Estimation of anisotropy coefficient and total attenuation of swine liver at 850 nm based on a goniometric technique: influence of sample thickness.

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Abstract— **Estimation of optical properties of biologic tissue is crucial for theoretical modeling of laser treatments in medicine. Tissue highly absorbs and scatters the light between 650 nm and 1300 nm, where the laser provides therapeutic effects. Among other properties, the characteristic of biological tissues to scatter the light traveling trough, is described by the anisotropy coefficient (g). The relationship between g and the distribution of the scattered light at different angles is described by Henyey-Greenstein phase function. The measurement of angular distribution of scattered light is performed by the goniometric technique.**

This paper describes the estimation of g and attenuation coefficient, μ_t, of swine liver at 850 nm, performed by an *ad hoc* designed goniometric-based system, where a spectrometer measures intensities of scattered light at fixed angles (0°, 30°, 45°, 60, 120°, 135° and 150°). Both one-term and two-term Henyey-Greenstein phase function have been employed to estimate anisotropy coefficient for forward (g_{fs}) and backward scattering (g_{bs}).

Measurements are performed on samples of two thicknesses (60 µm and 30 µm) to investigate the influence of this factor on g, and repeated 6 times for each thickness. The estimated values of g_{fs} were 0.947 and 0.951 for thickness of 60 µm and 30 µm, respectively; the estimations of g_{fs} were -0.498 and -0.270 for thickness of 60 µm and 30 µm, respectively. Moreover, μ_t of liver has been estimated (i.e., 90±20 cm^{-1}), through Lambert-Beer equation.

The comparison of our results with data reported in literature encourages the use of the *ad hoc* designed tool for performing experiments on other tissue, and at other wavelengths.

I. Introduction

Laser light has therapeutic effects on biological tissue, due to the conversion of its energy into heat. The energy of photons interacting with tissue depends on the wavelength (cfr. Plank Equation), and its conversion into heat is related to the capability of the tissue to absorb the light [1]. The interaction between tissue and laser is mostly described by three parameters, known as tissue optical properties: absorption, scattering and anisotropy coefficient [2]. In particular, the biological media are characterized by a strong scattering behavior, especially within the "therapeutic window", i.e., for wavelengths ranging from 650 nm to 1300 nm. Furthermore, the phenomenon of scattering depends on the anisotropy of the tissue [3]. The estimation of these properties is pivotal for the development of theoretical model describing the laser-tissue interaction; the model can be employed in medical field to predict effect of laser light on tissues, for several therapeutic application, such as laser ablation, among others [4-7].

In the last decades many approaches have been investigated, in order to experimentally estimate optical properties [2, 8, 9]. Due to the complexity of biological media, direct techniques for the determination of these properties are valid only under particular conditions. More precisely, according to the theory of laser-tissue interaction, the absorption coefficient can be estimated through the Lambert-Beer equation only if the tissue sample is "optically thin", and multiple light scattering is avoided. However, the experimental estimation of absorption coefficient is challenging, because of the strong contribution of the scattering coefficient, which is usually two or three orders of magnitude higher than the absorption coefficient. For example, Ritz *et al.* found, for native porcine liver, absorption coefficient of 0.7 cm^{-1}, and scattering one of 54 cm^{-1} at 850 nm [10]. Therefore the usual approach in the estimation of tissue optical properties requires employing indirect techniques, based on an iterative algorithm, which allows estimating optical coefficients from the measurements of the light transmitted and reflected by a tissue sample [11, 12].

Despite the complexity to implement these algorithms (e.g., inverse Monte Carlo), the anisotropy coefficient can be experimentally estimated from the intensity measurement of light scattered by tissue, thanks to a simple model (i.e., Henyey-Greenstein phase function) [13, 14].

This paper presents the estimation of anisotropy coefficient of ex vivo swine liver at 850 nm, with an *ad hoc* designed device, used to measure laser light scattered by liver at angles between 0° and 150° [15]. Furthermore, the measurements have been originally performed on samples with thickness (*d*) of 30 µm and 60 µm, and two anisotropy coefficients have been estimated: one to describe the forward scattering, the other one for the backward scattering. The set up allows also the estimation of total attenuation, and, consequently, of scattering coefficient of liver.

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II. Theoretical Background

The concept of anisotropy is strictly related to the scattering phenomenon, i.e. when traveling through a biological tissue, the photons change their propagation direction, because of the presence of particles with different dimensions. Since for each tissue a probability function p(θ) - also known as phase function- is defined, the anisotropy coefficient g is expressed, in polar coordinates, as:

$$g = \frac{\int_{4\pi} p(\theta)\cdot\cos(\theta)d\omega}{\int_{4\pi} p(\theta)d\omega} \quad (1)$$

where dω is the elementary solid angle, and θ is the angle between the axis of incident light, and the new direction after that scattering occurred. When g=0, the scattering is isotropic, while g=-1 means backward scattering and g=1 represents forward scattering. In biological tissues, the forward scattering is characterized by 0.5<g<0.99, within the therapeutic window [1-3].

The theoretical phase function p(θ), which can opportunely describe the anisotropy nature of tissue, is the Henyey-Greenstein function, implemented by the same authors in 1941 and commonly used in the field of astrophysics:

$$p(\theta) = b\cdot\frac{1-g^2}{\left(1+g^2-2g\cos(\theta)\right)^{3/2}} \quad (2)$$

where *b* is a normalization coefficient.

Equation (2) is an empirical model used to fit scattering behavior of media, and it is known as one-term Henyey-Greenstein (OTHG) phase function. As discussed by many authors [2, 3], OTHG is suitable for the fitting of phase function of biological tissues for angles smaller than 60°. To perform a more complete description, a two-term Henyey-Greenstein (TTHG) can be employed [16]:

$$p(\theta) = a\frac{1-g_{fs}^2}{\left(1+g_{fs}^2-2g_{fs}\cos(\theta)\right)^{3/2}} + c\frac{1-g_{bs}^2}{\left(1+g_{bs}^2-2g_{bs}\cos(\theta)\right)^{3/2}} \quad (3)$$

where *a* and *c* represent the probability of a photon to be scattered forward and backward by the tissue, g_{fs} is the anisotropy coefficient describing the phenomenon of forward scattering, and g_{bs} represents the backward scattering. The TTHG phase function was demonstrated to accurately describe anisotropy in biological tissues [13, 14].

III. Samples Preparation

Liver organ was collected and stored in freezer at -20 °C four hours after the swine was sacrificed. After 22 hours of storage, liver slices were cut by a traditional microtome, which is used for pathologic sample preparation. Six porcine liver samples have been prepared: three of them with *d*=60 μm, and three of them with *d*=30 μm. Liver samples were arranged between two laboratory glass slides, as shown in Fig. 1.A.

IV. Experimental Set Up

Experimental set up adopted is shown in Fig. 1. Swine liver samples (Fig. 1.A) were placed inside the goniometric holder shown in Fig. 1.B which has been *ad hoc* designed in order to accurately change the angular distance between the position of the fiber guiding the source LED light, and the fiber collecting the light transmitted by the sample. The emitting fiber has a fixed position, whereas the collecting fiber can be placed at different angles (0°, 30°, 45°, 60°, 120°, 135° and 150°) with respect to the axis of the emitting fiber.

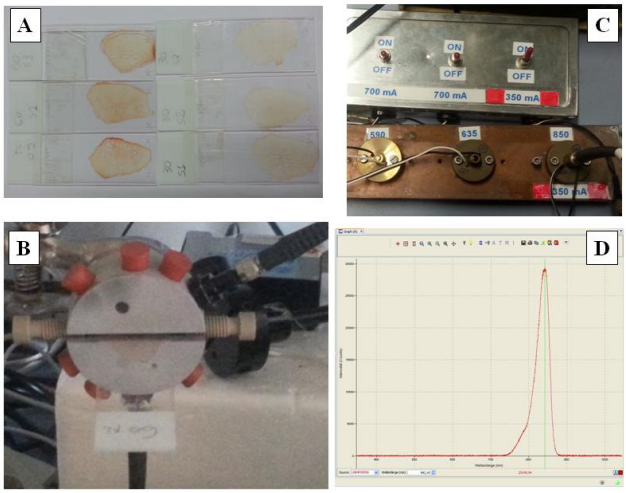

Figure 1. Experimental set up including: A) Swine liver samples: three with thickness of 60 μm and three with thickness of 30 μm, B) ad hoc designed holder for the placement of the sample and the measurement of light intensity at angles of 0°, 30°, 45°, 60°, 120°, 135° and 150° with respect to the axis of the source light, C) LED source at 850 nm. The spectrum of the transmitted light is shown in D.

The collecting fiber guides the light to a portable spectrometer (Ocean Optics USB4000), and the spectrum is visualized on a computer, through a dedicated software (Ocean Optics SpectraSuite). The wavelength of the light source was 850 nm (Fig. 1.C). At each angular position the spectrum of the light was collected (Fig. 1.D), and the peak value of the spectrum was recorded for the estimation of g.

The measurements of scattered light at 0°, 30°, 45°, 60° (defined transmittance angles) account for the forward scattering of the tissue, whereas the measurements performed at 120°, 135° and 150° (defined reflectance angles) refer to the backward scattering, as shown in the schematic in Fig. 2, where the ideal distribution pattern of the transmitted and reflected light is also presented.

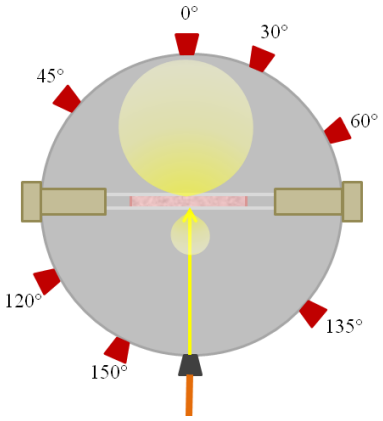

Figure 2. Schematic of holder, angular placement of collecting fiber and ideal distribution pattern of forward and backward scattered light.

Six liver samples were irradiated, each of them in two different positions of their surface: the probe was fixed between two screws in the holder, and the position of collecting fiber was changed at different angles.

V. Results

Light intensities, measured at the 7 angles, were normalized considering the measurement of the light transmitted by the glass slices in the absence of tissue. For each sample, two measurements were performed, and results were expressed as mean value ± standard deviation (Fig. 3).

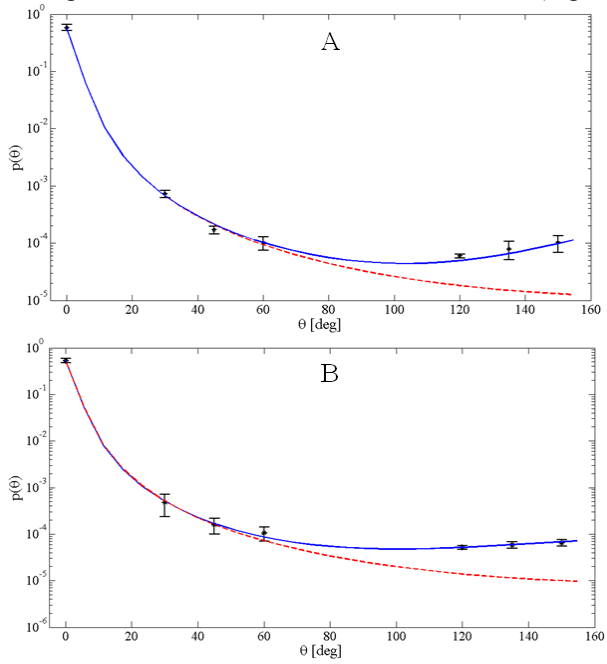

Figure 3. Phase function p(θ) between 0° and 150° for swine liver samples with thickness of A) 60 µm and B) 30 µm. In both A) and B) experimental data and their standard deviation (black asterisks), as well as the best fitting line according to (2) -red dashed line- and (3) -blue continuous line- are presented.

Fitting of experimental light scattering data in the framework of a Henyey-Greenstein model phase function was implemented in MatLab® environment. Both models of OTHG (2) and TTHG (3) phase functions have been used to fit the experimental data. The fitting parameters are listed in Table I:

TABLE I. Fitting parameters of (2) and (3) used to fit experimental data in Fig. 3.A and Fig. 3.B

	d=60 µm		*d=30 µm*	
Curve	**OTHG**	**TTHG**	**OTHG**	**TTHG**
a	-	0.00084	-	0.00067
b	0.00084	-	0.00067	-
c	-	0.00003	-	0.00003
g_{fs}	0.947	0.947	0.951	0.951
g_{bs}	-	-0.498	-0.270	-0.270
R^2	-	0.999	-	0.999

The OTHG phase function (2) used to fit the normalized light intensity values provides a value of g_{fs}=0.947 (red dashed curve in Fig. 3.A) for the samples with *d*=60 µm, and of 0.951 (red dashed curve in Fig. 3.B), which is slightly higher for the samples with *d*=30 µm. These values were obtained from the best fitting curve used only for the transmission angles (0°, 30°, 45° and 60°). Indeed, as observable in Fig. 3.A and 3.B, both curves provide an adequate description of the forward scattering, but their prediction capability is very poor regarding angles higher than 60°.

Therefore, the data have been fitted also using the TTHG defined in (3), which provides a good description of the phenomena of forward and backward scattering (Table I). Considering the values of the coefficients a and c, it is possible to find the percentage contribution of the forward and the backward scattering, respectively. For liver samples with *d*=60 µm, the forward scattering contributes to 96% of the scattering (g_{fs}=0.947), and the remaining 4% is represented by the backward scattering (g_{bs}=-0.498). The same percentage values were found for samples with *d*=30 µm, but values of coefficients g_{fs} and g_{bs} are different: forward scattering contributes for the 96% of the scattering (g_{fs}=0.951), and the remaining 4% is represented by the backward scattering (g_{bs}=-0.270).

It is relevant to discuss the influence of thickness in value of g. Whereas g_{fs} values are very close (0.947 *vs* 0.951, for *d*=60 µm and 30 µm, respectively), g_{bs} is significantly influenced by thickness. This finding can be explained considering that photons, during their traveling through a thin tissue, meet few tissue layers with respect to the phenomenon in thick tissue. Although the probability of scattering is the same (4% in both analyzed situations), photons undergo a weaker change of trajectory.

Another important finding from this experiment is the estimation of total attenuation coefficient (μ_t [cm^{-1}]) of liver tissue. The normalized intensity measured at 0° was considered as the collimated transmission (T_c) of the sample. The Lambert-Beer law (4) describes the relationship between the thickness of a medium and the attenuation of light in the tissue:

$$T_c = e^{-\mu_t \cdot d} \quad (4)$$

where $\mu_t=\mu_s+\mu_a$, μ_a is the absorption coefficient and μ_s is the scattering coefficient of the tissue. A value of $\mu_t=90\pm20$ cm^{-1} was estimated. Since it is known that μ_a of a biological tissue in NIR spectrum is about two orders of magnitude smaller than the value of μ_s, in first approximation it can be considered that $\mu_s \approx \mu_t$.

VI. Discussion

The results here presented are reasonable and comparable with theory and experiments already reported in literature.

As far as it concerns anisotropy coefficient, Marchesini *et al.* [14] found for liver tissue at 633 nm 86% of forward scattering (g_{fs}=0.85) and 14% of backward scattering (g_{bs}=-0.34), and, for lungs, 95% of forward scattering (g_{fs}=0.82) and 5% of backward scattering (g_{bs}=-0.54). Considering the results about scattering coefficient, Ritz *et al.* [10] proposed a μ_s value of about 60 cm^{-1} for porcine liver at 850 nm.

As deeply discussed before [1-3], in the field of optical properties measurement, it is quite challenging to establish comparisons with previously published data, even about the same tissue, because of many conditions, such as the intrinsic variability of biology, the inhomogeneity of tissue (as observable in Fig. 1) and the sample preparation. For example, Ritz *et al.* [10] homogenized the tissue with a pre-cooled mortar. Although Peters *et al.* [17] demonstrated, for breast tissue, that the homogenization procedure causes a maximum deviation for the 3.4% of reduced scattering and for the 5.9% of absorption, any manipulation of tissue lead to a change of its properties. Therefore the results obtained by our method are considered acceptable.

VII. Conclusion

This paper presents an *ad hoc* designed tool combined with mathematical theory to estimate the anisotropy coefficient of ex vivo swine liver at 850 nm. A further preliminary estimation of the scattering coefficient is also provided. The two-term Henyey-Greenstein phase function has been used to fit the angular distribution of the scattered light, providing anisotropy values for the forward and backward scattering.

The agreement of our results with data provided by literature encourage the employment of the tool to perform the experiments on other tissue, and at other wavelength. The information provided by such data are precious in medical field. Indeed, the physical and mathematical characterization of laser-tissue interaction is required to develop models, used to predict effects of laser light on tissues [4-6, 18]. In particular, the value of g and μ_s are needed to calculate the effective absorption of laser light by the tissue undergoing laser ablation. These models are employed in hyperthermia treatment planning (HTP) tools, which have already demonstrated their usefulness to improve the procedures outcomes. The knowledge of optical properties could improve the accuracy of HTP tools in tissue temperature prediction, in particular when coupled with thermometric techniques providing a temperature feedback [19, 20].

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