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Published in:
Proceedings of Photon Propagation In Tissues

DOI:
10.1117/12.228660

1995

Diffusely scattered femtosecond white light examination of breast tissue *in vitro* and *in vivo*

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ABSTRACT

Multispectral studies of light propagation in female breast tissue have been performed. Short pulses of white light were generated by using self-phase modulation of a high-power laser pulse focused into a cuvette filled with water. The white light pulses illuminated the tissue and the scattered light was recorded with time- and wavelength dispersion by a streak camera. Measurements were performed on breast mastectomies *in vitro* and measurements on healthy breast tissue *in vivo*. The reduced scattering coefficient and the absorption coefficient of breast tissue were obtained in different wavelength regions by fitting solutions of the diffusion equation to the experimental data. Significant variations in the magnitude of the optical properties could be seen between the different individuals. No characteristic spectral discrepance for tumour tissue was found.

Keywords: Tissue optics, white light, light diffusion, optical properties, optical mammography, tissue diagnostics, femtosecond pulses

1. INTRODUCTION

The rapidly increasing development of new medical laser applications has created a need to understand the propagation of light in living tissue. Tissue optical properties may be of interest for diagnostic purposes, e.g. laser Doppler flow studies, tissue oxygenation studies and laser-induced fluorescence studies for tissue diagnostics.[1-3] The knowledge of the optical properties are also important in light dosimetry and to understand the volume in interaction with light of various therapeutical laser applications, e.g. photodynamic therapy, laser photocoagulation, laser ablation and laser surgery.[4,5] The field of enhanced viewing in tissue transillumination for achieving optical mammography is currently also attracting much interest. Both tissue oxygenation studies and the technique for optical mammography are based on the measurement of spectral variations of the optical properties.

Optical mammography is a new and active field of research. There is a need to find additional approaches for detection of breast cancer, since the techniques used clinically today, mammography and ultrasonography, both have known limitations. One of the main drawbacks of ordinary mammography, is the use of ionising X-rays. The use of this radiation has a small but potential risk of being mutagenic. With this motivation, several new techniques to improve optical tissue transillumination imaging are under development.[6-8] The new modalities can be divided into two major groups: time- and frequency-domain methods. In time-domain methods, short light pulses illuminate the tissue and the transmitted photons are detected, using a time-resolved technique. By only using the very first arriving photons, an enhanced image of objects located deeply inside the tissue can be obtained. These photons have travelled the straightest and shortest path through the tissue and thus give a higher spatial resolution. The number of early photons depend on the optical properties of a volume close to a straight line through the medium, and is sensitive mainly to variations in the scattering properties.[9] Small objects with scattering properties other than those of the surrounding medium can thus be seen. The frequency-domain approach for tissue transillumination is based on irradiating the sample with intensity modulated light and detecting the demodulation of the intensity and the change of phase of the exiting light.[10,11] Also the frequency-domain method is based on the detection of photons with the straightest path, yielding an improved spatial resolution. The difference between the two methods is technological – how to best measure these photons.

The tissue properties of interest for light propagation are the absorption coefficient, μ_a, the scattering coefficient, μ_s, and the scattering phase function. To measure tissue optical properties *in vitro* several techniques can be used. A method based on measurements of the total diffusely reflected and transmitted light of a tissue sample using integrating spheres, has proved to give accurate results.[12-14] Some optical properties can also be extracted from indirect measurements of

homogeneous tissue, using either time resolved, frequency domain or spectrally resolved methods. These measurements can be performed both *in vivo* and *in vitro*.[7,15-19] The optical properties are then derived by fitting theoretical model curves to the experimental data. However, no model is available to determine the entire scattering phase function from such a measurement; only g, an anisotropy parameter equal to the average cosine of the scattering phase function, can be obtained. Furthermore, for conditions satisfying the diffusion approximation ($\mu_s >> \mu_a$), the optical parameters are reduced to μ_a and the reduced scattering coefficient, $\mu_s' = \mu_s (1 - g)$.

The wavelength region used for optical mammography is mainly the so called therapeutic window, between 600 nm and 1300 nm. In this region, the absorption of the tissue is low. The dominating interaction process between light and tissue is scattering, which reduces the contrast dramatically. To optimise the wavelength for optical mammography, spectroscopic studies of breast tissue have to be performed. Time-resolved spectroscopy can be accomplished by producing short pulses of white light using self-phase modulation. Self-phase modulation is a non-linear effect that occurs when short optical pulses propagate through a non-linear dispersive medium. Due to the non-linear part of the refractive index of the medium, the light pulses will experience an instantaneous frequency shift. This shift is proportional to the time derivative of the light intensity and will thus vary during the pulse.[20] If these white light pulses are detected with a time- and wavelength-resolved technique after propagating through tissue, the optical properties of the tissue can be determined in a broad spectral region in one measurement. Thus, it is possible to find spectral differences in different kinds of tissue, and to use this information to select useful wavelengths for optical mammography. Spectral differences of the optical properties between tumours and surrounding tissue could make it useful to use more than one wavelength to find tumours. Light at different wavelengths could also be used to certify the diagnosis.

In this paper, a technique with the potential of multispectral determination of tissue optical properties based on the time-resolved technique is described, using femtosecond white light pulses and time-resolved detection of multiply scattered light. This study is aiming at determining the optical properties of human breast tissue, both *in vivo* and *in vitro*, and thereby to find the optimal wavelengths for optical mammography.

2. EXPERIMENTAL SET-UP

In Fig. 1, a schematic of the experimental set-up is shown. As a light source, the table-top terawatt laser system in Lund was used.[21] 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 centred at 795 nm. To prevent damage to the optical components when these pulses were amplified, the peak power of the light must be reduced. This was done in a pulse stretcher based on a pair of gratings, giving a longer path length for longer wavelengths than for shorter wavelengths. In this way spectrally chirped pulses with a duration of 260 ps were obtained. As the pulses were stretched in time, the peak power was reduced from 120 kW to 8 W. An intracavity Pockels cell permitted ten pulses per second to enter the amplifier, consisting of two steps with Q-switched Nd:YAG-laser-pumped Ti:sapphire crystals. The amplifier increased the pulse energy from 2 nJ to a maximum of 450 mJ. Another pair of gratings compressed the pulses again to about 200 fs. Thus, pulses at a wavelength of 795 nm and with a peak power of 1.1 TW were generated with a 10-Hz repetition rate. In the recordings presented in this paper, a maximum pulse energy of 60 mJ was used.

The high power laser 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 wavelength region. The white-light pulses and the incoming laser light had similar pulse lengths and beam qualities. A 50-mm achromatic camera lens collimated the white light beam and a 15-cm focal-length lens focused it onto the end of an optical fibre. The other end of the fibre was mounted in a piece of black plastic (Delrin), with holes drilled every 5 mm. This fibre was used to illuminate the tissue. The average power of the white light out from the fibre was in the order of 1 mW. Another fibre, used for the detection of the diffusely scattered white light, was placed in one of the other holes. Thus the fibres were separated with a well-known distance that could be kept constant between the measurements.

The collected light was focused onto the entrance slit of a 27-cm polychromator (SPEX Model 270M). A 150-groove/mm grating gave wavelength-dispersed light in a window of about 250 nm over the 10 mm useful output at the exit plane of the polychromator. The image at the exit plane of the polychromator was resized to fit the useful length of 3.5 mm of the streak

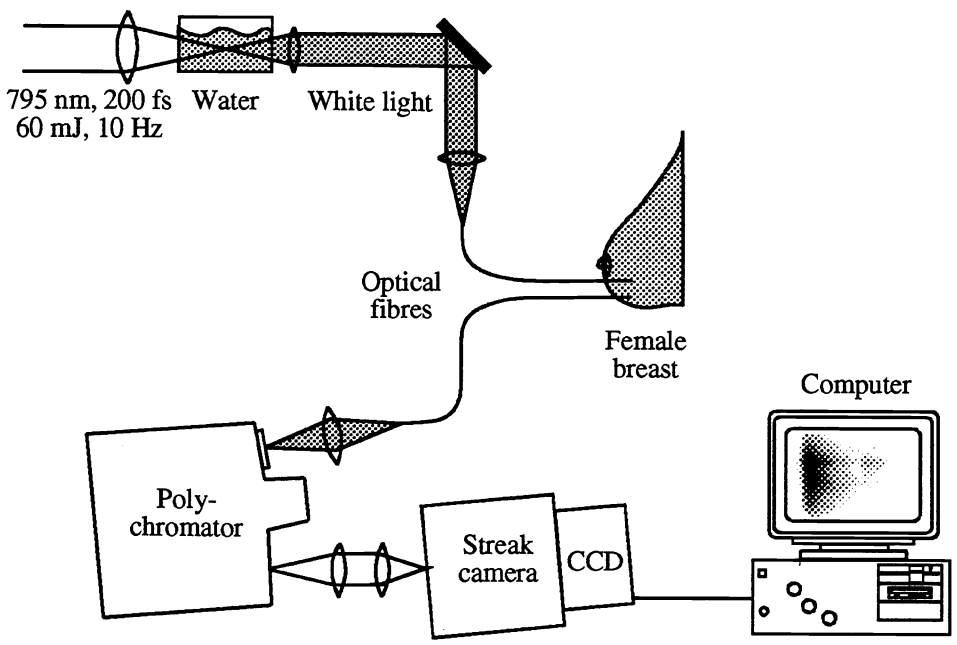

Fig. 1. Experimental set-up for multispectral measurements of breast tissue optical properties.

camera (Hamamatsu C1587) entrance slit by using two achromatic camera lenses (135- and 50-mm focal lengths, respectively). The streak-camera allowed time-resolved detection of the wavelength dispersed light. A two-dimensional CCD camera, thermoelectrically cooled to -30 °C, was used as a detector. It detected the various wavelengths (separated with the polychromator) along the y-axis, while light with various time delays from the source light pulse was obtained along the x-axis of the camera. In principle, it was thus possible to obtain time-dispersion curves of the diffusely scattered 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. The images from the CCD camera were read by and stored on a computer for further evaluation.

The spectral resolution in the measurements was 10 nm, while the streak camera gave a 2-ps resolution in single shots. In accumulation mode, when the signals from a large number of pulses were accumulated, the temporal resolution was much worse. This was mainly due to intensity variations and time jitter in the high power laser. To reduce the time jitter, the pulses from the Ti:sapphire laser were used as a trigger, since the oscillator signal is much more stable in time and intensity than the final high power pulse. A small fraction of the oscillator pulses was focused onto a photo diode and the resulting signal was amplified. In order to get triggering pulses with a repetition rate of 10 Hz, a high-speed switch was used. The switch was gated with a 10-ns pulse, which was triggered by the Pockels cell, allowing the oscillator pulses to enter the amplifier of the high-power laser. In this way only one pulse from the photo diode was allowed to pass through the switch for every high-power laser pulse. Thus a stable 10-Hz triggering signal was obtained, and the temporal resolution of the system working in accumulation mode was reduced to about 20 ps, best case.

3. MEASUREMENT PROCEDURE

3.1 *In vivo* measurements

In vivo measurements were performed on six volunteers, all between 25 and 30 years old. The piece of plastic with the two clear-cut optical fibres was attached to the breast, with the fibre ends in contact with the skin. In the *in vivo* measurements, the illuminating fibre was a fibre bundle with a diameter of 2.5 mm and the scattered light was collected with a 1-mm fibre.

In a typical measurement, the recordings covered the wavelength region from 600 nm to 850 nm. The laser pulse energy was about 60 mJ before the white light conversion, and the average power of white light illuminating the tissue was on the order of 1 mW. For every volunteer, two measurements were performed on each breast; one with an optical fibre separation of 10 mm and the other with a separation of 15 mm. The measurements were performed on corresponding locations on the two breasts. The results from a number of laser pulses, 100 for the smaller fibre separation and 200 for the larger, were added in the camera before the information was read out. This was repeated 30 times to create one image. The total accumulation time was thus 5 min or 10 min, depending on the distance between the fibres. This resulted in a good signal-to-noise ratio. Due to the relatively low intensity of the white light, it was not possible to have a fibre end separation between the source and the detector fibre of more than 15 mm.

A wavelength calibration of the system was obtained by putting different interference filters between the two fibres while recording apparatus functions. Gaussian peaks were fitted to the wavelength scale in the different images, and a linear interpolation between the transmission wavelength of the filters and the number of the centre channel in the fit resulted in an expression for the wavelength scale. From the recorded images, time dispersion curves were obtained in four different wavelength regions, each with a spectral width of 20 nm. These regions were centred on 660 nm, 710 nm, 760 nm and 810 nm. Below 650 nm the intensity falls of due to increased absorption in the tissue. Above 820 nm the detected signal decreases drastically, due to a decreasing quantum efficiency of the detector.

3.2 *In vitro* measurements

The *in vitro* measurements were performed on three breast mastectomies from three 56-year-old women. In two of these samples there was a ductal cancer and in the third there was a nectrotic area. Immediately following surgery, X-ray images were taken of the mastectomies. These images were used to identify the location of the anomalies. The samples were then brought to the high-power laser facility for measurements of tissue optical properties, using the above described technique. The measurements were performed within half an hour following mastectomy. The temperature of the sample had then decreased to room temperature. One of the *in vitro* measurements was performed in a transillumination geometry, with the illumination fibre above the sample and the detector fibre below. The two fibres were mounted on an xy-translator and could thus be moved without any change of the geometry. The two other samples were measured in the backscattering geometry with a 15-mm distance between the fibre ends.

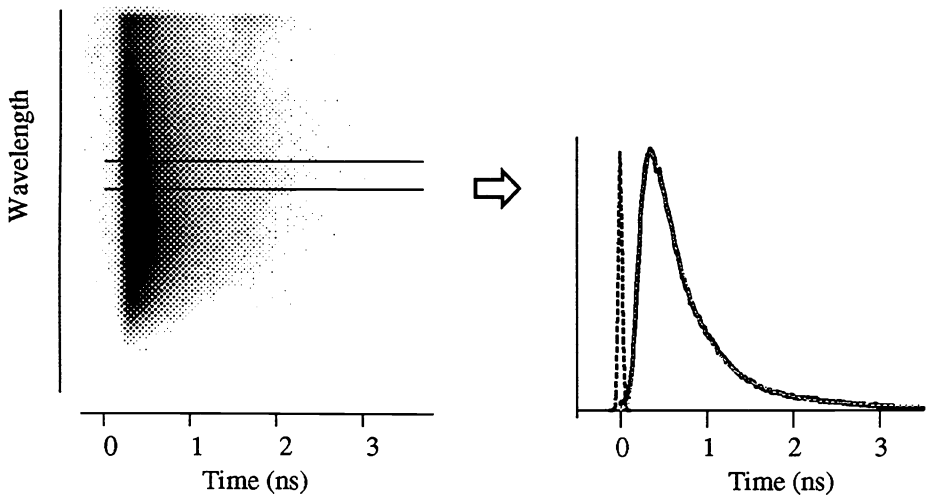

Fig. 2. Image obtained by recording diffuse backscattered light in female breast tissue *in vivo*. The distance between the fibres was 15 mm. The time dispersion curve obtained from the region marked with the two horizontal lines is shown to the right (solid curve). A fit of a theoretical curve (dotted line) and the apparatus function (dashed line) are included.

4. RESULTS

4.1 *In vivo* measurements

In the left hand part of Fig. 2 a recorded white light transmission image of female breast *in vivo* is shown. Two horizontal lines in the image mark the wavelength region used to obtain the time dispersion curve shown to the right. The curve was obtained by vertically summing the pixel values for all the wavelengths in the marked region for each time co-ordinate. Included in the graph is also the apparatus function obtained by having the ends of the transmitter and the receiver fibres facing each other with a neutral density filter in between. This shows the time resolution of the system. Values of μ_a and μ_s' were obtained by fitting solutions of the diffusion equation in a semi-infinite geometry, to the time dispersion curve, using a least squares fitting routine (see Fig. 2). These values represent an average of the optical properties in the probed tissue volume. As the distance between the fibres was increased, the main part of the detected light penetrated deeper through the tissue and could thus interact with tissue with other optical properties.

A significant variation in the optical properties from person to person could be seen. Similar measurements, with a fibre separation of 10 mm and 15 mm, were performed on both breasts of five volunteers. The results are shown in Fig. 3. In Figs. 3a and 3b the average values and the standard deviation of μ_s' in four different wavelength regions and with a fibre separation of 10 and 15 mm, respectively, are shown. The absorption coefficient is plotted in the same way in Figs. 3c and 3d. No significant change in optical properties could be found for the different wavelengths evaluated. There was, however, a reduction of both the scattering coefficient and the absorption coefficient as the separation between the transmitter and the receiver was increased. When the separation of the fibres is larger, the main part of the detected light has interacted with a volume deeper down in the tissue. Thus, variations of the optical properties as a function of the depth can be seen.

The correlation of the optical properties between the left and the right breast of the same volunteer can be seen in Fig. 4. Included in the data presented in Fig. 4, are also results from one volunteer when better white light conversion made it possible to use a separation between the fibre ends of up to 25 mm. The correlation of the scattering properties, which is shown to the left, is good, that is, the scattering properties are similar in the two breasts, even if the variation from person to person is large. This suggests that a method can be used to find tumours in one breast by using the other as an internal reference. The correlation in the absorption coefficient, shown to the left in Fig. 4, is weaker. This can be an artefact, since

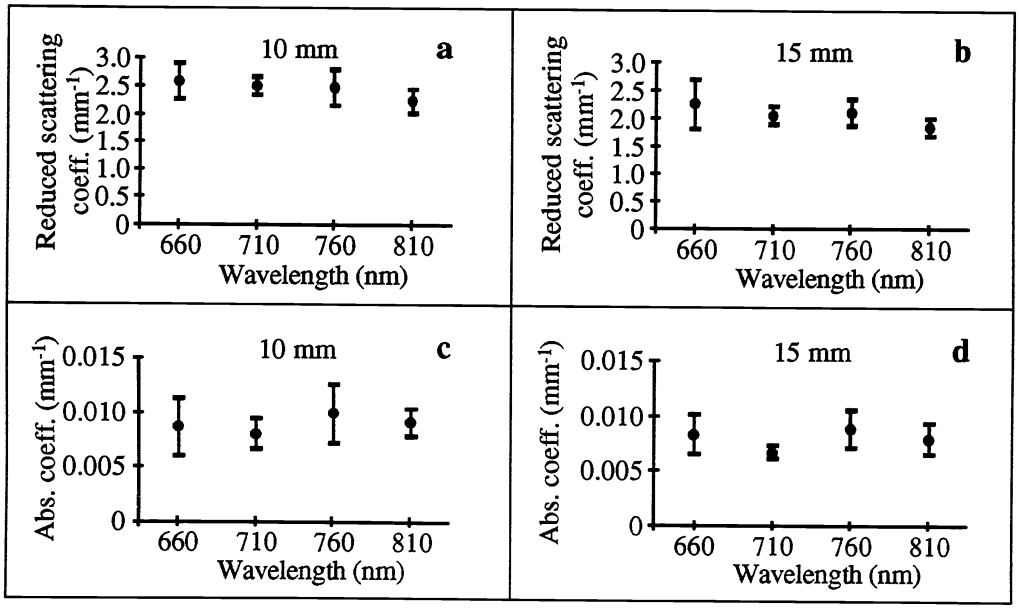

Fig. 3. The average values and standard deviation of the reduced scattering coefficient μ_s' (a and b) and the absorption coefficient μ_a (c and d) measured on female breast *in vivo* with 10 mm (a and c) and 15 mm (b and) between the fibres.

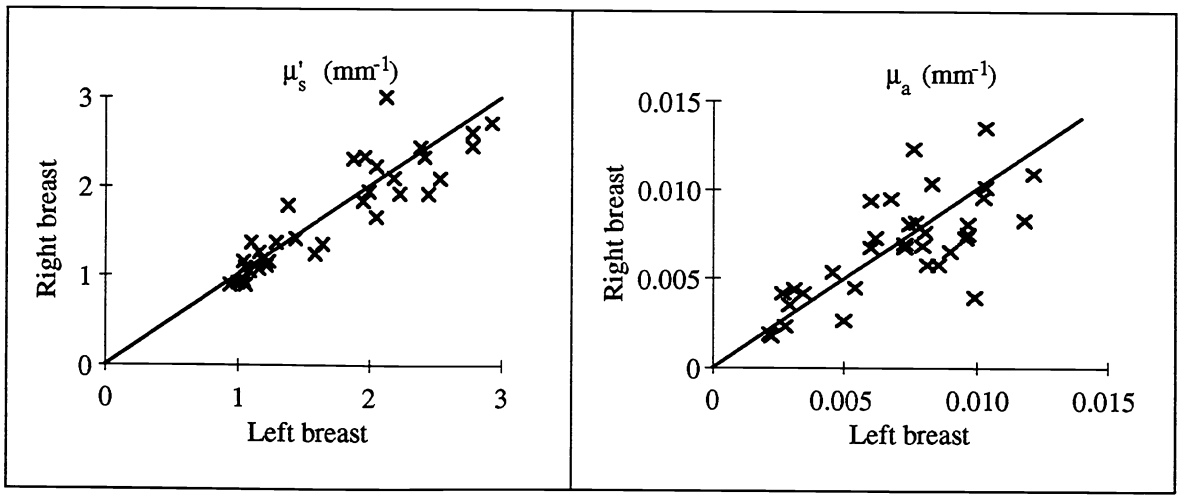

Fig. 4. The correlation between the two breasts of the examined volunteers. The correlation of the scattering properties is shown to the left and the absorption to the right. In the graphs, the optical properties for all wavelengths and different fibre separations are presented.

the evaluation routine used is not that sensitive for changes of the absorption coefficient. Fairly large errors of the absorption coefficient can be found in the evaluation procedure if a non-subtracted background remains as an offset of the signal. In the streak-camera images, a background often remained even after the subtraction of a dark measurement. This is a problem that needs further investigations.

4.2 *In vitro* measurements

One breast sample was compressed to a thickness of 19 mm between two plexy glass plates. Two measurements in the transillumination geometry were performed; one with the illumination fibre right over the location of the tumour and one 2 cm away from the tumour border in normal breast tissue. The tumour size was, according to the X-ray mammography image, 3 cm in diameter in the uncompressed state. As can be seen in the upper part of Fig. 5, an increase of μ_s' (62% - 67%) and μ_a (79% - 94%) could be seen when the tumour was between the fibres, compared to the surrounding tissue. The second breast studied was thicker, and almost no light could be detected in the transillumination geometry. Because of that, measurements were performed in a backscattering geometry, with a fibre separation of 15 mm. This tumour was 0.7 cm in diameter. The increase in μ_s' was not as pronounced as in the earlier experiment, only 11% to 17%, except for 760 nm, where there instead was a decrease of 7%. The rise of the absorption ranged from 40% to 84% for the different wavelengths (see middle part of Fig. 5). Measurements on the nectrotic area with a diameter of 1 cm in the third sample resulted in a reduction in the scattering coefficient with 1% to 32%, while the absorption increased with 67% to 129% (see lower part of Fig. 5).

5. DISCUSSION

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 and absorption properties by time-resolved recordings of diffusely scattered light.[22] One measurement covered about 250 nm with a spectral resolution of 10 nm. The time resolution was in these measurements approximately 20 ps. The time-resolved technique was previously shown to give accurate results for optical characteristics.[9,23,24] There are, however, some sources of errors when evaluating the time-dispersion curves by fitting solutions of the diffusion equation to the experimental data. When a small offset is superimposed on the curves, fairly large errors are obtained in the calculated absorption coefficients. Errors in the absorption coefficient will also occur when measurements are performed close to a border of the tissue sample. Due to photons leaving the tissue at the border, the absorption coefficient will be overestimated. Errors in the scattering coefficient will occur when the fit is shifted in time.

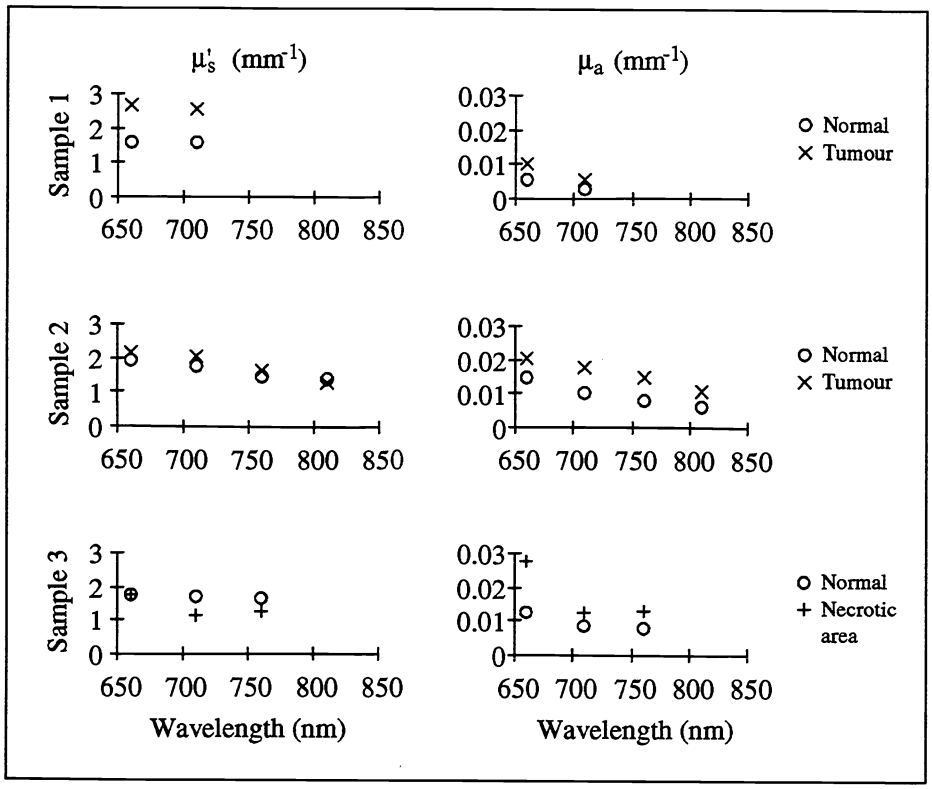

Fig. 5. The reduced scattering and absorption coefficients for normal breast tissue and tumours (sample 1 and 2) or a necrotic area (sample 3), obtained from measurements on three samples of female breast tissue *in vitro*.

This is a problem when there is an offset in the early part of the time dispersion curve, making it hard to exactly localise the leading edge in time.

From these first *in vivo* measurements, it seems that the scattering and the absorption do not vary very much in the wavelength range between 660 nm and 810 nm. However, significant variations could be seen from person to person. In our measurements, the reduced scattering coefficient ranged from 0.9 mm^{-1} to 3.0 mm^{-1} and the absorption coefficient from 0.002 mm^{-1} to 0.010 mm^{-1}, measured in the backscattering geometry with 10 and 15 mm between the fibre ends. Mitic *et al.* determined the reduced scattering coefficient ranging from $\mu_s' = 0.72$ mm^{-1} to $\mu_s' = 1.22$ mm^{-1} and the absorption coefficient ranging from $\mu_a = 0.0017$ mm^{-1} to $\mu_a = 0.0032$ mm^{-1}.[16] These measurements were performed on six volunteers in the transillumination geometry and at a wavelength of 800 nm. Since the geometries in the two different sets of measurements are different, it is hard to compare them with each other, but they are on the same order of magnitude. Suzuki *et al.* performed their measurements in the backscattering region and reported significant individual differences. Their results were also of the same order of magnitude.[15] Feng *et al.*[25] presented analytical expressions for the probing depth of the light in the backscattering geometry. Using these expressions, a maximum probing depth of the light in our experiments would be approximately 4 mm when the two fibre ends are separated with 10 mm, and approximately 5 mm when the separation is 15 mm. Thus, only a very superficial volume of the tissue, mostly consisting of fat, is probed. A transillumination geometry would then be preferable in order to localise tumours. We could also see a decrease in the scattering and absorption coefficients as the separation between the source and the detector increased, in agreement with the results presented by Suzuki *et al.*[15]

Even if the variations in optical properties from person to person is significant, there is a good correlation between the two breasts of the same person. Thus it might be possible to use one of the breasts as an internal reference, while looking for tumours in the other. Changes in the scattering and absorbing properties of tumours and necrotic area compared to the

surrounding tissue could be seen in breast tissue *in vitro*. The variations were most obvious in the sample measured in transillumination geometry. On a second sample, measured in the backscattering geometry, significant variations in the absorption coefficient, but not in the scattering coefficient, could be seen. This could be caused by two things. If the measurement on normal tissue was performed closer to a border, this would look like an increase of the absorption. It could also be that the absorption increases due to the vascularisation around the tumour. If the tumour is located deep down, the light might penetrate this area without entering the tumour volume, which should have a different scattering property.

6. ACKNOWLEDGEMENTS

The authors would like to thank Olof Jarlman at the Department of Diagnostic Radiology, Lund University Hospital, for the help with the tissue samples, and Anders Persson and Claes-Göran Wahlström for the help with the terawatt laser. The support of the Knut and Alice Wallenberg Foundation, the Swedish Council for Planning and Coordination of Research, and the Swedish Research Council for Engineering Sciences is gratefully acknowledged.

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