Noninvasive determination of absorption coefficient and reduced scattering coefficient of human skin tissues *in vivo* with oblique-incidence reflectometry

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A spatial distribution of diffuse reflectance produced by obliquely incident light is not centered about the point of light entry. The value of shift in the center of diffuse reflectance is directly related to the absorption coefficient μ_a and the effective attenuation coefficient $\mu_{\rm eff}$. μ_a and the reduced scattering coefficient μ_s' of human skin tissues *in vivo* are measured by oblique-incidence reflectometry based on the two-source diffuse theory model. For ten Chinese volunteers aged 15−63 years, μ_a and μ_s' are noninvasively determined to be $0.029-0.075$ and $0.52-0.97$ mm^{-1}, respectively.

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The noninvasive, *in vivo* and real-time measurement of tissue optical parameters, namely, absorption coefficient μ_a and reduced scattering coefficient μ_s', is a subject of considerable interest in a number of medical applications, such as photodynamic therapy and laser surgery[1−3]. The oblique-incidence reflectometry proposed and developed by Wang *et al.*[3−5] is a relatively simple method for measuring optical parameters, which makes clinical application feasible. Marquez *et al.*[5] applied this method to the chicken tissues *in vitro* firstly. But now, there are few reports on applications of human tissues *in vivo*. The aim of this study is to investigate μ_a and μ_s' of human skin tissues *in vivo* using oblique-incidence reflectometry at the wavelength of 650 nm. Differently, we use one detecting fiber scanning along a direction to collect enough raw data of diffuse reflectance of tissues. The advantage of using a single fiber optic scanning probe instead of an array of fibers like Marquez's setup[5] is that interference among contiguous fibers can be avoided. The reason of selecting the oblique incidence here is that it has an advantage over normal incidence by gathering information from superficial layers without penetrating deep tissue. This is of particular importance, since skin cancer information is usually present in the top layers of the skin tissues, and deeper layers only add to the background noise in the signals. The optical parameters measured in this letter show that the oblique-incidence reflectometry is valid to determinate human tissues *in vivo*.

The developed method of oblique-incidence reflectometry described by Lin *et al.*[4] is based on the two-source diffuse theory model of spatially resolved and steady-state diffuse reflectance[6]. This diffuse theory model is not accurate near the source, that is, reflectance that falls in the range of $1-2$ transport mean free paths (mfp$'$) of the source. We make up this drawback by measuring the reflectance beyond the range of $2-3$ mfp$'$. For normal incidence, Farrell *et al.* used two isotropic point sources, one positive photon source located 1 mfp$'$ below the tissue surface and one negative image source above the tissue surface[6]. If light is obliquely incident as shown in Fig. 1, the positive photon source is buried at the same path length into the tissue from the entry point as normal incidnece. The path length is modified by Snell's law and accurately determined by $3D=(0.35\mu_a+\mu_s')^{-1}$, where D is defined as the diffuse coefficient. Compared with normal incidence, the positive photon source of oblique incidence shifts a distance Δx in x direction and the shift is $3D\sin\theta_t$, where θ_t is the refractive angle of tissue. If the incident angle, the refractive indices of tissue, and ambient medium through which the light delivered are known, θ_t can be calculated according to Snell's law.

The diffuse reflectance profile for oblique incidence is centered about the position of the point source, so we can measure the shift Δx by finding the center of diffuse reflectance relative to the light entry point. The two-source model gives the expression of diffuse reflectance[6]:

$$R(r)=z_0(\mu_{\rm eff}+\frac{1}{\rho_1})\frac{\exp(-\rho_1\mu_{\rm eff})}{4\pi\rho_1^2}+(z_0+2z_b)(\mu_{\rm eff}+\frac{1}{\rho_2})\frac{\exp(-\rho_2\mu_{\rm eff})}{4\pi\rho_2^2}, \quad (1)$$

where $\mu_{\rm eff}$ is the effective attenuation coefficient defined

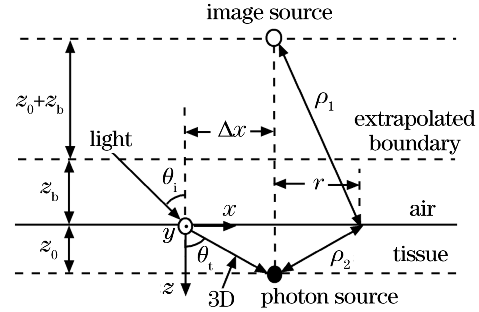

Fig. 1. Positions of point sources in the diffusion theory model for oblique incidence.

as $\mu_{\rm eff} = \sqrt{\mu_{\rm a}/D}$; ρ_1 and ρ_2 are the distances from the two point sources to the point of interest; $z_0 = 3D$ is the distance between the positive source and the tissue surface, $z_{\rm b} = 2AD$ is the distance between the extrapolated boundary and the surface of the tissue, and $z_0 + 2z_{\rm b}$ is the distance between the negative image source and the tissue surface, A is related to the internal reflection and has an empirical value $(1+r_{\rm i})/(1-r_{\rm i})$ with $r_{\rm i} = -1.440n_{\rm rel}^{-2} + 0.710n_{\rm rel}^{-1} + 0.668 + 0.0636n_{\rm rel}$. Here, $n_{\rm rel} = n_{\rm t}/n_{\rm a}$ is defined as relative index of the tissue-air interface[7].

We search for the center of diffuse reflectance by interpolating between the raw data points taken several mfp′ from the source and perform a nonlinear least-square fitting with the Levenberg-Marquardt method on Eq. (1) to determine $\mu_{\rm eff}$. $\mu_{\rm a}$ and $\mu_{\rm s}'$ are solved from[4]

$$\mu_{\rm a} = \frac{\mu_{\rm eff}^2 \Delta x}{3\sin\theta_{\rm t}}, \tag{2}$$

$$\mu_{\rm s}' = \frac{\sin\theta_{\rm t}}{\Delta x} - 0.35\mu_{\rm a}. \tag{3}$$

Δx in Eq. (2) can be practically measured and $\mu_{\rm eff}$ also be easily determined by fitting the diffuse reflectance profile. So this method is relatively simple.

The experimental setup is drawn schematically in Fig. 2(a) and the three-dimensional (3D) perspective view of the tissue surface is shown in Fig. 2(b). A semiconductor laser beam (20 mW, 650 nm, beam divergence< 0.3 mrad) was transmitted through an aperture with 1-mm diameter, and then was collimated with a lens. The beam was reflected by a mirror and then irradiates obliquely to the surface of tissue with the incident angle of 45°. The signals of diffuse reflectance scattered towards the exterior tissue surface were collected by a detecting fiber (65-μm diameter, numerical aperture (NA) = 0.3, multimode) and then coupled to a photomultiplier tube (PMT). The photoelectric signals of PMT were inputted to an analog-to-digital converter (A/D) and then saved in a personal computer. The detecting fiber was fixed on a 3D translation stage with the least step of 5 μm and used to scan the surface of tissue along the x direction shown in Fig. 2(b). When the electric translation stage moved a step, a value of diffuse reflectance related to a particular position was sampled process. Simultaneously, a computer controlled the moving of electric translation stage and sampling. Reflectance data were detected in another plane that was parallel to the incident plane, but the offset Δy in y direction is 1.3 ± 0.3 mm. The purpose was to avoid blocking off the entry light when the detecting fiber scanned.

Five male and five female subjects aged 15 – 63 years were selected. All of them are Chinese volunteers. The forearm of each subject was gently washed and dried. After a delay of 10 min, the subject was seated in a chair and the arm was placed in a pronated position on a horizontal surface with flexed elbow at the same body level. All the measurements were taken in a dark room maintained at 20 °C. The average skin temperature of ten subjects was 36.5 °C. The detecting fiber could be accurately adjusted along z axis. The gap between the surface of forearms and detecting fiber was as short as possible (∼ 1 mm). Five measurements for one subject were performed in the same location and each measurement took only 30 s. $\mu_{\rm a}$ and $\mu_{\rm s}'$ were evaluated for each diffuse reflectance measurement, and the final results were averaged. We used the refractive index $n = 1.45$ measured by Ding *et al.*[8] and chose the anisotropy factor $g = 0.95$ to fit the raw data. The raw data were fitted by a nonlinear least-square method according to Eq. (1).

We find that the deviation in optical properties for ten subjects studied here are fairly small. In shape, the distributions of diffuse reflectance of all subjects measured are similar. Moreover, their peak positions shift a little distance. We take the distribution of diffuse reflectance of volunteer No. 5 as an example, the shift $\Delta x = 0.5$ mm, as shown in Fig. 3. In experiments, we take the visual position of the light entry point as an initial value and then optimize it in the fitting program. The final results are compiled in Table 1. For all volunteers, $\mu_{\rm s}'$ ranges in $0.52 - 0.97$ mm^{-1}, and $\mu_{\rm a}$ ranges in $0.029 - 0.075$ mm^{-1}. The results show that $\mu_{\rm s}'$ ($\mu_{\rm a}$) values for different persons are at the same order. The optical parameters of human skin tissues were reported differently for different techniques, parts of body, and races[9−12]. Our experimental results of forearm skin tissues *in vivo* are in the same order as Moffitt's study using reflectometry[10].

From Table 1, the accuracy of $\mu_{\rm a}$ and $\mu_{\rm s}'$ depends on the directly measured parameters of Δx and $\mu_{\rm eff}$. $\mu_{\rm a} = D\mu_{\rm eff}^2$, so the error in $\mu_{\rm a}$ is of the same order of magnitude

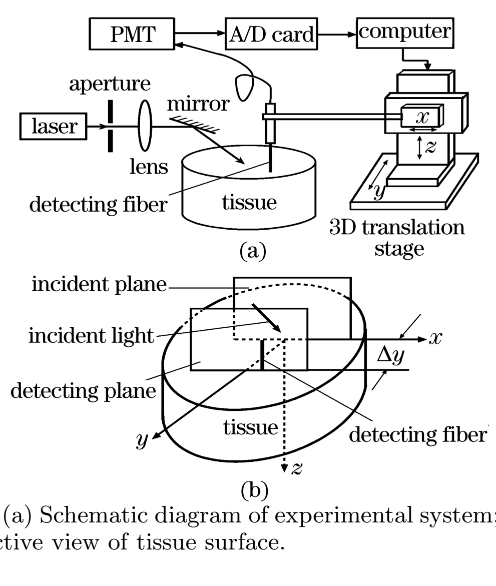

Fig. 2. (a) Schematic diagram of experimental system; (b) 3D perspective view of tissue surface.

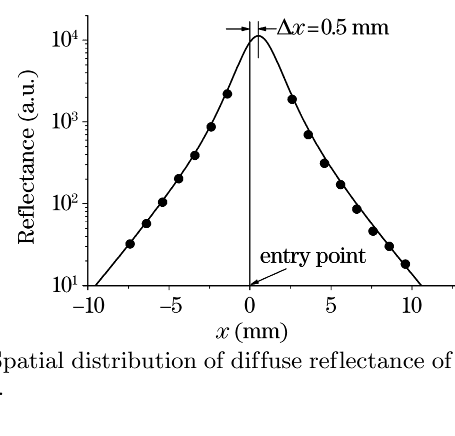

Fig. 3. Spatial distribution of diffuse reflectance of a 63-year-old man.

Table 1. Experimental Results of Human Skin Tissues

Subject	Gender	Age	Δx (mm)	μ_s' (mm^{-1})	μ_s (mm^{-1})	μ_a (mm^{-1})
1	Male	15	0.70	0.64	6.41	0.049
2	Male	24	0.63	0.70	6.99	0.075
3	Male	35	0.60	0.77	7.67	0.043
4	Male	45	0.65	0.70	7.00	0.051
5	Male	63	0.50	0.97	9.07	0.066
6	Female	24	0.84	0.55	5.52	0.029
7	Female	30	0.87	0.52	5.21	0.036
8	Female	40	0.55	0.85	8.49	0.033
9	Female	56	0.66	0.69	6.88	0.053
10	Female	60	0.65	0.68	6.83	0.071

as the error in D (or Δx) plus twice the error in μ_{eff} and can be derived by $\Delta\mu_a/\mu_a = \Delta D/D + 2\Delta\mu_{\text{eff}}/\mu_{\text{eff}}$. In contrast, the error in μ_s' is of the order of that in D (or Δx). Δx and μ_{eff} are calculated by fitting the measured diffuse reflectance to Eq. (1). However, it is practicable under the assumption of $\mu_s' \gg \mu_a$. The error will be enlarged if this assumption does not hold. In order to test this assumption, we measured the diffuse reflectance of a volunteer's forearm (No. 6) covered with a little black ink. The fitting result tended to be divergent, which meant that Eq. (1) was not suitable for the situation of weak scattering. Thus, this method is not suitable for brown people if the diffuse theory model is chosen. Moreover, the diameter of incident beam is still 1.0 mm, although we have used an aperture and a lens to narrow the incident beam of semiconductor laser. Consequently, the value of entry point is not strictly precise, which also expands the error of Δx. The errors for each optical parameter measured will be investigated quantificationally in our continued work.

In conclusion, we demonstrate that the oblique-incidence reflectometry can be exploited for the determination of optical properties of human skin tissues *in vivo*. If Monte Carlo simulation is selected to solve μ_s' and μ_a, the limit of $\mu_s' \gg \mu_a$ will be modified. The method has potential applications for various persons in medical field for the quick, inexpensive, and noninvasive advantages. Moreover, the results of this study will be helpful in further understanding of optical properties of human tissues and provide reference values for laser therapeutics.

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