Measurement of optical penetration depth and refractive index of human tissue

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Experimental techniques for measurement of optical penetration depth and refractive index of human tissue are presented, respectively. Optical penetration depth can be obtained from the measurement of the relative fluence-depth distribution inside the target tissue. The depth of normal and carcinomatous human lung tissues irradiated with the wavelengths of 406.7, 632.8 and 674.4 nm *in vitro* are respectively determined. In addition, a novel simple method based on total internal reflection for measuring the refractive index of biotissue *in vivo* is developed, and the refractive indices of skin from people of different age, sex and skin color are measured. Their refractive indices are almost same and the average is 1.533.

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The tissue characteristics play an important role in all kinds of medical laser applications, such as photodynamic tumor therapy and photothermal treatments, but also for cancer diagnostic techniques as fluorescence diagnostics and transillumination imaging[1,2]. In order to determinate the spatial and temporal distributions of light fluence rate in human tissues, the optical properties of human tissues should be primarily measured.

Human tissue is an optically turbid medium, and when photons propagate through such tissue they are scattered and absorbed by the particles and macromolecules inside the tissue. When the surface of tissue irradiated with a broad laser beam, the deep dosimetry of light in tissue is described by fluence rate ϕ in mW/cm^2,

$$\phi(z) = \phi_0 k \exp(-z/\delta), \tag{1}$$

where ϕ_0 is the incident irradiance, the parameter k accounts for the effect of back-scattered irradiance which equivalently augments of ϕ_0, δ is the optical penetration depth which describes the pathlength that causes a 1/e attenuation of light[3].

The experimental setup for measuring fluence rate in the normal and carcinomatous human lung tissues is shown in Fig. 1. The deflated normal and carcinomatous samples *in vitro* used in our measurements were obtained immediately after lobectomy of the whole left lung from a 51-year old female patient. The sample sizes of the normal and the carcinomatous were $5.0 \times 3.6 \times 1.1$ cm^3 and $5.0 \times 4.5 \times 1.1$ cm^3, respectively. The fresh samples were rinsed briefly with physiological saline to remove excess blood before measurements.

The *depthometer with isotropic fiber-optic probe*, developed by ourselves, mainly consists of a micrometer, an 18# 20-mm sterile syringe needle and a 400-μm-diameter fused quartz optic fiber with a 1.35-mm-diameter isotropic special probe (PDT Systems Co., USA). The isotropic probe was inserted into the needle, so it was possible to inject the probe into the tissue, position the probe and draw back the needle of 3 mm so that the distance from the sphere of probe to tip of the needle was 1.5 mm. The reading precision of the probe's position by the depthometer was 0.01 mm.

Kr^+ laser (Model 3500, Lexel Laser Inc., USA) operating at 406.7 – 415.4 or 676.4 nm and a He-Ne laser at 632.8 nm were used as the irradiation source. The output from the laser was collimated and expanded by an expander and perpendicularly irradiated upon the tissue surface. The beam diameter was 2.6 mm and irradiance ϕ_0 of the beam was 17, 17 and 35 mW/cm^2 for 406.7, 632.8 and 676.4 nm, respectively. The needle tip of the depthometer was also perpendicularly positioned in the center of light spot at the back surface of the tissue, and then penetrated into the tissue at the necessary depth z with high accuracy. The needle was drawn back 3 mm. Thus the probe was located directly under the center of the spot at the depth z. The output of the isotropic fiber-optic probe was fed to a photomultiplier and the photocurrent $I(z)$ can be read out by Model 7070 detection system (Oriel Co., USA). The light distribution

Table 1. The Optical Penetration Depth of Human Lung Tissues

λ (nm)	406.7	632.8	676.4
Lung Tissue		δ (mm)	
Normal	0.08	1.19	1.08
Adeno-Squamous Carcinoma	0.32	1.70	1.96

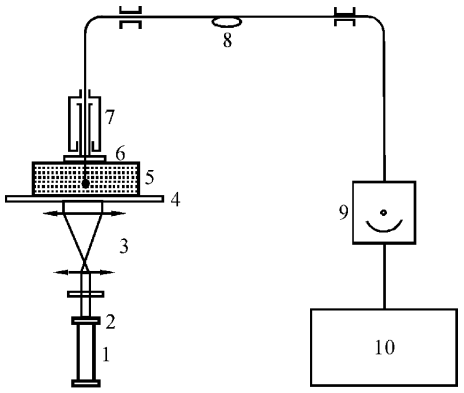

Fig. 1. Experimental setup for measuring fluence rate. 1: Kr^+/He-Ne laser; 2: attenuator; 3: beam expander; 4: glass platform; 5: tissue; 6: glass plate; 7: depthometer; 8: fiber probe; 9: photomultiplier tube; 10: reading system.

$\phi(z)$ inside the samples can be obtained by adjusting the probe's position in the tissue.

The measurements of $\phi(z)$ were performed for at least three locations on each sample tissue. For instance, light distribution in fresh human lung tissue *in vitro* under the irradiation of violet laser are shown in Fig. 2, denoted as the solid lines. The fluence rate in normal human lung (Fig. 2(b)) is much lower than in the malignant one (Fig. 2(a)) under the same input irradiance ϕ_0. This may be explained by the fact of different volumes of blood contained in them. There is less blood in poorly vascularized or necrotic tumor tissue, but rich blood in the normal lung tissue. However, the fluence rate $\phi(0)$ at surface within the adeno-squamous carcinoma lung tissue is 25.2 mW/cm^2, which is more than the delivered irradiance ϕ_0 of 17 mW/cm^2. This due to the relatively strong scattering of tumorous lung tissue, which causes light to be back scattered toward the tissue surface and increases the delivered irradiance and result in a higher fluence rate and greater penetration in it.

Optical penetration depth δ is available from the least square fitting with single exponential function as shown in Table 1.

The refractive index of biotissue is also an important optical parameter in tissue optics[4]. In spite of the refractive index is very important for determination of reflectance, angular change of light direction at tissue interface, but little experimental investigation *in vivo*, even *in vitro* has been performed for measuring the refractive indices of tissues[5]. Several investigators have attempted to improve the situation. F. P. Bolin *et al.* reviewed these attempts and put forward a method using an optical fiber with the cladding replaced by tissues. However, this method is still rather complicated, especially the calibration in a sucrose solution[6].

A new method based on total internal reflection is provided in this letter. The method comes from the well-known classical laws of reflection and refraction. Consider a monochromatic plane wave incident on the interface separating two different media. If the incident medium is denser ($n_i > n_t$), as the incident angle θ_i becomes larger the transmitted ray gradually approaches tangency with the boundary. When $\theta_i \geq \theta_c$ (θ_c is the critical angle), all the incoming light is reflected back into the incident medium in the process known as total internal reflection. The experimental device is shown in Fig. 3.

The key to the device is the semi-cylindrical lens. The lens is placed on the platform of a spectrometer that has 1′ scale interval. The center axis of the lens is made collinear with the axis of rotation of the spectrometer. The angle of incidence is read from a graduated disk on the spectrometer. A He-Ne laser producing TEM_{00} beam at 632.8 nm was used. A beam compressor was used to reduce the diameter of the light beam to 0.1 mm. The plano-concave cylindrical lens is to compensate for the aberration effects of cylindrical lens and keep the beam narrow. The photodetector is rotated to follow the reflected light beam for measuring.

Our previous study has proved that this method is practicable[7]. Recently the refractive indices of the palm's skin from people of different age, sex and skin color *in vivo* were measured. The measured targets of skin were located at side palm next to little finger. The glass index of semi-cylindrical lens used in the experimental device is 1.610 at 633 nm. The experimental results are included in Table 2.

The above results indicate that the refractive index of human skin is almost independent of age, sex and color of the skin. In addition, this method is much simpler than Bolin's approach and does not require the complicated calibration step, and the scattering effects from biological tissues can be also avoided. The measurement area is so small that it is possible to determine the distribution of the refractive index of biological tissues.

The scattering characteristics of human tissues are caused by microscopic fluctuations in the refractive index, and play an important role in the determination of the basic optical properties, which result in the variation of optical penetration depth of human tissues. Therefore, one of the further studies will focus on the relationship between optical penetration depth and refractive index of human tissues in the near future.

Table 2. Refractive Index of Human Skin *in vivo*

Man Kind	θ (deg.)	N_T
Man, 55 Years Old (Chinese)	72.3	1.533
Man, 26 Years Old (Indian)	72.5	1.535
Woman, 33 Years Old (Oriental)	72.2	1.532

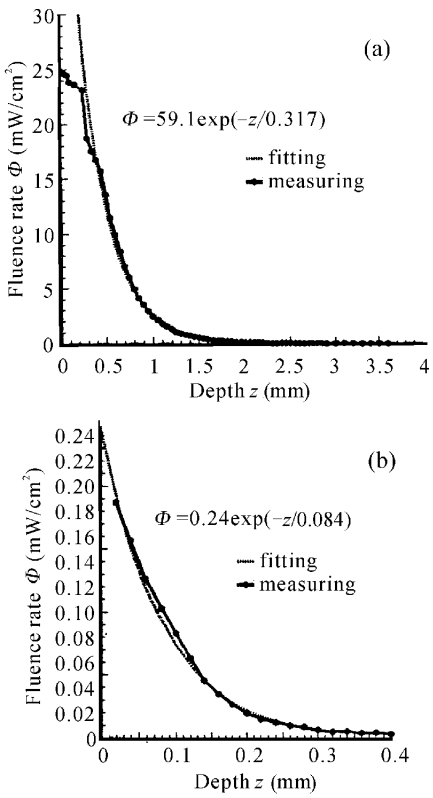

Fig. 2. The fluence rate distribution of Kr^+ laser in human lung tissue *in vitro* with ϕ_0 =17 mW/cm^2. (a) Adeno-squamous carcinoma tissue. (b) Normal tissue.

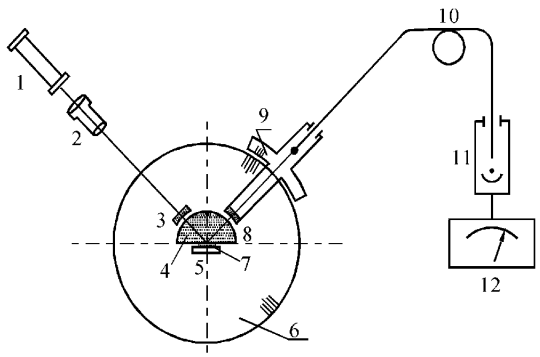

Fig. 3. Experimental setup for determining index of refraction of tissue. 1: He-Ne laser; 2: reverse expander; 3, 8: plano-concave lens; 4: semi-cylindrical lens; 5. sample holder; 6: graduated disk of spectrometer; 7: sample; 9: vernier; 10: fiberoptic probe; 11: photon detector; 12: readout and display.

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