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In vitro optical characterization and discrimination of female breast tissue during near infrared femtosecond laser pulses propagation

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Abstract. Ultrashort infrared laser pulses were transmitted through excised female breast tissue. The resulted signal was recorded by a streak camera with a time resolution of the order of a few ps. Experimental data of the temporal spread of the ultrashort pulse during the transmission through the tissue have been analyzed using the Patterson analytical expression derived from the diffusion theory. This resulted in the calculation of the absorption and reduced scattering coefficients, which are related to the optical characteristics of each type of tissue. The goal of the study was to use the theoretical values of the coefficients to discriminate different kinds of tissue. [DOI: 10.1117/1.1412223]

Keywords: ultrashort breast transillumination; diffusion theory; optical characterization; tissue discrimination.

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1 Introduction

Optical tomography has been a subject of extensive study by many research groups. Over the last years the goal has been the replacement of ionizing radiation, such as x rays, with visible or near infrared (NIR) light. This breakthrough will provide a noninvasive imaging technique using nonionizing radiation in contrast with the commonly used x rays. The large penetration depth of NIR photons owed to the scattering mechanism taking place inside the tissue is the most important reason for using this type of light for imaging purposes. Early arriving photons of a transmitted ultrashort laser pulse can carry the image information while late-arriving photons, due to their extensive scattering, will bear little information.[1–4] Various time-resolved techniques have been used in order to detect objects hidden in such turbid media. Enhancement of the early part of the propagating pulse via nonlinear techniques or time gating imaging has been used in order to discriminate between these photons.

On the other hand, the temporal spreading of a short light pulse as it propagates through the scattering medium may also provide further information about the optical coefficients of the medium,[5] taking advantage of the extensive scattering. In previous published works measurements have been made both *in vitro* and *in vivo* in human volunteers, both in the temporal and spectral domains.[6–12] The results have been based on the known topography of the sample under investigation, which supported the extrapolation of the properties of the different kinds of tissue making up the sample. In the literature, measurements of optical coefficients have been made using the entire female breast. The fact that lipid tissue scatters more effectively than the potentially pathogenic fibrous has been extrapolated from the geometry of the measurements, knowing the histology of the female breast. However, this is not a conclusion one can rely on since no distinct measurements of specific types of tissue have been made.

This is the subject of this work where different kinds of tissue are being optically characterized using the transmitted part of the diffused photons. Since this part of the propagating pulse was more affected by the extensive scattering, the photon path inside the tissue was longer. Therefore, the provided information is statistically better than that corresponding to early arriving photons. The diffused part of the temporal distribution was described very accurately by the analytical expression used for the calculation derived from diffusion

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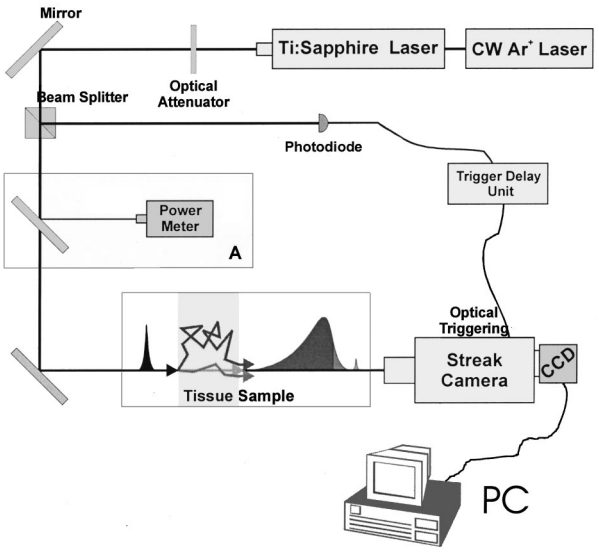

Fig. 1 Experimental setup of transillumination system.

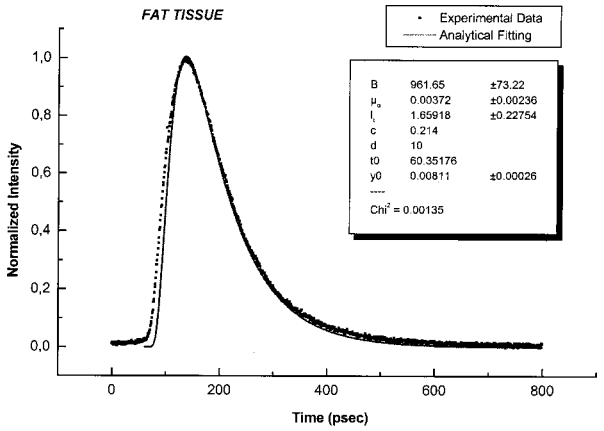

Fig. 2 Characteristic temporal profile of fat tissue obtained from the three-dimensional (3D) streak image. The dashed curve corresponds to the experimental data, whereas the solid curve represents the analytical fitting.

theory. The variability of the calculated optical parameters of one type of tissue among different tissue samples was very small.

The tissue samples were obtained from female breast during tumor extraction or biopsy operations. The study concerned tissue from 20 cases in total. Twelve of them were macroscopically characterized as fat deposited and eight of them as fibrous. All tissues were obtained 1/2 h after excision and were returned after the measurement to the Department of Gynecology of the University Hospital for histologic analysis. The thickness of the samples varied from 5 to 30 mm and they were placed on an $X-Y$ translation stage in order to take measurement from more than one point on each tissue. For biopsy samples with thickness less than 5 mm, where the diffusion theory fails to describe the temporal spread, the calculation of the optical properties was based on the Laguerre expansion of the kernels method reported elsewhere.[13] The distance of the backside of the tissue to the front face of the detector was kept constant at 1 cm, thus keeping the observation angle constant.

2. Experiment

A schematic representation of the setup used for the transmission measurements is shown in Figure 1. The source of the femtosecond (fs) pulses is a mode-locked Ti:sapphire laser emitting at 800 nm, pumped by a Spectra Physics Ar^+ laser operating with a power of 9 W. The repetition rate of the Ti:sapphire was 82 MHz with an average power of $\sim$1 W. A Hamamatsu C 5680 Streak Camera with a temporal resolution of 2 ps was used as the detector. A charge coupled device (CCD) camera operating at $-50°$C for reduced dark noise was used for the recording of the signals. The resulting signal was fed to a PC for further processing. Two different signals were simultaneously recorded: The diffused pulse emerging from the sample and a small portion of the original pulse, which provided the time reference. This part of the pulse was coupled into an optical fiber and directed to the entrance slit of the camera. The position of the coupler was adjusted so that the two signals arrive at the same time without the sample. The width of the entrance slit of the streak camera was 28 mm and during the experiments it was opened 5–10 μm in order to achieve the maximum temporal resolution. A Hamamatsu $p-i-n$ photodiode provided the optical trigger to the streak camera using a very small part (few mW) of the original pulse. The diameter of the beam was about 3 mm while the samples were irradiated with a power of 100 mW.

Time profiles were obtained in order to extract the temporal information. Intensity versus time graphs could then be constructed. The optical parameters (absorption and reduced scattering coefficients) of the samples were calculated by fitting the diffusion curves with the Patterson analytical expression[14] (derived from the diffusion theory), modified to fit the data from the streak camera

$$
\begin{aligned}
T(d,t) = {} & (4\pi Dc)^{-1/2} t^{-3/2} e^{-\mu_a ct} \\
& \times \{(d-z_0)e^{-(d-z_0)^2/4Dct} - (d+z_0)e^{(d+z_0)^2/4Dct} \\
& + (3d-z_0)e^{(3d-z_0)^2/4Dct} \\
& - (3d+z_0)e^{-(3d+z_0)^2/4Dct}\},
\end{aligned}
$$

where

$$
t = t - t_o, \quad z = 1_t = [(1-g)\mu_s]^{-1} = 1/\mu_s' \quad \text{and}
$$

$$
D = [3(\mu_a + (1/z))]^{-1}.
$$

The fixed parameters of the equation were the thickness of the sample (d), the time corresponding to the position of the maximum of the reference pulse (t_o) and the speed of light (c). The value of the latter was calculated for water (0.214×10^9 m/s, with $n_{\text{water}} = 1.4$).[15] B and y_o, were parameters that had to do with specific characteristics of each curve and are important for the adjustment of the theoretical curve to the data. The output of the calculation was the absorption and the inverse reduced scattering coefficient (μ_a and $1/\mu_s'$, respectively). Since the scattering coefficient cannot be calculated

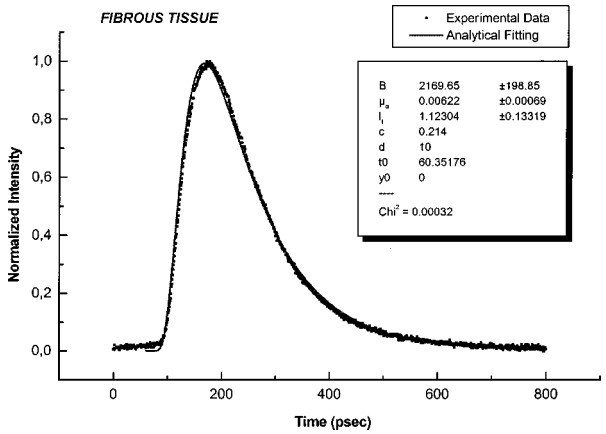

Fig. 3 Characteristic temporal profile of fibrous tissue obtained from the 3D streak image. The dashed curve corresponds to the experimental data, whereas the solid curve represents the analytical fitting.

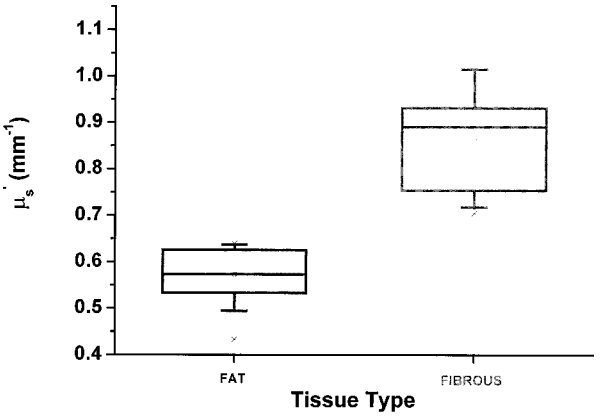

Fig. 5 Statistical plot constructed using the values calculated from the theoretical fitting. The horizontal lines from bottom to top represent the 25th, 50th, and 75th percentile, respectively. The 5th and the 95th percentile are used as errors bars. Finally, the 1st and 99th percentile are depicted as stars.

independently of the anisotropy factor g, the transport mean free path $(1/\mu_s')$ was used for the characterization of each type of tissue.

3 Discussion

Characteristic profiles for lipid and fibrous tissue are presented in Figures 2 and 3, respectively (scatter graph), where the results of the analytical fitting are also given in the inset as well as the fitted curves (solid line). To make the differences more pronounced the two curves are plotted in the same graph (Figure 4). The larger temporal spreading and the longer delay of the curve corresponding to fibrous tissue suggest that fibrous tissue has higher scattering properties compared to lipid tissue. This is in contradiction with already published results. However, as mentioned in the introduction part the investigation was made in the entire breast and not in separate tissue samples.

The difference of the mean values of the reduced scattering coefficient was used in order to discriminate the different types of tissue as described below. The absorption coefficient was not taken into account since it did not significantly affect the fitting of the theoretical curve and, furthermore, its values were at least two orders of magnitude smaller and had a large deviation for the same tissue. This conclusion is also supported by the fact that the absorption of tissue in the near infrared is associated with the presence of blood and when dealing with tissues *in vitro* the blood content varies between tissue samples. Moreover, the absorption is negligible because of the minimum of hemoglobin absorption around 700 nm. Therefore, it could not be connected to the characterization of the tissue. The values obtained were 0.0040 ± 0.0010 mm^{-1} for lipid tissue and 0.0045 ± 0.0021 mm^{-1} for fibrous tissue. These values are in good agreement with already published work even if slightly different wavelengths or techniques might have been used for the investigation of the tissue samples.[16,17] Even in the case of one sample of fibrocystic tissue the values obtained were in perfect agreement with the values previously reported by Peters et al.[17]

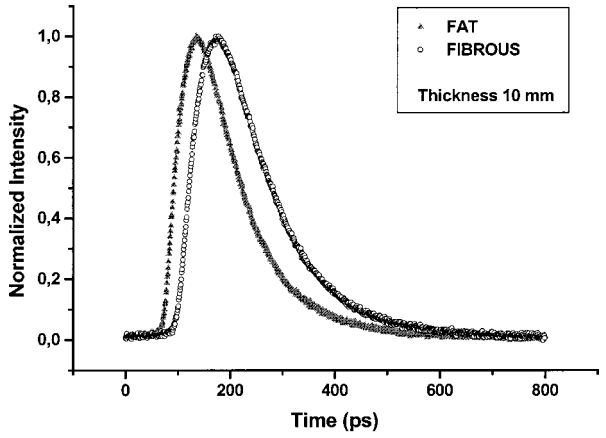

Fig. 4 Curves corresponding to fat (solid triangles) and fibrous tissue (open circles) with the same thickness and recorded under the same conditions (laser intensity, observation geometry and time scale).

The values obtained from the fittings were plotted in statistical graphs as the one shown in Figure 5, where the percentile distribution was plotted. In this graph the horizontal lines from bottom to top represent the 25th, 50th, and 75th percentile, respectively. This meant that, for example, the values above the 25th percentile were larger than the 25% of the range of the values. The error bars represented the 5th and the 95th percentile while the stars the 1st and 99th (meaning, in this case, the first and last value of the distribution), respectively. The square inside the box was the mean value of the distribution. It could be seen clearly from the plot that using as criterion the reduced scattering coefficient, the discrimination between lipid and fibrous tissue was possible since a different range of values corresponded to the two types of tissue studied here. For the lipid tissue the values lay in the interval [0.43–0.64] mm^{-1} and for the fibrous tissue in the interval [0.71–1.1] mm^{-1}. Values, which were between the two distributions, were assumed to correspond to tissue with different percentage of fat and fiber. This classification was based on the corresponding histologic analysis. The same method could also be used for the discrimination of the other components of the breast.

4 Conclusions

The result of the study was the discrimination between lipid, fibrous and benign tissue with the latter being a sensitive indicator of the presence of a histological deviation (precancerous lesion, benign or malignant tumor). This could lead to a noninvasive technique, which may eventually operate synergistically with biopsy, which requires the extraction of tissue sample from the patient. Optical topography, meaning calculation of the scattering coefficient in specific and very distinct positions of the tissue, could assist in achieving that goal.

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