample, called sample A, two resolution targets were stacked with an air gap and facing each other as shown in Fig. 4. The glass substrate of the target was 1.585
mm thick and had a reported RI of 1.5078. The air gap was 0.153 mm wide and was maintained by placing pieces of microscope cover glass between the resolution targets. At first, the FF-OCT system was adjusted to have both the IP and the FP at the top surface of sample. Then the sample was moved up so that the chromium-coated test bars of the upper and bottom resolution targets were successively imaged through the top substrate. Finally, the CCD plane is numerically shifted to have well-focused images of both target patterns. From these sample moving distances and their corresponding numerical CCD shifting distances, the thickness and RI of the top glass substrate and the ones of the air gap layer were extracted.

For the sample A, with a sample movement $d_{s1}$ of 1.795 mm the image of the top target pattern was obtained as in Fig. 5(a). As shown with its enlarged figure of Fig. 5(b), the image of the bar pattern was highly blurred due to the sample movement. The same blurring phenomenon happens with the bottom target pattern of Fig. 5(d) and its enlarged image of Fig. 5(c), obtained at $d_{s2} = 0.115$ mm. The dark bars in Fig. 5(d) are the shadow of the upper target pattern. With the process summarized in Fig. 2, the refocused image of the upper target pattern was obtained at a numerically adjusted CCD position of $Z = 58.55$ mm as shown in Fig. 5(e). Successively, at $Z = 45$ mm, it was possible to refocus the blurry image of the bottom target pattern as shown in Fig. 5(h). The enlarged Figs. 5(f) and 5(g) show that focusing of the images was highly improved. The corresponding AMP value variation, which has a minimum at the optimized $Z$, is shown in Figs. 5(i) and 5(j). We also calculated $\ell_1' = 0.403$ mm and $\ell_2' = 0.313$ mm with the empirical equation of Fig. 3. Finally, with Eq. (14), the RIs of the upper resolution target substrate and the air layer were extracted as 1.5092 and 0.9954, respectively. The thicknesses of these layers were calculated as 1.5807 mm and 0.1535 mm.

In the same manner, several kinds of samples, samples B–K, were prepared. Each of them was made by stacking two transparent plates with a combination of fused silica, BK7 glass, and the resolution test target. By filling the air gap between the plates with an index matching oil of known RI, we were able to make a sample having a hidden layer of a fixed thickness but with various RI. For each sample the measurements were performed five times and averaged. Finally, the measured RI and thickness were compared with the reported ones in Table 1. The reference RIs of the glass plates and oils (or air) are the ones measured at 830 nm and 840 nm, respectively. The average errors of the measured RIs at both layers are 0.062% and 0.128%. In the case of thickness measurement, average errors of them are respective 0.180% and 1.394%.
Table 2. RI and thickness of the same oil layer hidden in several kinds of multi-layered samples

| Sample# | Stacked layers on the top of the oil layer | Measured RI and thickness (mm) | Reference RI and thickness (mm) | Standard deviation | Error (%) |
|---------|-------------------------------------------|-------------------------------|---------------------------------|-------------------|-----------|
| L       | Three cover glasses, one resolution target | 1.3743, 0.1551               | 1.3759, 0.153                  | 2.605 × 10⁻³      | 0.116     |
| M       | Two cover glasses, one resolution target   | 1.3750, 0.1526               | 1.3759, 0.153                  | 1.894 × 10⁻³      | 0.065     |
| N       | One cover glass, one resolution target     | 1.3750, 0.1521               | 1.3759, 0.153                  | 1.219 × 10⁻³      | 0.065     |
| O       | Three cover glasses, one BK7 glass         | 1.3732, 0.1540               | 1.3759, 0.153                  | 2.237 × 10⁻³      | 0.196     |
| P       | Two cover glasses, one BK7 glass           | 1.3750, 0.1559               | 1.3759, 0.153                  | 1.83 × 10⁻³       | 0.065     |
| Q       | One cover glass, one BK7 glass             | 1.3767, 0.1567               | 1.3759, 0.153                  | 2.394 × 10⁻³      | 0.058     |
| R       | Four 34 µm thick films, one cover glass, one resolution target | 1.3745, 0.1538               | 1.3759, 0.153                  | 1.694 × 10⁻³      | 0.102     |
| S       | Two 34 µm thick films, one cover glass, one resolution target | 1.3734, 0.1533               | 1.3759, 0.153                  | 1.306 × 10⁻³      | 0.182     |
| T       | Four 27 µm thick films, one cover glass, one resolution target | 1.3754, 0.1558               | 1.3759, 0.153                  | 2.562 × 10⁻³      | 0.036     |
| U       | Two 34 µm thick films, four 27 µm thick films, one cover glass, one resolution target | 1.3770, 0.1528               | 1.3759, 0.153                  | 1.145 × 10⁻³      | 0.079     |
| Average | -                                         | 1.3750, 0.1542               | 1.3759, 0.153                  | 1.986 × 10⁻³      | 0.065     |

Moreover, it has been confirmed that the RI and the thickness of a particular layer within a sample could be extracted regardless of the conditions at other sections or layers of the sample. For this measurement, ten more samples were prepared by stacking some number, maximum 7, of several kinds of transparent plates on a resolution target as shown in Fig. 4. At the gap between the bottom target plate and the stacked front plates, an index matching oil of RI 1.3759 was applied. For the RI and the thickness measurements of the oil layer, the top and bottom interface boundaries of the oil layer were imaged and numerically focused. For the top interface boundary imaging, some scratches or dusts were made or located on the bottom surface of the top plate. Table 2 shows that the RI of the oil layer is in good agreement with the reference RI even with harsh conditions at the front layers of various samples. The error and deviation of the measured RIs were ranged from 0.036% to 0.196%, and had an average of 0.065%. Besides, those thicknesses also were computed as shown in Table 2 with an average error 0.990%.

4. Summary and conclusion

It is known that the FF-OCT image of the inner structure of a sample is blurred or defocused due to the refractive index (RI) of the sample itself. The image plane (IP) is separated from the focal plane (FP) of the system; the former one is determined by the optical path length difference in the OCT interferometer but the latter one is determined solely by the imaging optics of the sample arm. With a sample having higher RI than its surrounding medium, the physical path length for coherent gating becomes shorter but the focal length for imaging becomes longer. Therefore, obtaining well-focused FF-OCT images on both sides of a sample layer, especially the bottom side, is impossible without doing something. One of the simplest
things for having the well-focused image is moving the CCD plane for the imaging of each side, or we can move the sample position. Fortunately both things are numerically possible with FF-OCT since it gives the complex field of the sample. With the complex field taken at a distance we can calculate the field at any distance. Further, since the separation length between the IP and the FP is given as a function of the RI of the sample, we can extract the RI and the thickness of the sample with the parameters used for the numerical focusing.

In the experiment, since the sample such as a glass plate has fine optical surfaces without any pattern the resolution target was used for getting focused FF-OCT image. Or intentionally, some scratches or dusts were made or located on the surfaces. However, with a practical biomedical sample, it is expected that some layer boundaries have some patterns on which the image focusing can be made. With the samples having no appreciable patterns, we also think that by making a patterned illumination on the layer boundary and getting focused pattern image, we could get the position of the layer boundary. Further, when the sample has non-flat layer boundaries, we cannot use the AMP method directly for the whole area of an OCT image. For that case, we might apply the AMP to a small region of the image. By scanning the AMP applied region across the OCT image, we might get the topological structure of that particular layer. We are planning experiments for implementing these ideas in near future.

We have presented the RI measurement scheme for each layer in multilayered samples based on the numerical refocusing of FF-OCT images. The RI and the thickness of a particular layer of a multilayered sample could be simultaneously extracted from the numerically adjusting the CCD plane location. With each numerically adjusted position of the CCD plane, in the background medium, the position of its corresponding conjugate sample plane could be calculated from the optical system parameters or by forming an empirical equation. Since the separation distance between the IP and the FP is related with the sample movement distance and the RI of that particular layer, the RI and the thickness could be extracted. Interestingly, the RI extraction of a particular layer was possible only with the FF-OCT images, and their numerically refocused images of course, taken at both interface boundaries of that particular layer. As the RI measurements, several samples were prepared by stacking two transparent plates with a gap between them. By changing the top plate material and filling the gap with oil of various RIs, the RIs of the top plate and the oil were measured. The average measurement error was ranged from 0.062% to 0.128%. From those RIs, it was possible also to get the layer thicknesses with an average error of 0.394%. Furthermore, we were able to extract the RI of a particular layer hidden in a multilayered sample regardless of the conditions or the number of its front layers. With stacking various combinations of plates and films, up to 7 layers, on a 0.153 mm thick oil layer, we were able to extract the oil RI with an average error of 0.065%. The thickness of the oil layer was obtained with an average error of 0.990%. The proposed scheme does not require supplementary equipment or labor-intensive mechanical alignment; only numerical calculation is enough. Therefore, it is expected that this method can lead to many interesting applications in which non-invasive RI depth distribution is required.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2008-0062606, CELA-NCRC).