In vitro double-integrating-sphere optical properties of tissues between 630 and 1064 nm

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Abstract. The optical properties (absorption and scattering coefficients and the scattering anisotropy factor) were measured *in vitro* for cartilage, liver, lung, muscle, myocardium, skin, and tumour (colon adenocarcinoma CC 531) at 630, 632.8, 790, 850 and 1064 nm. Rabbits, rats, piglets, goats, and dogs were used to obtain the tissues. A double-integrating-sphere setup with an intervening sample was used to determine the reflectance, and the diffuse and collimated transmittances of the sample. The inverse adding–doubling algorithm was used to determine the optical properties from the measurements. The overall results were comparable to those available in the literature, although only limited data are available at 790–850 nm. The results were reproducible for a specific sample at a specific wavelength. However, when comparing the results of different samples of the same tissue or different lasers with approximately the same wavelength (e.g. argon dye laser at 630 nm and HeNe laser at 632.8 nm) variations are large. We believe these variations in optical properties should be explained by biological variations of the tissues. In conclusion, we report on an extensive set of *in vitro* absorption and scattering properties of tissues measured with the same equipment and software, and by the same group. Although the accuracy of the method requires further improvement, it is highly likely that the other existing data in the literature have a similar level of accuracy.

1. Introduction

Optimization of therapy or devices for laser applications in medicine often requires knowledge of the light fluence rate distribution inside the target tissue. It is generally accepted that such information can be obtained from solving the equation of radiative transfer (Chandrasekahar 1960). This integro-differential equation requires the optical properties of the tissue as input parameters, that is, the absorption coefficient, μ_a, the scattering coefficient, μ_s, and the phase function for scattering. For tissues, this latter function is often represented by the Henyey–Greenstein relation which requires only one additional parameter, the scattering anisotropy factor, g (Henyey and Greenstein 1941, Flock *et al* 1987, Jacques *et al* 1987).

A double-integrating-sphere setup was developed for measurement of *in vitro* tissue optical properties (μ_a, μ_s, g) (Beek 1993, Pickering *et al* 1993, Prahl *et al* 1993). An intervening sample is illuminated with a collimated beam and the reflectance measured in the first sphere, the diffusely transmitted light measured in the second sphere and the collimated transmission at some distance from the sample through a hole in the second

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sphere (Beek 1993, Pickering *et al* 1993, Roggan *et al* 1993). The inverse adding–doubling algorithm was used to determine the optical properties from these measurements (Prahl *et al* 1993). This paper compiles all measurements on several tissues (cartilage, liver, lung, muscle, myocardium, skin, and tumour) at a number of wavelengths (630, 632.8, 790, 850, and 1064 nm). These measurements were performed as part of various projects, including pneumothorax monitoring by remittance measurement (Beek 1993). Our list supplements the extensive list by Cheong *et al* (1990). However, we emphasize that our data refer to *in vitro* optical properties measured with the same equipment and software, and by the same group.

2. Materials and methods

Tissues were taken from New Zealand white rabbits, Wagrij rats, piglets, Saane goats, and mongrel dogs, between 0 and 24 h after sacrificing the animal (all animals were used for other experiments). Immediately after excision the tissues were sectioned at 120–840 μm thickness.

   We tried to cut fresh tissue samples with an estimated optical thickness τ ($\tau = (\mu_a + \mu_s)d$, where d is the sample thickness) of $1 < t < 10$ (Beek 1993, Pickering *et al* 1993). The total attenuation coefficient ($\mu_t = \mu_a + \mu_s$) was found to be in the range of 100 to 800 cm^{-1}, implying that the actual sample thickness should be between 100 μm and 1 mm for samples with a low total attenuation coefficient ($\sim$100 cm^{-1}) and between 15 and 150 μm for samples with a high total attenuation coefficient ($\sim$800 cm^{-1}). Cutting non-frozen tissue in slices smaller than half a millimetre was found to be difficult, especially for highly elastic tissues. We used a vibratome and tissue glued to a table with cyanoacrylate to cut tissue slices >0.5 mm thickness and we used a hand-held microtome knife in combination with a custom-built stand that supported the tissue on all sides to cut the tissue slices <0.5 mm thickness. For preparing the skin samples of piglets, an electrical dermatome was used (Aesculaap), and the skin was shaved before cutting if necessary. Two skin samples were taken from each location, one consisting of epidermis and dermis (a full-thickness skin graft), and one of dermis only.

   Immediately after cutting, the sample was placed between two glass slides that served to minimize rough surface effects. A small quantity of phosphate buffered saline was added to the sample to prevent refractive index mismatches due to air bubbles. The two slides were clamped together whilst aluminium spacers of approximately the same thickness as the sample were used between the slides to prevent compression of the sample. The sample thickness was determined by measuring the thickness of the slide/sample/slide arrangement with calipers and subtracting the thickness of the slides. Only samples that entirely covered the sample port of 25 mm were used.

   The experimental setup is schematically shown in figure 1.

   The sample was placed between two identical integrating spheres of 70 mm diameter with a circular sample port of 25 mm diameter and illuminated with collimated light from (i) a 2 mm beam diameter, 630 nm argon-pumped dye laser (SpectraPhysics model 2030, Eindhoven, The Netherlands) operating at 0.5–1 W, (ii) a 1 mW, 1 mm beam diameter, 632.8 nm HeNe laser (Polytec model PL650, Waldbronn, Germany), (iii) a 1 mW, 1 mm beam diameter, 790 nm semiconductor laser (Philips CQL73, Eindhoven, The Netherlands), (iv) a 5 mW, 1 mm beam diameter, 850 nm semiconductor laser (Philips CQL13, Eindhoven, The Netherlands), or (v) a 2 mm beam diameter, 1064 nm Nd:YAG laser (SLT CL60, Malvern, AR, USA) operating at 1 to 5 W. The output of the argon-pumped dye laser and the Nd:YAG laser were collimated and attenuated (to a power < 20 mW) by respectively
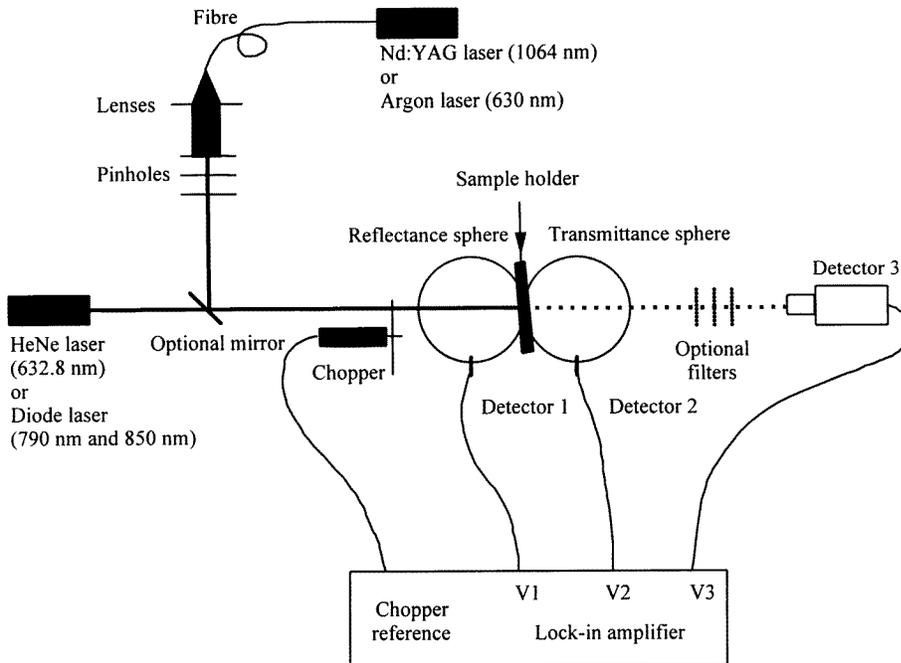
Figure 1. The experimental setup consisting of two integrating spheres with an intervening sample. An argon-pumped dye laser, an HeNe laser, a semiconductor laser (790 or 850 nm), or an Nd:YAG laser were used to direct the light onto the sample through a chopper. The signal was detected by photodiodes placed on the walls of the two spheres and at a distance of 70 cm from the exit port of the transmittance sphere. The signals from the photodiodes and the frequency of the chopper were fed into a lock-in amplifier.

lenses and pinholes. The light fluences within each sphere (reflectance in the first sphere, the diffusely transmitted light in the second sphere), and the collimated transmittance (at a distance of 70 cm beyond the second sphere) were measured using standard light measuring techniques. The input light beams were chopped mechanically at 600 Hz (EG&G Princeton Applied Research, Wellesley, MA, USA, model 196 light chopper) and the signals measured using BPW 34 photodiodes (Telefunken, Hannover, Germany) and a lock-in-amplifier (model 5210, EG&G Princeton Applied Research). All measurements within the spheres were made relative to the signal when a 99% reflecting plate (Labsphere SRS-99-010, North Sutton, NH, USA) was placed at the sample aperture. The collimated transmission measurement was made relative to a measurement with no sample. Attenuation of the beam, using filters with varying attenuation factors, was required to avoid saturation of the photodiode. In addition, background measurements were made (Beek 1993, Pickering *et al* 1993).

A total of 236 combinations of sample/wavelength were used. For each sample, a full set of measurements was made at one to six different positions of the sample. For most samples four measurements were made, i.e. two on each side. After each set of measurements the sample was displaced by a few millimetres to allow the incident beam to fall on a different part of the tissue.

Determination of the optical properties from the three measurements and the sample thickness by the inverse adding–doubling numerical procedure has been described elsewhere

(Prahl 1988, Prahl *et al* 1993). If the programme did not converge to a unique set of optical properties the results were discarded. The programme converged for 770 sets of measurements and did not converge for 32 sets.

3. Results

Table 1 summarizes the results of our measurements for the various tissues at the five wavelengths. The optical properties are expressed as the mean ± standard error of the mean (SEM) for all measurements within one group of samples (e.g. piglet lung at 850 nm). The reduced scattering coefficient ($\mu'_s = \mu_s(1 - g)$), and the effective attenuation coefficient ($\mu_{\text{eff}} = (3\mu_a(\mu_a + \mu'_s))^{1/2}$), were calculated from the measured optical properties.

Table 1. *In vitro* optical properties measured with double integrating spheres (mean ± standard error of the mean). The reduced scattering coefficient ($\mu'_s = \mu_s(1 - g)$), and the effective attenuation coefficient ($\mu_{\text{eff}} = (3\mu_a(\mu_a + \mu'_s))^{1/2}$), were calculated from the measured optical properties.

Tissue	λ (nm)	Species	Number of (a)	(b)	(c)	g	μ_a (cm^{-1})	μ_s (cm^{-1})	μ'_s (cm^{-1})	μ_{eff} (cm^{-1})
Cartilage	632.8	rabbit	1	1	4	0.909±0.005	0.33±0.05	214±0.2	19.4±1.1	4.4±0.4
Liver	632.8	rabbit	2	4	5	0.934±0.023	11.3±5.2	190±41	8.9±3.9	21.9±8.7
		goat	1	3	3	0.980±0.011	12.3±9.0	491±72	8.7±4.6	26.7±13
		rat	7	14	28	0.952±0.004	3.8±0.2	280±10	13.0±1.0	13.8±0.9
	1064	rat	7	14	28	0.948±0.005	2.0±0.3	151±6	7.9±0.7	7.5±0.8
Lung	632.8	rabbit	3	3	6	0.904±0.012	2.8±0.2	330±21	30.8±3.2	16.5±1.0
		piglet	3	15	48	0.933±0.003	2.0±0.1	301±22	19.7±1.4	11.3±0.6
		dog	1	2	8	0.935±0.017	3.2±0.7	230±5	15.4±4.3	11.6±1.5
	790	piglet	2	11	44	0.926±0.004	2.4±0.3	263±18	20.0±1.7	12.4±1.3
	850	piglet	2	11	43	0.957±0.002	0.76±0.07	278±21	10.9±0.7	4.8±0.3
Muscle	630	rabbit	3	7	19	0.846±0.009	1.4±0.2	110±5	16.5±0.7	8.4±0.8
		piglet	1	3	12	0.732±0.013	1.2±0.1	239±16	62.1±2.0	15.0±0.7
	632.8	rabbit	8	16	40	0.968±0.002	0.74±0.06	140±6	4.4±0.3	3.3±0.2
		piglet	1	2	7	0.858±0.012	0.59±0.01	179±12	24.7±0.7	6.7±0.1
	790	rabbit	3	9	34	0.950±0.005	2.3±0.2	157±11	6.8±0.7	7.8±0.8
Myocardium	630	dog	1	1	4	0.854±0.015	2.0±0.2	159±6	23.0±1.4	12.1±0.1
	632.8	dog	3	3	9	0.940±0.004	2.1±0.1	191±16	11.3±1.2	9.1±0.6
	790	dog	2	4	12	0.943±0.004	0.98±0.2	164±10	6.0±1.6	3.4±1.0
Skin epi+der	630	rabbit	1	2	8	0.812±0.017	0.94±0.13	213±21	40.0±2.2	10.7±1.4
	632.8	rabbit	6	9	36	0.898±0.007	0.33±0.02	306±12	31.6±2.2	5.4±0.2
		piglet	2	14	64	0.953±0.001	1.0±0.1	492±17	22.7±0.8	8.3±0.4
	790	rabbit	2	3	11	0.940±0.003	0.70±0.07	321±8	18.4±0.5	5.9±0.4
		piglet	2	14	64	0.952±0.001	2.4±0.2	409±14	19.3±0.6	12.3±0.6
	850	piglet	1	10	40	0.962±0.005	1.6±0.1	403±20	14.3±1.5	8.5±0.6
Skin dermis	632.8	piglet	2	11	58	0.926±0.002	0.89±0.10	289±7	21.1±0.4	7.1±0.5
	790	piglet	2	11	44	0.945±0.001	1.8±0.2	254±5	13.9±0.3	8.8±0.6
	850	piglet	2	11	35	0.968±0.001	0.33±0.03	285±5	9.0±0.2	3.0±0.1
Tumour[a]	632.8	rat	7	14	28	0.946±0.004	1.4±0.2	280±20	15.0±2.0	8.3±0.9
	1064	rat	7	14	28	0.952±0.005	2.5±0.5	180±10	9.0±1.0	9.0±1.0

[a] Colon adenocarcinoma CC 531.
(a) = animals.
(b) = samples.
(c) = measurements.

4. Discussion

In this study the *in vitro* optical properties of cartilage, muscle, myocardium, liver, lung, skin, and tumour (colon adenocarcinoma CC 531) were determined at 630, 632.8, 790, 850, and 1064 nm. The measurements were performed using a double-integrating-sphere setup and the optical properties were assessed from these measurements using the adding–doubling solution of the transfer equation, a method that was considered reliable (Prahl 1988, Beek 1993, Pickering *et al* 1993, Prahl *et al* 1993). Overall, no statistical differences were found between various locations of harvesting, nor did we find in this study a difference in measurements on various orientations within the sample holders of structurally anisotropic tissue.

Our data compare well with values from the literature, although large variations in numbers are well known to occur (Cheong *et al* 1990) and only limited data are available at 790–850 nm. In our study, it is interesting to note the large differences in optical properties measured at 630 nm and 632.8 nm. We believe these variations in optical properties should be explained by biological variations of the tissues. At 630 nm, tissues used were harvested from other animals than at 632.8 nm. The only other difference between the two sets of measurements was (the coherence length of) the laser source that was used, the argon dye laser at 630 nm having a long coherence length and the HeNe laser at 632.8 nm having a short coherence length. However, we do not believe that the differences found can be explained by coherence length differences. Variations in optical properties at 630–635 nm as found in the literature are also large. For instance, values for muscle are $0.2 \leqslant g \leqslant 0.97$, $4.3 \leqslant \mu_t \leqslant 345$, $0.12 \leqslant \mu_a \leqslant 1.7$, $4.1 \leqslant \mu_s \leqslant 45$ (μ_s was not measured separately for $\mu_t = 345$ by Karagiannes *et al* (1989)), $1.2 \leqslant \mu'_s \leqslant 7.0$, and $1.1 \leqslant \mu_{\text{eff}} \leqslant 12.5$ (Doiron *et al* 1982, 1983, Preuss *et al* 1982, Wilksch *et al* 1984, Wilson *et al* 1985, 1986, Marijnissen *et al* 1985, Bolin *et al* 1987, Flock *et al* 1987, Marijnissen and Star 1987, McKenzie and Byrne 1988, Karagiannes *et al* 1989) versus $0.732 \leqslant g \leqslant 0.968$, $111 \leqslant \mu_t \leqslant 240$, $0.59 \leqslant \mu_a \leqslant 1.4$, $110 \leqslant \mu_s \leqslant 239$, $4.4 \leqslant \mu'_s \leqslant 62.1$, and $3.3 \leqslant \mu_{\text{eff}} \leqslant 15.0$ in this work.

For liver, values are $g = 0.95$, $\mu_t = 417$, $2.3 \leqslant \mu_a \leqslant 3.2$, $\mu_s = 414$, $5.2 \leqslant \mu'_s \leqslant 17.0$, and $6.8 \leqslant \mu_{\text{eff}} \leqslant 26.6$ (Doiron *et al* 1983, Preuss *et al* 1982, Wilson *et al* 1985, 1986, Andreola *et al* 1988, Karagiannes *et al* 1989, Marchesini *et al* 1989, Arnfield *et al* 1992) versus $0.934 \leqslant g \leqslant 0.980$, $201 \leqslant \mu_t \leqslant 503$, $3.8 \leqslant \mu_a \leqslant 12.3$, $190 \leqslant \mu_s \leqslant 491$, $8.7 \leqslant \mu'_s \leqslant 13.0$, and $13.8 \leqslant \mu_{\text{eff}} \leqslant 26.7$ in this work.

For lung, values are $0.75 \leqslant g \leqslant 0.95$, $44.3 \leqslant \mu_t \leqslant 332$, $8.1 \leqslant \mu_a \leqslant 8.4$, $35.9 \leqslant \mu_s \leqslant 324$, $1.8 \leqslant \mu'_s \leqslant 81$, and $11 \leqslant \mu_{\text{eff}} \leqslant 46.6$ (Doiron *et al* 1982, Andreola *et al* 1988, Marchesini *et al* 1989) versus $0.904 \leqslant g \leqslant 0.935$, $233 \leqslant \mu_t \leqslant 333$, $2.0 \leqslant \mu_a \leqslant 3.2$, $230 \leqslant \mu_s \leqslant 330$, $15.4 \leqslant \mu'_s \leqslant 30.8$, and $11.3 \leqslant \mu_{\text{eff}} \leqslant 16.5$ in this work.

We emphasize that a comparison of optical properties measured by different investigators is in general difficult, because of differences in the materials used. For instance, a comparison of the *in vitro* absorption coefficient of a black, tar-containing deflated lung of a male adult, with the *in vivo* absorption coefficient of the whitish lung of a piglet is, obviously, of little use. In addition to differences in materials, differences in methods influence the results as well. Measurements on bulk tissue are considered to be the best method to determine the effective attenuation coefficient. If we limited our comparison at 630 and 632.8 nm to interstitial fibre measurements, μ_{eff} for muscle, liver, and lung is 2.7–12.5, 11.0, and 12.5–13.0 respectively (Doiron *et al* 1982, 1983, Preuss *et al* 1982, Wilson *et al* 1985, Bolin *et al* 1987, Flock *et al* 1987, Marijnissen and Star 1987). Our calculated μ_{eff} is comparable for muscle and lung and is a factor of two higher for liver. However, there is now strong evidence that absorption is overestimated when spectrophotometric

techniques are used, especially when a broad light beam and a low port-to-beam-size ratio is used (Beek 1993, Pickering *et al* 1993, Torres *et al* 1994). We used a small spotsize and a high port-to-beam-size ratio, which limits light losses through the side of the sample. Nevertheless, absorption could have been overestimated and, as a result, the calculated values for μ_{eff} should be considered high estimates. Also, we found that measurement of the collimated transmission should be made at a sufficient distance from the second sphere to avoid measurement of diffuse light. We are convinced that, if this and other requirements are met (Pickering *et al* 1993), double integrating spheres combined with an inverse adding–doubling algorithm produce accurate values of μ_s and g. However, other measurements are probably required for producing adequate results of μ_a. Such other methods may include thermal instead of optical techniques (Prahl *et al* 1992, Torres *et al* 1994).

In conclusion, we report on an extensive set of *in vitro* absorption and scattering properties of tissues measured with the same equipment and software, and by the same group. Although the accuracy of the method requires further improvement, it is highly likely that the other existing data in the literature have a similar level of accuracy.

Acknowledgments

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