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Application of matching liquid on the refractive index measurement of biotissue: A theoretical and experimental study

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ABSTRACT

The application of matching liquid on the measurement of the refractive index (RI) of biotissue using total internal reflection (TIR) method is investigated in detail. A theoretical model describing samples with different absorbing and scattering ability is given based on Fresnel formula. The theoretical calculation is verified by experimental results of three simulation samples (transparent plexiglass, white plexiglass and ZB3 glass) and cedar wood oil as the matching liquid. Reflectance curves of porcine tissue samples were recorded and systematically studied using two kinds of matching liquid (cedar wood oil and adipose oil) at the incident of TE and TM wave, respectively. Method for proper selection of matching liquid under different conditions is discussed.

1. Introduction

Knowledge of the refractive index (RI) of biotissue would facilitate the study of the other optical parameters of biotissue, for example, the scattering coefficient μ_s and the absorption coefficient μ_a [1]. Except for some medium membrane with high absorption, most biotissue are optically turbid, highly scattering, and nonhomogeneous. Using diffusion equation for accurate calculations of the light fluence rate in tissue during photodynamic therapy or to measure the absorption and scattering properties of tissue depend on the boundary conditions, which is actually the RI match condition at the interface of tissue and surrounding medium [2].

For the highly scattering character of most biotissue, the traditional Abbe refractometer [3] is not applicable for RI measurement anymore. The total internal reflection (TIR) method is becoming widespread as a basic method for the RI measurement of bio-optical material [4] and biotissue [5–9]. Using TIR for RI measurements, the main obstacle encountered by most researchers is the contact between the prism and biotissue, which has a crucial impact on the measuring results. Normally, surface roughnesses of biotissue as well as air gap at the prism–tissue interface make intimate contact hard to achieve. Previous experimental investigations have presented several methods to solve the problem [5–9].

First, homogenize the sample. Bolin et al. resorted to homogenization [5]. Tissue sample was made in a moderate speed blender and then substituted the cladding of a commercial fiber. They assumed that the intracellular and extra cellular fluids expressed from the homogenate have a RI closely to the overall tissue. Although they proved the homogenization effect is subtle and credible in principle, the method is experimentally laborious. What is more, the method is invalid for small size biotissue or loose contact occurs at the fiber core and sample interface. Second, apply a pressure on the sample [6–8]. Li et al. emphasized that liquid sample is sandwiched between a flat glass and a semicylindrical len, while solid sample must have intimate contact with the surface of the lens [6]. Ding et al. have proved that the RI of the skin sample changed dramatically with the pressure applied on the surface of the sample, for example, RI equals to 1.355 at 0.1 MPa and 1.388 at 0.55 MPa [7]. Fast dehydration of tissue and significant reduction of tissue thickness were observed when the pressure was higher than 0.4 MPa. Bolin et al. [5] also found that the output pattern changed with the tightness of packed tissue. So over high pressure on the sample surface is forbidden to avoid discernible change of the real cellular environment of biotissue. Third, add adhesive. Sun et al. daubed the surface of prism with animal adipose and then pressed thin section of animal tissue on the prism, so as to obtain intimate contact [9]. The adipose has a RI value (≈ 1.46) much larger than the muscle tissue, which makes the problem complicated.

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Actually, RI matching liquid can be added at the prism–tissue interface to replace the role played by animal adipose, which makes a close contact as the adhesive and brings no impairment to the biotissue. As an old technique, RI matching method has been wildly applied in a large area, including Abbe refractometer [3], liquid flow investigations [10], aberration correction of single-photon and two-photon confocal fluorescence microscopy [11,12], enhancement the fluorescence sensor signal location of human skin [13] and the optical clarity of Interferometric technique [14], determination the RI of small particles [15], living cells [16] and rough solids [17] et al. While some insights have been gained through the application of matching liquid, there is little empirical information available especially for the RI measurement of biotissue. As a practical problem of interest to optical sensing, it is quite unsatisfactory to leave this important problem unnoticed.

In this paper, we aim to investigate the application of matching liquid in the RI measurement of biotissue using TIR method. For tissue with different absorbing and scattering ability, adequate model was built. The theoretical analysis of light propagation based on Fresnel formula, coupled with polarized optical reflectance measurements performed on three simulation samples (including transparent plexiglass, white plexiglass and ZB3 glass), leads to the verification of the theoretical model. Performances of two different matching liquid (cedar wood oil and adipose oil) on biotissue samples are compared and examined. Experimental observations of biotissue ('Double Peaks' and 'Triple Peaks' on derivative curves) are successfully explained by the theoretical model. At the end, rules for preparation of samples and selection of matching liquid for biotissue RI measurement are summarized.

2. Theoretical analysis

The experimental setup is shown in Fig. 1. Matching liquid is daubed on the surface of the sample and then sandwiched between the sample and the prism. The equilateral prism is fixed on a rotation stage (M-038, PI), with apex angle $\beta=60^\circ$. α, θ, φ are the incident angles at the air–prism (A–P), prism–matching liquid (P–M) and matching liquid–sample (M–S) interfaces, respectively. A He–Ne laser (632.8 nm), a polarizer P and a half-wave plate H placed after the beam splitter are used to generate light with different polarization. D_1 and D_2 are two aperture diaphragms. A beam splitter M and a dual-channel power meter (PM320E, Thorlabs) with two detectors PD1 and PD2. PD1 is used to monitor the light source fluctuation, while PD2 is used to record the reflectance.

The complex RIs of matching liquid and sample can be defined as $n'_m = n_m + i\kappa_m$ and $n' = n_s + i\kappa_s$, respectively. The relationship between the extinction coefficient κ_s and the total attenuation coefficient μ_t can be described as $\kappa_s = \mu_t\lambda/4\pi n.\mu_t = \mu_s + \mu_a$. μ_s and μ_a are the scattering and absorption coefficients, respectively. n_p is the RI of prism. When light came from an optically denser medium enter into an optically thinner medium, TIR will occur at the critical angle θ_c. When TIR occurs at the M–S interface, we have

$$n_s = n_p \sin\theta_c \tag{1}$$

According to the schematic diagram in Fig. 1, we have

$$n_s = n_p \sin(\beta \pm \operatorname{asin}(\sin\alpha_c/n_p)) \tag{2}$$

here, n_p and n_m are considered to be known parameters. Commonly, we need to measure the angle dependent reflectance curve to determine θ_c and then solve n_s by Eq. (2).

The reflectance changes with the incident angle at the interface of two media, which can be described by Fresnel Formula [18]. The reflection coefficients at the A–P interface for TE ($Rs_{a,p}$) and TM ($Rp_{a,p}$) waves can be described as

$$\begin{cases} Rs_{a,p} = \left(\dfrac{\cos\alpha - n_p\cos(\operatorname{asin}(\sin\alpha/n_p))}{\cos\alpha + n_p\cos(\operatorname{asin}(\sin\alpha/n_p))}\right)^2 \\ Rp_{a,p} = \left(\dfrac{n_p\cos\alpha - \cos(\operatorname{asin}(\sin\alpha/n_p))}{n_p\cos\alpha + \cos(\operatorname{asin}(\sin\alpha/n_p))}\right)^2 \end{cases} \tag{3}$$

When the light beam reaches the P–M interface, some of the light is reflected back into the prism (Part I light) and some is multi-reflected in the matching liquid (Part II light). The reflection coefficient at the P–M interface for TE ($Rs_{p,m}$) and TM wave ($Rp_{p,m}$) can be described as

$$\begin{cases} Rs_{p,m} = \dfrac{(n_p\cos\theta - u)^2 + v^2}{(n_p\cos\theta + u)^2 + v^2} \\ Rp_{p,m} = \dfrac{[n_m^2(1-\kappa_m^2)\cos\theta - n_p u]^2 + [2n_m^2\kappa_m\cos\theta - n_p v]^2}{[n_m^2(1-\kappa_m^2)\cos\theta + n_p u]^2 + [2n_m^2\kappa_m\cos\theta + n_p v]^2} \\ u^2 = \left\{ n_m^2(1-\kappa_m^2) - n_p^2\sin^2\theta + \sqrt{[n_m^2(1-\kappa_m^2) - n_p^2\sin^2\theta]^2 + 4n_m^4\kappa_m^2} \right\}/2 \\ v^2 = \left\{ -[n_m^2(1-\kappa_m^2) - n_p^2\sin^2\theta] + \sqrt{[n_m^2(1-\kappa_m^2) - n_m^2\sin^2\theta]^2 + 4n_m^4\kappa_m^2} \right\}/2 \end{cases} \tag{4}$$

where parameter u, v are the intermediate variables. When Part II light reaches the M–S interface, the reflection coefficients for TE ($Rs_{m,s}$) and TM ($Rp_{m,s}$) waves can be described as

$$\begin{cases} Rs_{m,s} = \dfrac{(n_m\cos\varphi - \varepsilon)^2 + \sigma^2}{(n_m\cos\varphi + \varepsilon)^2 + \sigma^2} \\ Rp_{m,s} = \dfrac{[n_s^2(1-\kappa_s^2)\cos\varphi - n_m\varepsilon]^2 + [2n_s^2\kappa_s\cos\varphi - n_m\sigma]^2}{[n_s^2(1-\kappa_s^2)\cos\varphi + n_m\varepsilon]^2 + [2n_s^2\kappa_s\cos\varphi + n_m\sigma]^2} \\ \varepsilon^2 = \left\{ n_s^2(1-\kappa_s^2) - n_m^2\sin^2\varphi + \sqrt{[n_s^2(1-\kappa_s^2) - n_m^2\sin^2\varphi]^2 + 4n_s^4\kappa_s^2} \right\}/2 \\ \sigma^2 = \left\{ -[n_s^2(1-\kappa_s^2) - n_m^2\sin^2\varphi] + \sqrt{[n_s^2(1-\kappa_s^2) - n_m^2\sin^2\varphi]^2 + 4n_s^4\kappa_s^2} \right\}/2 \end{cases} \tag{5}$$

where parameter ε, σ are the intermediate variables. When Part II light reflected from the M–S interface comes back to the P–M interface, the intensity reflection coefficients for TE ($Rs_{m,p}$) and TM ($Rp_{m,p}$) waves are

$$\begin{cases} Rs_{m,p} = \left(\dfrac{n_m\cos\varphi - n_p\cos\theta}{n_m\cos\varphi + n_p\cos\theta}\right)^2 \\ Rp_{m,p} = \left(\dfrac{n_p/\cos\theta - n_m/\cos\varphi}{n_p/\cos\theta + n_m/\cos\varphi}\right)^2 \end{cases} \tag{6}$$

We assume that the reflection loss occurring at the incident A–P interface is almost equal to the loss at the emergent A–P interface. Considering the multi-reflection at the M–S interface, the final

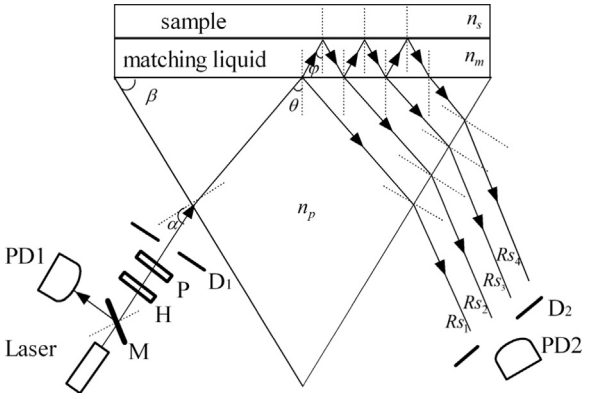

Fig. 1. Schematic diagram of the experimental setup for reflectance measuring.