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Abstract. Refractive index of biotissue is a useful optical parameter in the biomedical field. An extended differential total reflection method is introduced to determine the complex refractive index. The real part is directly determined by differential of the reflectance curve, and the imaginary part is obtained from nonlinear fitting. The method is verified by a series of tissue-mimicking phantoms, porcine muscle and porcine adipose. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3615657]

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1 Introduction

Refractive index (RI) is an important optical parameter of biotissue, which can be defined in terms of a real part \( n_r \) and an imaginary part \( n_i \) as \( n = n_r + i n_i \), where \( n_r \) is equal to the extinction coefficient \( \kappa \), which means energy loss per unit at certain direction caused by absorption and scattering. When using a diffusion-approximation-based inverse model to determine the tissue optical parameters, RI is used as a known parameter and therefore its measurement accuracy affect the precise determination of other optical parameters. When the Monte Carlo method is used to simulate the light propagation in biotissues, RI determines the photons directions and boundary mismatch. It has been proved theoretically and experimentally that measurement of optical properties can be substantially affected by RI.

The extinction coefficient should satisfy \( \kappa = \mu_t \lambda / 4\pi \), \( \mu_t \), \( \mu_s \), and \( \mu_a \) are the total attenuation coefficient, the scattering coefficient, and the absorption coefficient, respectively. \( \mu_t = \mu_s + \mu_a \). In the biomedical field, except for some weakly scattering tissues such as the cornea and lens in the anterior eye chamber, most biotissues have strong scattering. In the UV and visible regions of the electromagnetic spectrum, tissue absorption coefficient \( \mu_a \) varies from 0.02 to 2.5 mm\(^{-1}\), while scattering coefficient \( \mu_s \) varies from 2.5 to 40 mm\(^{-1}\). So the traditional method such as Abbe refractometer is not applicable, and a new method named the optical fiber cladding method was developed. The main drawback of this method is the time-consuming procedure of substituting the cladding with biotissue and invalidity when the tissue sample is heterogeneous or distributed, or much smaller than the length of the fiber.

Several other methods have been developed to measure the RI of biotissue, such as minimum deviation angle method, optical coherent tomography (OCT), surface plasmon resonance method (SPR), and total internal reflection method (TIR). The differential total reflection method (DTRM) is a modified version of TIR. When the sample has weak absorption or little scattering, an inflexion point of the reflectance curve at the critical angle makes it easy to obtain \( n_r \). When the absorption or scattering is obvious, the reflectance curve will become smoother and no inflection point appears, so the differential of the reflectance curve is required to find the critical angle.

One of the authors of this paper used DTRM to obtain RI of a strong absorption medium film. So far as we know, RI measurement of media with weak absorption or scattering have already been studied, but the study of strong scattering media is still in the exploration stage. All the methods mentioned above can only determine the real part of RI, and the imaginary part \( \kappa \) remains unsolved. Ding et al. first introduced the conception of complex refractive index in the measurement of human skin tissue and they used a nonlinear regression to obtain \( n_r \) and \( \kappa \) simultaneously.

In this paper, an extended differential total reflection method (EDTRM) is proposed to determine the complex refractive index of strong scattering media. Using this method, \( n_r \) is directly determined by a differential of the reflectance curve, and \( \kappa \) can be obtained from nonlinear fitting. We proved that EDTRM is reliable for measuring RI of high scattering media such as biotissue. The usefulness and reliability of this method is verified by measuring the complex refractive indices of a series of high scattering tissue-mimicking phantoms and biotissues.

2 Material and Methods

Eight kinds of tissue-mimicking phantoms are chosen: India ink solution (Solarbio Co.) of 3.3%, 5%, and 10% concentration, Intralipid-10% solution (Sino-Swed Pharmaceutical Co., Ltd.), white room temperature vulcanized silicon rubber (RTV No. 704), transparent RTV (No. 705) mixed with Al2O3 particles.
In general, we need to measure the intensity of reflection light at different incident angle as to determine the critical angle. According to the schematic diagram in Fig. 1, the mathematical formula of \( n_r \) is

\[
n_r = n_1 \sin \left( \frac{\beta \pm \arcsin\left(\sin \alpha_c/n_1\right)}{2} \right)
\]  

(2)

Where \( \alpha_c \) is the critical incident angle. According to electromagnetic theory, when total internal reflection occurs, the energy of the incident light decays rapidly and some light does penetrate into the less dense medium over relatively short distances, but usually do not exceed about several wavelengths. The mean free length, which describes the distance between scattering and absorption events for photons in medium, is about 10 to 100 \( \mu \)m and far larger than the distance where the light interacts with the medium. So the amplitude of the light field decays exponentially in the medium, and can approximately be described using the Lambert–Beer’s law as follows:

\[
E = E_0 e^{-\mu m \epsilon_i (kz - \omega t)}.
\]  

(3)

We eliminate the influence of time variation, and introduce \( n \) as complex refractive index, then \( E \) can be described as:

\[
E = E_0 e^{2\pi \gamma/\lambda n}
\]  

(4)

where \( E \) is the amplitude of the light field.

Based on the Fresnel formula, \(^1\) when the light is reflected at the prism-sample interface, we obtain

\[
2u_1^2 = n^2(1 - k^2) - n_1^2 \sin^2 \theta
\]  

\[
+ \sqrt{n^2(1 - k^2) - n_1^2 \sin^2 \theta}^2 + 4n^4k^2
\]  

(5)

\[
2v_1^2 = -[n^2(1 - k^2) - n_1^2 \sin^2 \theta]
\]  

\[
+ \sqrt{n^2(1 - k^2) - n_1^2 \sin^2 \theta}^2 + 4n^4k^2
\]  

(6)

where parameters \( v_2 \) and \( u_2 \) are the intermediate variables. For \( s \)-polarized light, the amplitude reflection coefficient \( r_{1,2} \) at the prism-sample interface can be written as:

\[
r_{1,2} = \frac{n_1 \cos \theta - (u_2 + iv_2)}{n_1 \cos \theta + (u_2 + iv_2)} - i
\]  

(7)

The intensity reflection coefficient \( R_{1,2} = (r_{1,2})^2 \) is

\[
R_{1,2} = \left(\frac{n_1 \cos \theta - u_2}{n_1 \cos \theta + u_2}\right)^2 + \left(\frac{v_2}{n_1 \cos \theta + u_2}\right)^2
\]  

(8)

When emergent light leaves the prism and enters into the air, the reflection loss occurs at the prism-air interface, which is approximately equal to the loss of incidence at the air-prism interface. The reflectance of the \( s \)-polarized light at the air-prism interface (or the prism-air interface) is

\[
R_{2,3} = \frac{\cos \alpha - n_1 \cos\left[\arcsin\left(\sin \alpha/n_1\right)\right]}{\cos \alpha + n_1 \cos\left[\arcsin\left(\sin \alpha/n_1\right)\right]}^2
\]  

(9)

Finally, the measured reflectance should include the loss between the two interfaces mentioned above. For \( s \)-polarized light, the measured reflectance is given by

\[
R_i = R_{1,2}^2(1 - R_{2,3})^2
\]  

(10)
The consistency between the measured curve and fitting curve is described by $E_r^2$, defined as

$$E_r^2 = 1 - \frac{\sum_{i=1}^{N} (R_{m,i} - R_{s,i})^2}{\sum_{i=1}^{N} (\bar{R} - \bar{R})^2},$$

(11)

where $R_{m,i}$ is the $i$'th measured reflectance, $R_{s,i}$ is the $i$'th calculated reflectance, $\bar{R}$ is the mean value of measured reflectance over $N$ values of incident angle. The value of $E_r^2$ ranges from 0 to 1 and it is closer to 1 when we obtain a reliable fitting. There are similar formulas for $p$-polarized light. When light with different polarization enters into the prism at different incident angles, the theoretical value of reflectance can be calculated using the equations depicted above.

In our experiment, the reflectance curves of the sample as a function of incident angle were measured for $p$- and $s$-polarized incident light, respectively. We use the EDTRM to obtain $n_i$ and $\kappa$. The reflectance curves change most rapidly near the critical angle. By differential of the reflectance curve, we obtained the value of critical angle $\alpha_c$, whose position corresponds with the peak of the differential curve. The real part $n_i$ is calculated using Eq. (2).

Here we use a nonlinear fitting program based on the Nelder–Mead simplex method to solve the imaginary part $\kappa$, which is a popular direct search method for multidimensional unconstrained minimization. For $s$-polarized light, the value of incident angle and the real part $n_i$ are substituted into Eqs. (5)–(10) to obtain the calculated data. By fitting the calculated data to the experimental data in the nonlinear fitting program, $\kappa$ is solved when we get a smallest fitting error in Eq. (11). For $p$-polarized light, the procedure is similar.

3 Results and Discussion

The reflectance curves of deionized water and ink solution for $p$-polarized light are shown in Fig. 2(a). For $p$-polarized light, the real part $n_i$ of the deionized water is 1.3324 and the extinction coefficient $\kappa$ is smaller than $1 \times 10^{-4}$. The complex refractive indices are 1.3344 ± 0.0012i, 1.3359 ± 0.0018i, and 1.3391 ± 0.0041i for 3.3%, 5%, and 10% ink solution, respectively. It is clearly seen from Fig. 2(b) that the extinction coefficient increases nearly linearly with the ink concentration, which agrees well with the previous work.21 This verifies the reliability of our measurement setup. As shown in Fig. 2(a), an obvious difference exists among samples with different absorption. The smoothness of the reflectance curve increases with the absorption of the sample, so the differential of the reflectance curve is needed to find the critical angle.

The RI of Intralipid-10% solution is 1.3496 ± 0.0022i for $p$-polarization and 1.3500 ± 0.0024i for $s$-polarization. When $\kappa = 0.0024$, $\lambda = 632.8$ nm, the total attenuation coefficient $\mu_t$ is about 47.6 mm⁻¹. Staveren et al.22 reported that $\mu_t$ of Intralipid-10% solution varies from 34 to 55 mm⁻¹ at the wavelength of 632.8 nm. Considering the differences of recipe and manufacturing procedure between different brands, our results are acceptable.
The measured extinction coefficient $\kappa$ of porcine muscle and porcine adipose are about 0.002, which exhibits biotissue to have the character of high light scattering. The increase of the scattering coefficient will make the reflectance curve become smoother near the critical angle, which will lead to the decrease of measurement accuracy using EDTRM. So when $\kappa$ is larger than 0.004, the difficulty of using EDTRM to determine RI arises. Fortunately, most of the biotissue has a $\kappa$ smaller than 0.002, so EDTRM is generally applicable.

The parameter $E^2$, defined as the fitting quality, is larger than 0.992 for deionized water and ink solution. For Intralipid-10% solution, $E^2$ is about 0.980. The results are similar to Ding et al.’s20. For the other six types of tissue mimicking phantoms, $E^2$ ranges from 0.975 to 0.999. Each of the samples was continuously measured 4 times to calculate the standard deviation. The standard deviation of $n_r$ is smaller than 0.0001 for the tissue-mimicking phantoms and smaller than 0.0005 for the measured porcine tissue, which is much smaller than Ding et al.’s20. The main difference may be caused by the difference of tissue, for we measured the same sample 4 times and they measured 4 or 6 skin samples 12 or 18 times.

Table 1 Rs for tissue-mimicking phantoms and biotissues.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$n_r \pm \Delta n_r$ $s$-polarized</th>
<th>$\kappa$ $s$-polarized</th>
<th>$n_r \pm \Delta n_r$ $p$-polarized</th>
<th>$\kappa$ $p$-polarized</th>
</tr>
</thead>
<tbody>
<tr>
<td>704 RTV</td>
<td>1.4112 ± 0.003</td>
<td>0.0036 ± 0.0008</td>
<td>1.4112 ± 0.003</td>
<td>0.0031 ± 0.0008</td>
</tr>
<tr>
<td>705RTV (polystyrene added)</td>
<td>1.4048 ± 0.001</td>
<td>0.0002 ± 0.00005</td>
<td>1.4048 ± 0.001</td>
<td>0.0002 ± 0.00005</td>
</tr>
<tr>
<td>705RTV (Al$_2$O$_3$ added)</td>
<td>1.4053 ± 0.002</td>
<td>0.0021 ± 0.0008</td>
<td>1.4053 ± 0.002</td>
<td>0.0019 ± 0.0008</td>
</tr>
<tr>
<td>Gelatin (Al$_2$O$_3$ added)</td>
<td>1.3835 ± 0.002</td>
<td>0.0009 ± 0.0007</td>
<td>1.3835 ± 0.002</td>
<td>0.0008 ± 0.0007</td>
</tr>
<tr>
<td>Agar (Al$_2$O$_3$ added)</td>
<td>1.3352 ± 0.001</td>
<td>0.0009 ± 0.0003</td>
<td>1.3352 ± 0.001</td>
<td>0.0009 ± 0.0003</td>
</tr>
<tr>
<td>Epoxy resin (Al$_2$O$_3$ added)</td>
<td>1.5532 ± 0.002</td>
<td>0.0022 ± 0.0008</td>
<td>1.5532 ± 0.002</td>
<td>0.0022 ± 0.0008</td>
</tr>
<tr>
<td>Porcine adipose</td>
<td>1.4663 ± 0.003</td>
<td>0.0016 ± 0.0010</td>
<td>1.4676 ± 0.003</td>
<td>0.0013 ± 0.0010</td>
</tr>
<tr>
<td>Porcine muscle</td>
<td>1.3671 ± 0.002</td>
<td>0.0021 ± 0.0009</td>
<td>1.3676 ± 0.002</td>
<td>0.0022 ± 0.0009</td>
</tr>
</tbody>
</table>
The experimental error of $n_r$ can be calculated by the differential of Eq. (2), which is

$$\Delta n_r = \left| \frac{\partial n_r}{\partial \beta} \right| \Delta \beta + \left| \frac{\partial n_r}{\partial \alpha} \right| \Delta \alpha + \left| \frac{\partial n_r}{\partial n_c} \right| \Delta n_c. \quad (12)$$

The possible sources of error include errors of the vertex angle ($\Delta \beta$), the measured critical incident angle ($\Delta \alpha$), and the refractive index of prism ($\Delta n_c$). The high resolution rotation stage has a minimum incremental motion of 3.5 $\mu$rad and a divergence angle of laser and the precision of the detector can be neglected. For high scattering media, the minimum angle interval in our experiment is about 0.1 deg. For porcine muscle, $n_r = 1.514, \Delta \beta = 0.0002$ rad, $\Delta \alpha = 0.1^\circ$, by substituting these values into Eq. (2), we get the total experimental error $\Delta n_r = 0.002$. By changing the value of $n_r$ in the range of error, we calculate the error of $\kappa$ by the fitting program. The experimental error of the other phantoms and biotissues can be estimated by the same way and the results are listed in Table 1. The main experimental error originates from the shift of the critical incident angle $\Delta \alpha_c$, which is influenced by the extinction coefficient of the sample. In conclusion, a new method of determining refractive indices is introduced. Our experimental results prove that EDTRM is applicable for RI measurement of high scattering media such as tissue and tissue-mimicking phantoms. Considering the importance of RI and tissue-mimicking phantoms in biomedical fields, we intend to make a further study of this method.

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