Measurements of optical properties of pig brain tissue in vitro using a novel compact device

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ABSTRACT

In numerous medical and scientific fields, knowledge of the optical properties of tissues can be applied. Among many different ways of determining the optical properties of turbid media; integrating sphere measurements are widely used. However, this technique is associated with bulky equipment, complicated measuring techniques, interference compensation techniques, and inconvenient sample handling. This paper describes measurements of the optical properties of porcine brain tissue using novel instrumentation for simultaneous absorption and scattering characterization of small turbid samples. The system used measures both angularly and spatially resolved transmission and reflection and is called Combined Angular and Spatially-resolved Head (CASH) sensor. The results compare very well with data obtained with an integrating sphere for well-defined samples. The instrument was shown to be accurate to within 12 % for μ_a, and 1 % for μ_s' in measurements of intralipid-ink samples. The corresponding variations of data were 17 % , and 2 %, respectively. The reduced scattering coefficient for porcine white matter was measured to be 100 cm^{-1}, while the value for coagulated brain tissue was 65 cm^{-1}. The corresponding absorption coefficients were 2 and 3 cm^{-1}, respectively.

Keywords list: Optical properties, Turbid media, Optical device

1. INTRODUCTION

Optical properties of tissue are important both for dosimetry planning of laser treatments, especially photodynamic therapy and laser-induced thermotherapy, as well as for various optical diagnostic measurements. Quantative physiological information about different turbid media, e.g. tissues can be obtained measuring the optical properties of the materials. The optical properties of a turbid medium, i.e. the absorption coefficient, μ_a, the scattering properties (reduced scattering coefficient, μ_s', or scattering coefficient, μ_s), and anisotropy factor, g, can thus provide important information on the composition and the dynamics of a medium. While μ_a provides information on the concentration of various chromophores, the scattering properties provide information on the form, size, and concentration of the scattering components in the medium [1,2]. Thus, accurate and fast determination of μ_a and the scattering properties of turbid media are useful and important in numerous fields of science and medicine, as well as in industry and environmental monitoring, etc. In this study we focused on biomedical applications, and measurements of the optical properties of brain tissue. In biomedicine the distribution of light in tissue depends on the optical interaction coefficients and the illumination geometry. Knowledge of these coefficients is therefore essential for optically based diagnostic and therapeutic procedures [3,4]. Optical measurements of brain tissue have recently attracted much interest, especially for brain activation studies [5-9], and for planning photodynamic therapy in the treatment of brain tumours. In this paper results are presented from optical measurements on normal and coagulated porcine brain tissue. Measurements of the optical properties of tissue can be performed *in vivo* or *in vitro*. Most *in vivo* measurements performed to date rely on frequency domain measurements [10], or time or spatially resolved measurements [11,12] utilizing an inverse algorithm to extract the optical properties. This algorithm is usually based on the diffusion approximation and the fact that the tissue is relatively homogeneous within a relatively large probe volume. These requirements are not always completely fulfilled and the techniques used in such cases are thus limited to providing relative rather than absolute values, such as spatial or

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temporal variations in the optical properties. Novel techniques to measure absorption and scattering spectra in a much smaller probe volume have been suggested[13-17]. A disadvantage of these techniques is that they cannot accurately measure small absorption coefficients. The most frequently used method of measuring the optical properties of small tissue volumes is the integrating sphere (IS) which is used to probe a thin slice of tissue. This can be used as either a three-parameter technique for extracting μ_a, μ_s, and g from measured total diffused reflectance (R_{tot}), total diffused transmittance (T_{tot}) and collimated transmittance (T_{col}), or as a two-parameter technique for measuring μ_a and μ_s' from the recorded values of R_{tot} and T_{tot}. The technique has been shown to provide relatively accurate results for tissue phantoms [18]. It can, however, be debated how the optical properties are affected by tissue excision and sample preparation. Also, the sample thickness most suitable to obtain an optimal signal-to-noise ratio in the evaluation differs for R_{tot}, T_{tot} and T_{col}. Therefore, a compromise must be made, and a thickness that is reasonably suitable for all measurements has to be used. This usually results in a poor signal-to-noise ratio in the measured value of T_{col}. In addition, bulky instrumentation and sample handling make the measurements complicated. To circumvent some of these problems, we have developed a novel system for measuring the optical properties of tissue presented by Dam et al.[19]. This system is constructed to enable real-time simultaneous determination of μ_a, μ_s, and g from thin turbid samples using a continuous wave (i.e. steady-state) light source. In this study we focused on measuring the absorption (μ_a) and reduced scattering (μ_s') coefficient, using both the new system and an integrating sphere as a reference system. To extract these coefficients in the new system, we measured the spatially resolved diffuse reflectance (R), and angularly resolved diffuse transmittance (α). Both the integrating sphere system and the new sensor were calibrated and optimized for the measurements using suspensions of intralipid and ink.

The aims of this work were to measure the optical properties of porcine brain tissue before and after coagulating with a monopolar radio frequency electrode and to evaluate whether our newly developed instrument could provide accurate results for real tissue measurements.

2. MATERIALS AND METHODS

2.1 CASH sensor measurements

The Combined Angular and Spatially-resolved Head (CASH) sensor is a compact device. It may prove to be a good alternative to the integrating sphere system for measurements of optical properties in certain situations. The device, designed and constructed in collaboration between Bang & Olufsen Medicom A/S, Denmark and the Department of Physics, Lund University is illustrated in Figure 1.

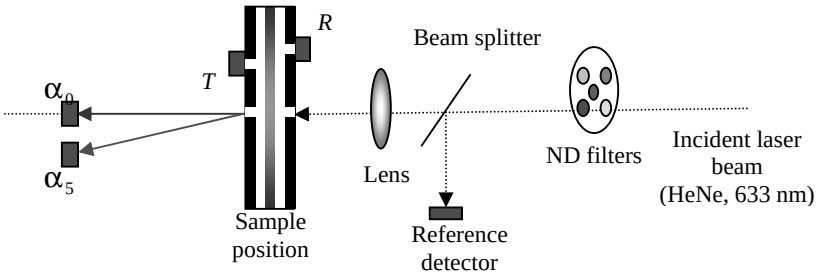


Figure 1: CASH sensor set-up. R, T, α_0 and α_5 denote light referred to as reflectance, transmittance, and angular transmittance at 0˚ and 5˚ to the detectors, respectively. The light is collected by optical fibres. Neutral density (ND) filters were used to increase the dynamic range of the measurements.

A 2 mW HeNe laser (633 nm) with a beam diameter of 1.0 mm was used as a light source. Part of the beam was split off to a reference detector to check that the output from the laser remained constant throughout the measurements. The rest of the light beam was aligned and sufficiently focused to pass through a ϕ=1.0 mm hole in a black metal sheet to irradiate the central part of the cuvette. The cuvette was mounted between two black metal sheets. In the centre of the cuvette, transmitted light could escape through a ϕ=1.0 mm hole. Two 400 μm core diameter optical fibres were placed at a distance of 75 mm from the cuvette surface to collect light escaping at two angles of 0^o and 5^o, the signals being denoted

α_0 and α_5, respectively. The flat polished ends of two other 400 µm fibres were mounted in holes next to the cuvette at radial distances of 2.5 mm (R) and 2.0 mm (T). Only the α_0 and R signals were used in the subsequent analysis, as we only aimed to extracting μ_a and μ_s'. The optical fibres were terminated on Si detectors controlled by a Labview© PC card. The detectors were used to read the light intensities from each fibre. All signals were recorded simultaneously using an exposure time of 10 ms.

2.2 Integrating sphere measurements

In Figure 2 the arrangement used for the IS method is illustrated [18,20]. A 75 W Xe lamp is coupled into one 600 µm fibre, providing light in a broad spectral region. The light is formed into a ~2 mm parallel light beam using a positive lens with a 10 mm focal length. By placing the sample at position A or B, the transmitted and the diffusely reflected light flux, I_T and I_R, respectively, can be acquired [21]. The light was spectrally dispersed in a spectrometer (SPEX Industries Inc. 270M) with a 150 grooves/mm grating. A liquid nitrogen cooled CCD camera (EG&G) connected to the spectrometer was used to capture the spectra. The data were subsequently transferred to a computer for storage and analysis.

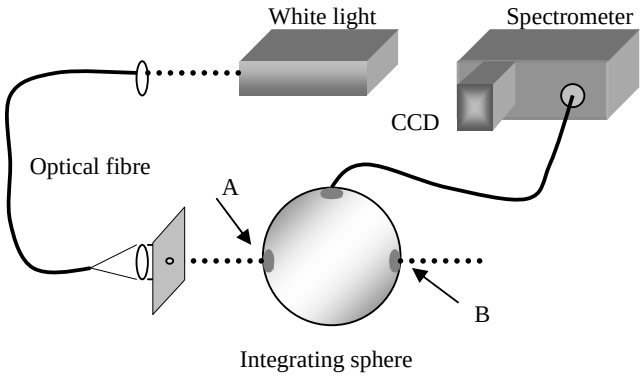


Figure 2: Integrating sphere set-up. A and B are the sample positions for measurements of T and R, respectively.

The total reflectance, R_{tot} and transmittance, T_{tot} were obtained from the measured data by applying the following equations,

$$R_{tot} = R_{BS} \cdot \frac{I_R}{I_{ref}} \quad ; \quad T_{tot} = \frac{I_T}{I_{ref}}$$

where I_R is the measured intensity of the reflectance flux with the sample at position B; I_T is the measured intensity of the transmittance with the sample at position A and a barium sulphate plug (standard sample) at position B; and I_{ref} is the reference signal with the standard sample placed at position B. The barium sulphate plug is a calibration standard with a certified reflectance factor, R_{BS}.

2.3 Samples and sample preparation

Liquid samples containing a mixture of intralipid, ink, and water were prepared in ten groups in order to facilitate calibration of both systems. Each group contained ten samples with fixed intralipid concentration, providing a constant scattering coefficient, while the concentration of ink was varied in between 0.001 % - 0.1 % to provide ten different absorption coefficients. Likewise, the intralipid concentration was varied between groups within the range 0.4 % - 6.8 % to provide ten different scattering coefficients. In this way a matrix of samples was formed with optical properties covering the typical biological range of absorption and scattering coefficients.

$$0.1\ \text{cm}^{-1} \leq \mu_a < 10\ \text{cm}^{-1} \quad ; \quad 5\ \text{cm}^{-1} \leq \mu_s' < 85\ \text{cm}^{-1}$$

Identical cuvettes were made for the measurements, consisting of two glass microscope slides (1 mm thickness) glued with an air gap of 0.3 mm between them. One end was left open so that the liquid sample could be introduced.

This study, with the aim to measure optical properties of brain tissue, is part of a larger study approved by the local Ethical Committee for animal research (Project no. 42-01, Linköping University). Brain tissue samples were taken from five Swedish native-bred pigs with a body weight of 20.5 kg ± 0.5 kg. Small slices were cut to a thickness of 0.3 mm, 2 cm in diameter. These slices were placed in between two glass plates with 0.3 mm spacers at each side for measurements.

2.4 Measurement procedure

The prepared intralipid-ink samples were used in ten sets of ten samples to calibrate and evaluate the performance of both systems. The 0.3 mm thick cuvettes were first filled with samples 1-10 from group one. For the IS measurements a standardized acquisition time of 10 second were employed. Then, the reference intensity (I_{ref}) of the lamp through port A of the sphere the transmission of light through the sample (I_T), and the reflected light from the sample (I_R) were measured. Further on, measurements were conducted with the CASH sensor. First the background was recorded with all detectors by blocking the laser light, followed by a laser intensity measurement by the reference detector. The laser light was then attenuated with a neutral density filter (1.0 mm NG1, Schott, Germany), and the intensity at the α_0 detector was recorded to ensure the stability of the system. The cuvettes were then placed in the cuvette holder, one by one, for the recordings. All samples were measured both with and without different combinations of neutral density filters to improve the dynamic range of detection. The acquisition time for each measurement was 1 ms. Following these measurements, the pig brain samples were measured with the same procedure, except that several measurements were performed at different positions on each sample with the CASH sensor.

2.5 Data processing

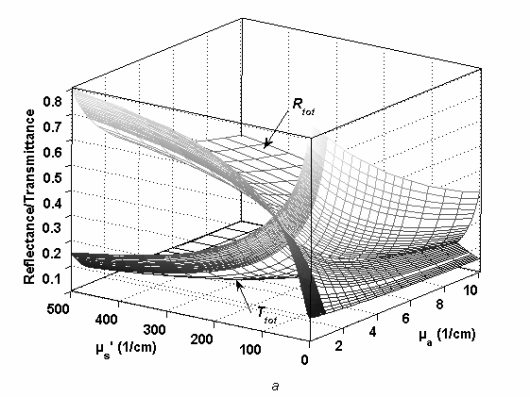
Optical properties were extracted from the recorded data. Three different algorithms were employed sequentially in this analysis. Briefly, a multiple polynomial regression (MPR) technique was used, employing fitting of an analytical expression to a discrete number of measured or modelled data points [18]. This provides a fast and accurate method for applications involving real-time, multi-parameter extraction problems. Rapid data processing is of particular importance for spectral recordings including data from a large number of wavelengths. The MPR method was used to extract μ_a and μ_s' from IS measurements of R_{tot} and T_{tot} on thin turbid samples. In MPR, the first step (calibration) is to perform two objective mappings of relevant subsets of the [μ_a, μ_s'] space onto their images in R_{tot} and T_{tot} space, respectively. Monte Carlo simulations using the code provided by Wang and Jacques [22], were employed for this purpose. The R_{tot} and T_{tot} values were simulated for a 38 x 38 matrix of μ_a and μ_s'. The values in these matrices were chosen so that their distributions were wider than their typical biological ranges [23,24]:

$$0.01\ \text{cm}^{-1} \le \mu_a \le 10\ \text{cm}^{-1};\ 3\ \text{cm}^{-1} \le \mu_s' \le 500\ \text{cm}^{-1};\ g = 0.74;\quad n = 1.33$$

The second step (fitting) involves creating a calibration model describing R_{tot} (μ_a, μ_s') and T_{tot} (μ_a, μ_s') by polynomial regression. Here a fifth degree polynomial was used for both μ_a and μ_s'. Finally, in the last step (evaluation), the Newton-Raphson algorithm was applied to extract μ_a and μ_s' from integrating sphere measurements of R_{tot} and T_{tot} by solving the inverse problem. This was performed both for intralipid-ink samples and brain tissue samples. Evaluations were conducted for all wavelengths between 520 and 770 nm.

The CASH sensor was also calibrated and evaluated using the MPR technique. In this case we chose to limit the evaluation to two measured properties, R and α_0. The database was generated from values measured in the intralipid-ink samples with known values of μ_a and μ_s' [25]. We generated in this way 10 x 10 matrices for R and α_0. The values of μ_a and μ_s' in these matrices were chosen to be somewhat wider than their typical biological ranges:

$$0.1\ \text{cm}^{-1} \le \mu_a < 10\ \text{cm}^{-1};\quad 5\ \text{cm}^{-1} \le \mu_s' < 85\ \text{cm}^{-1};\quad g = 0.74;\quad n = 1.33$$


Reflectance/Transmittance
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0.7
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R_{tot}
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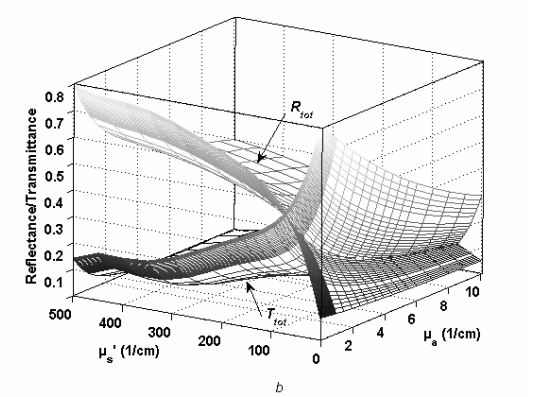
μ_a (1/cm)

a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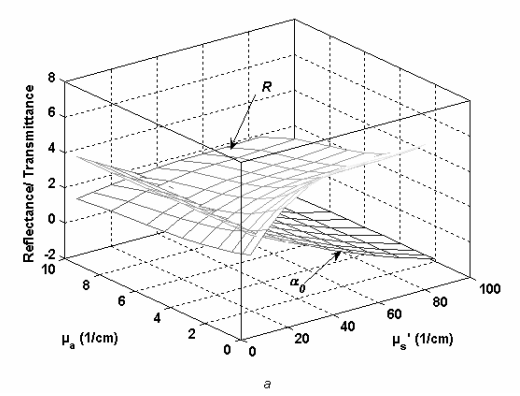
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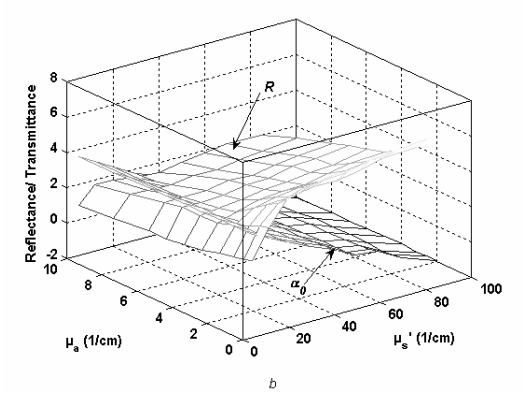
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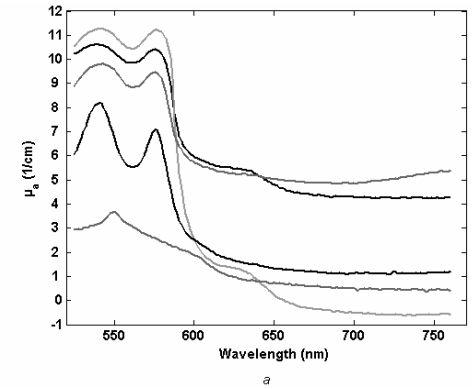
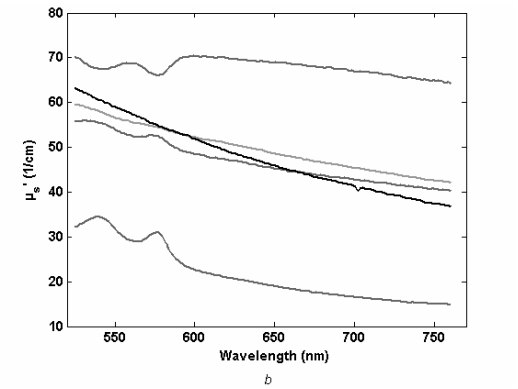

μa (1/cm)
Wavelength (nm)
a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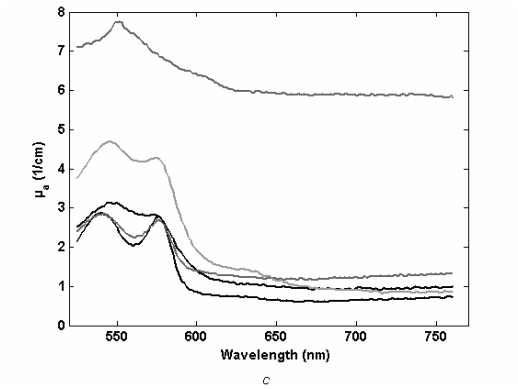
μa (1/cm)
Wavelength (nm)
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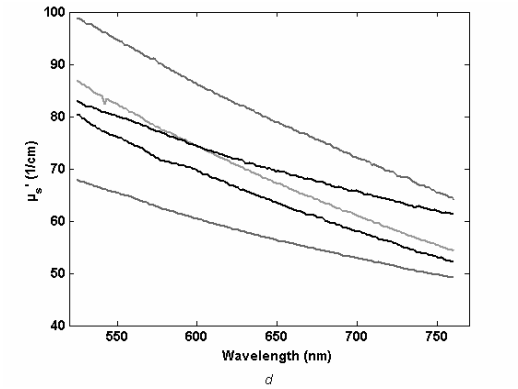
μs' (1/cm)
Wavelength (nm)
d

visually similar tissue. Results for absorption and scattering at 633 nm are presented in Figures 6 and 7 for white matter and coagulated brain tissue, respectively. The reference values in the figures are derived from measurements on human brain tissue performed, previously [3].

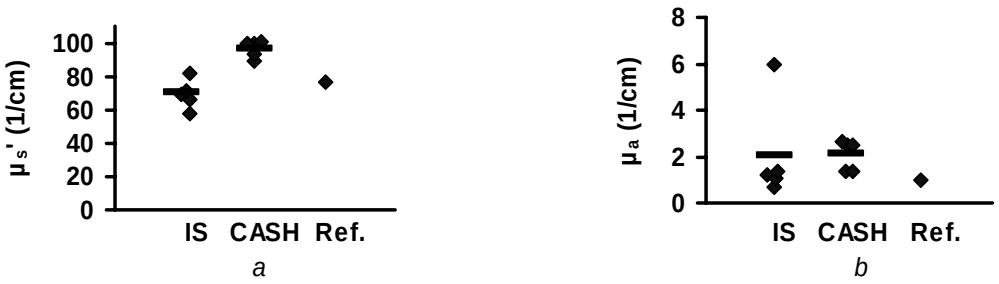

Figure 6: Results from measurements on white matter at 633 nm performed with the IS and CASH sensor. Published data for human brain are denoted Ref. a) Reduced scattering coefficient measurements, and b) absorption coefficient measurements.

Figure 7: Results from measurements on coagulated pig brain tissue at 633 nm performed with the IS and CASH sensor. Published data for human brain are denoted Ref. a) Reduced scattering coefficient measurements, and b) absorption coefficient measurements.

CV of neighbouring areas measurements within the same brain tissue sample with this sensor produced values within 7 % of each other for μ_s' of white matter, and 4 % for μ_s' of the coagulated one. The corresponding values for μ_a measurements were 17 % and 17 % for the white matter and the coagulated tissue, respectively. Figure 8, shows these neighbouring area measurements for white matter and, Figure 9, shows the same measurements for coagulated tissue.

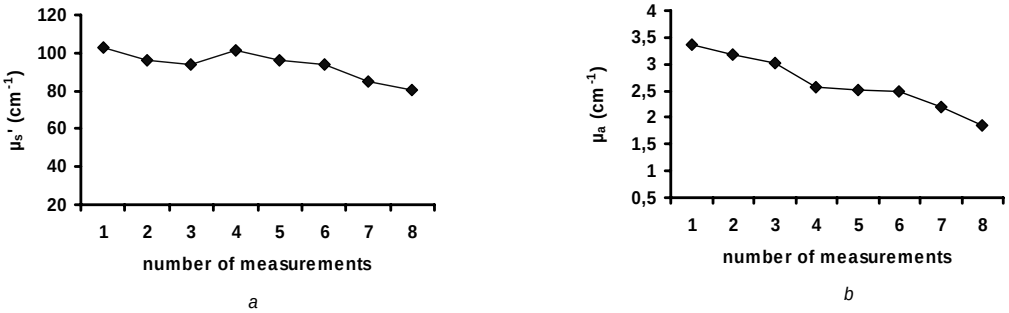

Figure 8: Neighbouring areas measurements within the same brain tissue sample- white matter. a) μ_s', and b) μ_a measurements.

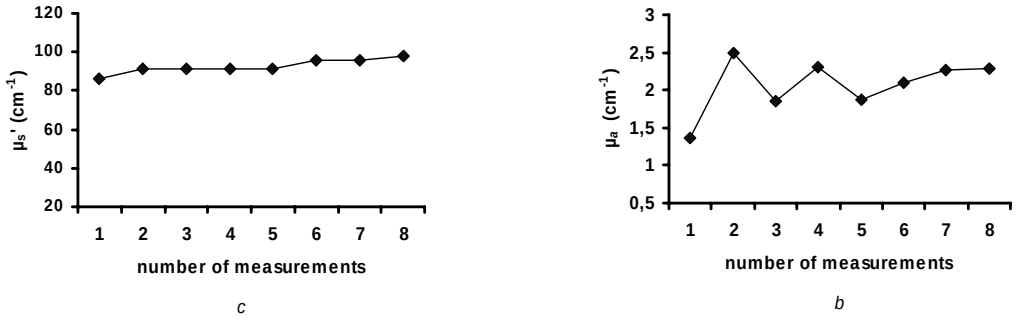

Figure 9: Neighbouring areas measurements within the same brain tissue sample- coagulated matter. a) μ_s', and b) μ_a measurements.

Interestingly, the optical properties for the coagulated brain tissue varied considerably more than those for the normal white matter. This might be due to from pieces of white matter intermixed with gray matter inside the coagulated volume. The targeted area for coagulation was always gray matter. Other factors that may cause variations in measured results are slight variations in sample preparation, sample thickness, sample positioning, etc.

4. DISCUSSION AND CONCLUSIONS

In this work we have introduced a novel technique for determination of the optical properties of turbid media from spatially and angularly resolved measurements on a small, thin sample, i.e. a solid slice or liquid sample in a cuvette, using simple, continuous-wave, non-coherent light sources. The compact instrument developed allows rapid measurements without repositioning the sample, providing the possibility for real-time determination of optical properties. The acquisition time in this study was only 1 ms. This may be of special interest for certain measurements of liquids in process or food industries.

In this study we have used the instruments to measure on brain tissue samples. All results and conclusions presented are based on calibrations with Monte Carlo simulation or real measurements using especially prepared liquid samples of known optical properties. As the presented results show, there is a very good correlation between the expected and the experimental results for these samples. In the evaluation of the system, the results for scattering agreed especially well with the expected values. The absorption varied more, probably due to the small sampling volume, leading to low sensitivity to absorption in a medium with a relatively low absorption coefficient. The evaluated new technique is interesting due to the advantages it has over IS method: the sample does not have to be moved during the measurements, no bulky spheres are needed, and there is the possibility of sampling smaller volumes [19].

The results from the liquid samples measurements were promising for the new sensor and we thus continued with real tissue measurements. The results from these measurements show much larger variations between the samples than for the liquid samples used in the evaluation of the system. The variations observed from the tissue measurements are thus not believed to be due to insensitivity of the system, but rather due to variations between the samples. This is also suggested by the low observed variations within the same sample (data not shown) and that results from the IS set-up varied more than for the CASH sensor. A probable explanation of these variations is the heterogeneity of brain tissue. In these measurements, it was sometimes difficult to measure white matter or coagulated tissue only, as light was collected out to a distance of 12 mm from the optical axis in the IS set-up. This was sometimes sufficiently large to cover not only the targeted tissue, but also other parts of the samples, areas not intended to be included in the measurement. It is thus important to conduct local measurements of optical properties, as brain tissue is very heterogeneous. The CASH sensor could be considerably better than the IS in this respect, as the probed volume is smaller. For this sensor, only light at a radius of 2.5 mm is detected.

It is known that in measurements on very thin samples, such as those in our experiments, tissue preparation affects the measured optical properties. Mainly the absorption coefficient is supposed to alter, due to loss of liquid constituents in tissue. For example it is shown that the effective light attenuation of tissue may change by a factor of two or more in the visible wavelength range [26,27]. Also, the transmission of light through tissue may be altered by freezing and thawing [27]. The structural changes resulting from freezing and the reduction in tissue water content upon thawing effect the optical response of tissue. A 39 % and 160 % change in transmission of thick sample at 488 and 514 nm, respectively; due to prefreezing has previously been reported [23]. Also, it has been demonstrated that the optical properties measured from thick samples are generally smaller than those measured from thin samples [28]. These observations suggest that one should be sceptical to measurements of especially the absorption coefficient of tissue from all ex vivo measurements. In these experiments the samples were snap frozen in liquid nitrogen and kept frozen until the measurements. The samples were stored in relatively large chunks (several centimetres in diameter) and thawed wrapped in plastic foil to minimize the effects of loss of liquid. The samples were sliced just prior to the measurements, again to minimize alterations in optical properties due to the sample preparation. The results from brain sample measurements are in good agreement with previous ex vivo observations [3]. These values need to be reconfirmed by in vivo measurements, e.g. by elastic scattering probes for the scattering properties [13-15] or time-resolved measurements for absorption properties [29,30].

The knowledge of optical properties in normal and coagulated brain tissue is of most importance, especially for improving devices used in minimally invasive intervention and target localisation during stereotactic neurosurgery. There exists a large amount of research for the treatment of motor disorders such as ex. Parkinson's disease. By new knowledge it might be possible to perform surgery safer and with higher accuracy in the future.

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