Multispectral Tissue Characterization With Time-resolved Detection of Diffusely Scattered White-light

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Multispectral tissue characterization with time-resolved detection of diffusely scattered white light

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A novel technique for the noninvasive measurement of tissue optical properties simultaneously at all visible and near-infrared wavelengths is presented. The technique is based on the time-resolved detection of multicolor diffusely scattered light. Short pulses of white light are produced by using self-phase modulation by focusing the light from a short-pulsed high-power laser into a cuvette filled with water. After spectral dispersion in a polychromator and temporal dispersion in a streak tube, a two-dimensional CCD camera was used as a detector, with one dimension used for time resolution and the other one for wavelength separation.

The recent development of many new medical laser applications has made it increasingly interesting to understand light propagation in tissue. Tissue optical properties are of interest for dosimetry and for understanding the volume in interaction with light in various therapeutical laser applications, e.g., photodynamic therapy, laser photocoagulation, laser ablation, and laser surgery, but are also important for diagnostic purposes, e.g., laser Doppler flow studies,[1] tissue oxygenation studies,[2] and laser-induced fluorescence studies for tissue diagnostics.[3] The field of enhanced viewing in tissue transillumination for achieving optical mammography is currently also attracting much interest.[4] Both tissue oxygenation studies and the technique for optical mammography directly use tissue optical properties in the diagnosis and are based on their spectral variations.

The tissue properties of interest for light propagation are the absorption and scattering coefficients, μ_a and μ_s, and the scattering phase function. Direct measurements of these parameters can be performed with excised thin tissue samples.[5] Some optical properties can also be extracted from indirect measurements of homogeneous tissues. Such measurements can be performed either *in vivo* or *in vitro*. The optical properties are then derived by fitting light-transport model results to the experimental data.[6–8] However, no model is available to obtain the entire scattering phase function from such measurements; only the mean cosine, g, can be obtained. Furthermore, for conditions satisfying the diffusion approximation ($\mu_s \gg \mu_a$), the optical parameters are collapsed to μ_a and the reduced scattering coefficient, $\mu_s' = \mu_s(1 - g)$. For *in vitro* measurements of tissue optical properties a method based on measurements of the total diffusely reflected and transmitted light of a tissue sample has proved to give accurate results.[9,10] A method better suited for *in vivo* characterization of tissue optical properties is based on time-resolved diffusely scattered light following short pulse illumination.[6] Spatially resolved measurements[11] will give information similar to that from time-resolved measurements, and simpler instrumentation can be used for such recordings. This method also permits direct measurements at all wavelengths. However, a disadvantage with the spatially resolved method is that only a limited number of spatial separations can be easily measured. Also, although it is as accurate as the time-resolved technique, the method tends to be more sensitive to noise and thus less robust in realistic measurements.

In this Letter we describe a novel technique with the potential of multispectral determination of tissue optical properties based on the time-resolved technique. To produce white light in short pulses, self-phase modulation of an incident high-power laser pulse in water was used.[12,13] Self-phase modulation is a nonlinear optical effect that can occur when short optical pulses propagate through a dispersive nonlinear optical medium. The nonlinear property of the refractive index of the medium results in an instantaneous frequency shift of the light.[14] This shift is proportional to the time derivative of the light intensity and will thus vary during the pulse.

A schematic of the experimental setup is shown in Fig. 1. The tabletop terawatt laser system in Lund was used as a light source.[15] The oscillator of the system is an argon-ion-laser-pumped passively mode-locked Ti:sapphire laser, which gives approximately 100-fs-long pulses with a repetition rate of 76 MHz. The average power was 1 W in a Fourier-limited spectral profile peak centered around 792 nm. To permit simple amplification of these pulses, we have to reduce the peak power of the light to prevent damage to the optical components. This was performed in a pulse stretcher based on a pair of gratings, giving a shorter path length for shorter wavelengths than for longer wavelengths. Spectrally chirped pulses with a duration of 260 ps resulted. By stretching of the pulses, the peak power of the pulses was reduced from ~120 kW to 8 W. Ten pulses per second were amplified in two steps with Q-switched Nd:YAG-laser-pumped Ti:sapphire crystals. In this way the pulse energy was elevated from 2 nJ to a maximum of 450 mJ. A fast photodiode was placed to record scattered light at the first amplifier to produce an accurate trigger signal for the detection

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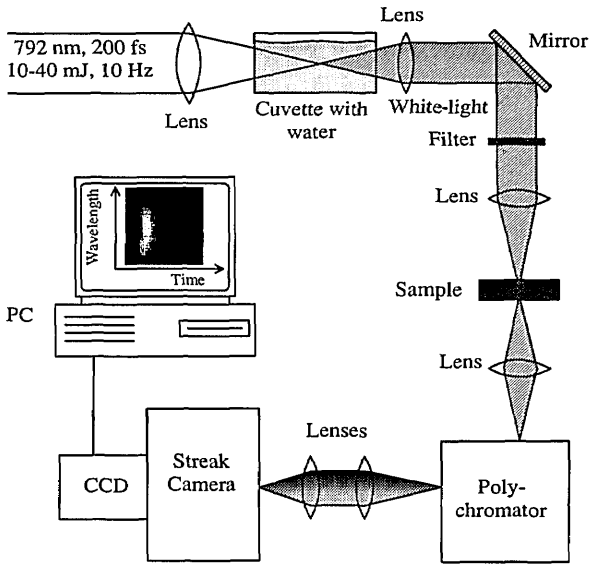

Fig. 1. Experimental setup for multispectral measurements of tissue optical properties.

electronics. Another pair of gratings compressed the pulses again to ~200 fs. In this way pulses at a wavelength of 792 nm with a peak power of 1.1 TW were generated with a 10-Hz repetition rate. In the recordings presented in this Letter we used a maximum pulse energy of 35 mJ.

When these pulses were focused with a 15-cm focal-length lens into a 30-mm-thick cuvette filled with water, self-phase modulation of the refractive index resulted in structureless light pulses in the entire visible and near-infrared range. The white-light pulses and the incoming laser light had similar pulse length and beam qualities. The white light was collected and focused onto the sample by two 50-mm achromatic camera lenses. A filter was placed in the white-light beam to filter out all wavelengths shorter than the ones under study, to minimize any influences of induced fluorescence light interfering with the measured signals. For the samples studied, recordings were made both with and without this filter, and no significant differences between the recordings could be seen. The light transmitted through the sample was detected in a modified confocal geometry, for which the x and y coordinates were identical for the source and detected light but with the z coordinate at the first and second surfaces of the sample. The transmitted light was collected with a 50-mm lens and was focused onto the entrance slit of a 27-cm polychromator. A 150-groove/mm grating gave wavelength-separated light in a window of ~210 nm over the 10 mm useful output at the exit plane of the polychromator. The spectral resolution of this light was 10 nm with the width of the entrance slit used in the recordings. We resized the image at the exit plane of the polychromator to fit the useful length of 3.5 mm of the streak camera (Hamamatsu C1587) entrance slit by using two achromatic camera lenses (135- and 50-mm focal lengths, respectively). The streak camera gave a 2-ps time resolution in single shots. However, we were able to obtain only ~50-ps time resolution in the accumulation mode, when the signals from a large number of pulses were accumulated. This was due to time jitter in the system, partly in the trigger signal from the laser and partly in the streak camera itself.

We used a two-dimensional CCD camera, thermoelectrically cooled to −30 °C, as a detector. It detected the various wavelengths separated with the polychromator along the y axis, while light with various delays from the source light pulse was obtained along the x axis of the camera. In this way we could, in principle, obtain time-dispersion curves of the transmitted light for all visible and near-infrared wavelengths in one single laser pulse. However, the electron current through the streak camera tube is limited, and thus the signals from a large number of laser pulses must be accumulated if useful signals with a high dynamic range are to be obtained.

Results from a porcine muscle *in vitro* are presented in Fig. 2. The light was focused on one side of the 100 mm × 100 mm × 14 mm sample, and the light was collected from the other side. The recording covered the wavelength region from 450 to 660 nm, and the laser pulse energy was 35 mJ. The gray-scale image shown is a result of an accumulation from 1000 laser pulses. The results from 10 sequential laser pulses were added in the camera before the information was read out. The results from 100 readouts were accumulated in the computer. The recording time was thus 1 min and 40 s. No correction for the spectral sensitivity of the camera was performed, since only the shapes of the time-dispersion curves are of interest in evaluation of the tissue optical properties. However, one can easily notice that below 470 nm and between 540 and 580 nm much less light is transmitted through the sample. This is due to the hemoglobin absorption.

In Fig. 3 the results from a measurement through a finger are shown. The light was focused on the nail of the index finger, and the light was collected from

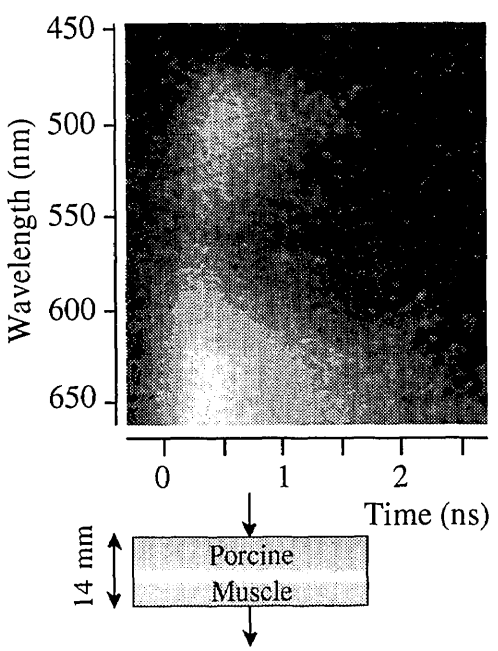

Fig. 2. Recording image through 14 mm of porcine muscle *in vitro*.

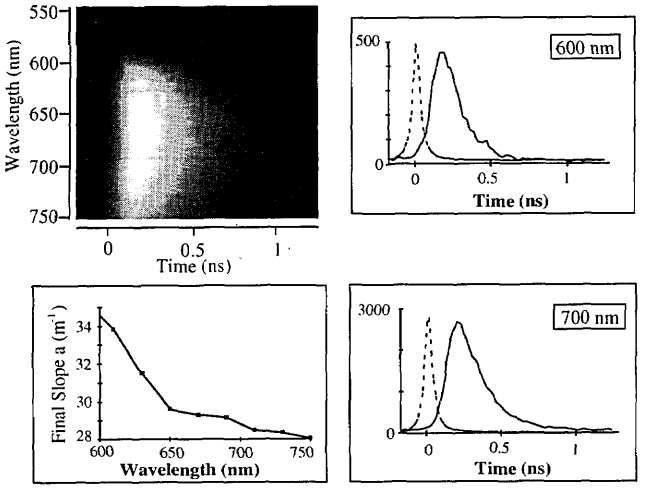

Fig. 3. Image obtained by recording diffuse light transmitted through a 12-mm-thick finger *in vivo*. The time-dispersion curves at two selected wavelengths are shown (solid curves) with the apparatus function at that wavelength (dashed curves). The final slopes of the curves obtained at different wavelengths are also shown (lower-left plot).

the other side of the finger, 12 mm from the source spot. The recording covered the wavelength region from 550 to 750 nm. The gray-scale image shown is a result of an accumulation from 4000 laser pulses. The time-dispersion curves at two wavelengths are shown separately, together with the laser pulse. No evaluation of the tissue optical properties has been made of this sample, owing to its inhomogeneous nature and the complex measurement geometry. However, the later part of the curves was found to follow an exponential decay, $I = I_0 \exp(-act)$, where a is a coefficient, c is the speed of light in the tissue, and t is the time. For a homogeneous medium in a slab geometry, the a coefficient would have been equal to μ_a. However, in this case a will also depend on the boundaries and any inhomogeneity. A plot of the a coefficient as a function of wavelength is shown at the lower left of Fig. 3. This curve shows that the final slope is higher at 600 nm than for the other wavelengths, in agreement with values from the hemoglobin absorption.

The potential of this method is that short pulses at all visible and near-infrared wavelengths can be produced. It can be used for indirect measurements of tissue scattering as well as absorption characteristics by time-resolved recordings of diffusely scattered light. The time-resolved technique was previously shown to give accurate results for optical properties. Difficulties in producing short pulses in a practical measurement application have, however, limited the use of this technique. Self-phase modulation in water of short intense laser pulses is one way to produce short pulses of all wavelengths of interest. As is seen from the recordings, it might be difficult to obtain a high enough dynamic range in the detection to evaluate all wavelengths from one single recording. In that case it is possible to filter the white light and measure several wavelength regions sequentially.

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