Temperature-dependent optical properties of Intralipid® measured with frequency-domain photon-migration spectroscopy

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Abstract. We present the temperature dependence of absorption and reduced scattering coefficients of 1.8% Intralipid measured by frequency-domain photon-migration spectroscopy between 710 and 850 nm. These measurements were made in the physiologically relevant 30 to 40°C temperature range. The temperature coefficients for absorption were consistent during heating and cooling and follow closely other reported results. The change in absorption coefficient at 740 nm suggests that a minimum temperature change of 4°C is observable within the error limits. We found that the reduced scattering coefficient shows a hysteresis with temperature at 740 nm. The temperature coefficient for reduced scattering determined from heating cycle measurements agrees with theory and other measurements within the error limits. © *2010 Society of Photo-Optical Instrumentation Engineers.*
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1 Introduction

Accurate knowledge of the optical properties of turbid media, such as tissue, is important in many clinical applications that use light for diagnosis and treatment. The optical properties depend on the physiological state of the tissue and are influenced by many parameters. One such parameter is temperature, which can substantially alter measured optical properties. The effect can be quantified as a temperature coefficient, the rate of increase (or decrease) in optical absorption (α) and scattering (β) coefficients with temperature.

Temperature-induced change in the optical properties can, for example, limit the application of photodynamic therapy.[1] Other applications include the estimation of temperature from the change in optical, property measured. Temperature can be estimated from changes in the absorbance[2] however, in turbid media, variation in scatter introduces an uncertainty in the path length, making it difficult to reliably estimate temperature from optical extinction coefficients. Uncoupling the effect of scattering on absorption may reduce this obscurity, offering the potential for accurate measurements of temperature in turbid solutions.

Kelly et al.[3] measured the near-infrared (NIR) spectra of chicken, bovine, and porcine tissues from 17 to 45°C with a spectrophotometer and reported that the measurements can predict tissue temperature with a standard error less than 0.2°C. Laufer et al.[4] measured the change in optical properties of human dermis and subdermis from 25 to 40°C. They observed positive and negative temperature coefficients of

scattering for dermis and subdermis, respectively, but no change in absorption with temperature.

A common phantom to simulate optical properties of tissue is Intralipid. Temperature-induced changes in the optical properties of Intralipid have been described previously. Kakuta et al.[2] reported the temperature dependence of absorbance at 1440 nm using a spectrophotometer and proposed a method to measure the temperature of turbid aqueous solutions, but they did not recover the absorption coefficient. McGlone et al.[5] determined the temperature dependence of absorption and reduced scattering coefficients of Intralipid using continuous wave photon migration measurements from 700 to 1000 nm. The authors relied on measurement of attenuation close to the source to separate scattering and absorption. The changes in absorption coefficients due to temperature were attributed to water,[6,7] the main absorbing species in Intralipid.[8,9] Kakuta et al. found a discrepancy in absorption between heating and cooling cycle measurements and linked it to irreversible changes in the scattering properties of the micelles. McGlone et al. observed a lack of repeatability, during heating and cooling, in temperature-induced changes in the reduced scattering properties.

The frequency-domain technique uncouples absorption from scattering[10,11] when determining the optical properties of turbid media, which potentially allows more accurate recovery of optical coefficients.[12] Our work is a preliminary step toward monitoring these subtle changes. We have investigated the optical properties of a tissue-simulating phantom and present, for the first time, frequency-domain measurements of the temperature dependence of the absorption (μ_a) and re-

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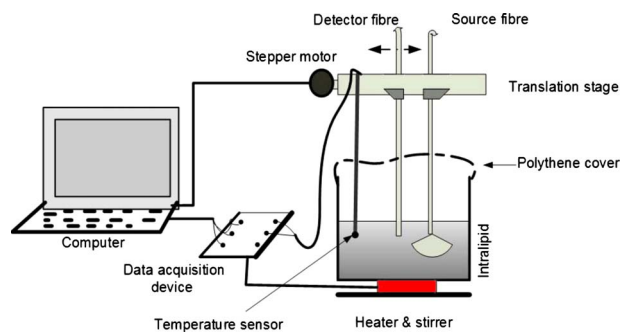
Fig. 1 Experimental setup.

Fig. 2 Absorption coefficients measured at 22°C (triangle),[19] 30°C (hexagram), 35°C (circle), and 40°C (diamond) plotted against wavelength. The changes in absorption around 740, 800, and 840 nm are indicated by arrows.

duced scattering (μ_s') coefficients of Intralipid in the wavelength range from 710 to 850 nm. We have used a 1.8% (w/w) solution of Intralipid as the sample to simulate the reduced scattering properties of human brain and breast tissues.[13] Measurements were made in the physiologically relevant temperature range of 30 to 40°C while heating and cooling the liquid.

2 Method

Optical properties can be determined by measuring the amplitude attenuation and phase shift of intensity-modulated light passing through a turbid medium.[14] The propagation of intensity-modulated light through turbid media has been extensively discussed in the literature.[15–18]

We employed the multidistance method, where the amplitude and phase of a photon density wave is measured at different separations from the source. An outline of our experimental setup is shown in Fig. 1 and has been described in detail previously.[19] Using this configuration, we measured absorption (0.009 to 0.045 cm^{-1}) to an agreement with reported literature of better than 10% in the presence of reduced scattering coefficients between 10 and 35 cm^{-1}. Briefly, the instrumentation employed a tunable Ti: Sapphire laser as the light source, which was intensity modulated by an acousto-optic modulator. A lock-in amplifier in conjunction with an avalanche photodiode (APD) module constituted a phase-sensitive detection system. Optical fibers, suspended from a translation stage in the middle of the Intralipid solution, were used to couple the light into and out of the medium.

The temperature of the medium was maintained using a closed-loop control system incorporating a heated plate (VELP Scintifica, Italy) equipped with a magnetic stirrer and a semiconductor temperature sensor (LM35, National Semiconductor, Santa Clara, California) immersed in the solution. The temperature sensor was located so that it would not affect the photon density wave. The temperature sensor and the solution heater were connected via a data acquisition device (U 12, LabJack Corporation, Lakewood, Colorado) to a computer and controlled by a Labview (National Instruments, Austin, Texas) program. A loose polythene cover minimized water evaporation during the experiment. A 1.8% (w/w) solution of Intralipid was obtained by diluting 500 mL of Intralipid-20% (Pharmaco, New Zealand) in 5 L of distilled water.

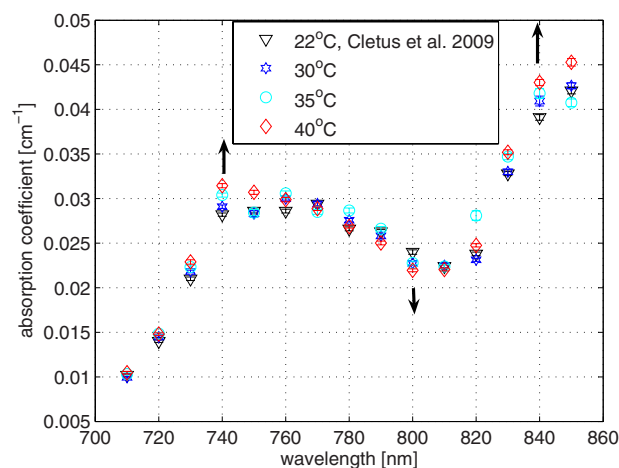Amplitude and phase measurements were initially collected, at 10-nm intervals, between 710 and 850 nm at three different set-point temperatures (30, 35, and 40 ± 0.5°C—the "low-temperature resolution" data) as the distance between the source and detector fibers was increased from 10 to 30 mm (0.8-mm steps). At each stage-position, five amplitude and phase measurements were recorded to estimate the errors in our estimated absorption and reduced scattering coefficients. The temperature control system was allowed to stabilize for at least 30 min before collecting each set of measurements. Additional amplitude and phase measurements—the "high-resolution" data—were made at 740, 800, and 840 nm at room temperature (22°C), then as the solution was first slowly heated (over 2 h) from 30 to 40°C, then again as it cooled (over 4 h), and finally again at room temperature. These measurements were collected every 1.1 ± 0.1 °C between 30 to 40 °C over the heating and cooling regimes. Replicate heating and cooling measurements were collected at 740 nm and 840 nm with similar solutions prepared, on different days; a single set of measurements was recorded at 800 nm. Last, optical measurements were recorded when the solution reached 22°C after a cooling cycle, then again at 22°C the next day.

As Intralipid is known to decay over time,[20] a fresh solution was prepared for each set of measurements.

3 Results

Figure 2 shows the absorption coefficient of Intralipid, calculated from the amplitude and phase measurements, over the wavelength range 710 to 850 nm measured at the three different temperatures. The absorption coefficient from previous measurements made by us at 22 ± 0.5°C is included.[19] The error bars at each measurement illustrate the measurement uncertainty (±1 standard deviation). The uncertainty in the optical coefficients was estimated from the replicate error in phase and amplitude measurements through error propagation.[21] Arrows on the plot show the trend in absorption coefficient with temperature. The absorption coefficient increases around 740 and 840 nm and decreases around 800 nm.
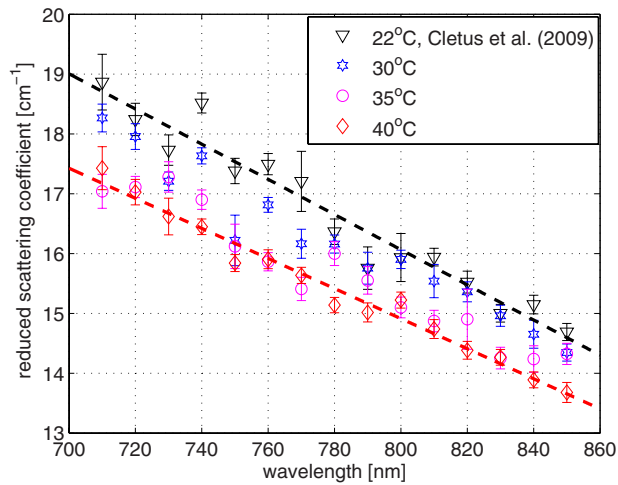
Fig. 3 Reduced scattering coefficient measured at different temperatures plotted against wavelength. Linear, least-squares fits at 40°C (red) and 22°C (black) show the trends (dashed lines). (Color online only.)

The reduced scattering coefficient, again calculated from the amplitude and phase measurements, is illustrated in Fig. 3 for the three temperatures measured in this study and at 22°C (Ref. 19). As predicted by Mie theory,[22] the reduced scattering coefficient decreases, nearly linearly, as wavelength increases. Moreover, these measurements show that the reduced scattering coefficient decreases as temperature increases.

The temperature trend is more clearly illustrated for the absorption and reduced scattering coefficients using the data measured continuously as the sample was heated and cooled (Fig. 4 and Fig. 5, respectively). Each plot illustrates an approximately linear relationship between the optical coefficient and temperature at 740 and 840 nm for two replicate measurements during heating and cooling of the sample. Results at 22 ± 0.4°C are included for comparison from earlier work.[19] Each data point between 30 and 40°C represents the

average of five measurements with one standard deviation error bars estimated by propagating the noise in the amplitude and phase measurements through the calculations for the optical coefficients.

As Fig. 4 shows, the absorption coefficient increases with temperature at both 740 and 840 nm. This is consistent with the change in absorption coefficient of pure water,[7] the main absorbing species in Intralipid.[8] Lipid, the other absorbing species in Intralipid, has an absorption coefficient of less than 0.0005 cm^{-1} but is present only at 1.8% by volume, so its contribution will be negligible.[9] The absorption coefficients over the heating and cooling period match well at 740 nm. However, at 840 nm, absorption was generally higher during the cooling period. This may be due to a variation in dilution across the different batches of Intralipid.

A temperature coefficient, α, has been estimated using a linear, least-squares fit to the measurements made in the heating and cooling periods. The discrepancy between heating and cooling at 840 nm had no significant effect on the slope, so data from the two periods has been combined in all cases. These measurements are in good agreement with results collected at 22°C both during this work and in previous work.[19]

Generally, the reduced scattering coefficient decreased as temperature increased at 740 and 840 nm (Fig. 5). However, at 740 nm, a hysteresis was observed whereby the reduced scattering coefficient decreased during heating yet remained reasonably constant during cooling, even down to 22°C. This behavior was observed in both replicates but was not a result of the self cooling, as the measurement time for a single set (a detector fiber scan from 10 to 30 mm) is about 7 min and the change in temperature in this interval is only of the order of 0.2°C. Earlier data at 22°C and a subsequent measurement at 22°C (green square) on the following day (15 h later), indicate that the scattering coefficient recovers to its original value eventually. This behavior was observed in both replicates at 740 nm, but at 840 nm, although the slope is slightly lower during the cooling period, no significant difference was observed between the heating and cooling periods.
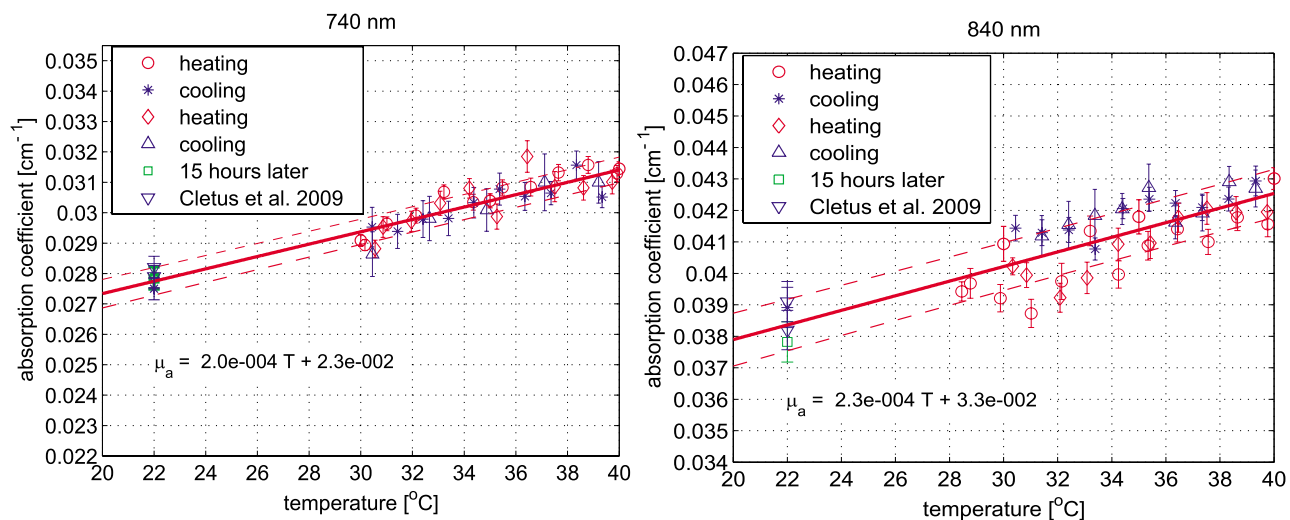
Fig. 4 Absorption coefficient at 740 and 840 nm plotted against temperature. The red circles correspond to the heating cycle data, and the blue asterisks to the cooling cycle. The solid line is a least-squares fit to all the data, and the dashed lines indicate 68% confidence interval. The error bars give ±1 standard measurement error. (Color online only.)
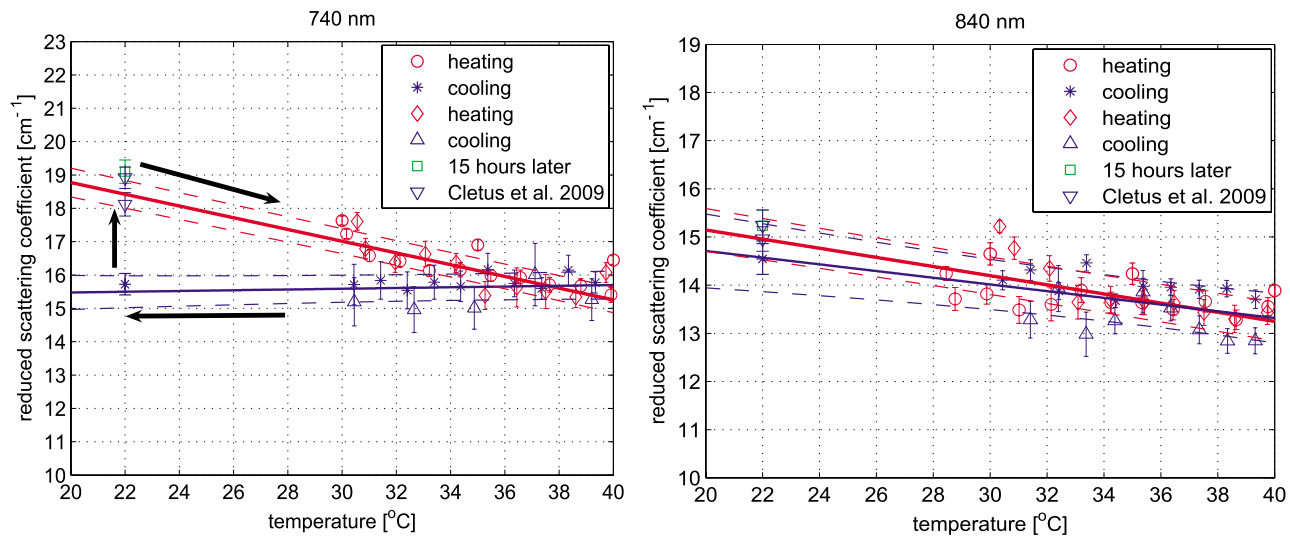
Fig. 5 Reduced scattering coefficients at 740 and 840 nm plotted against temperature. The red circles indicate heating, and the blue asterisks cooling cycles. The solid lines are least-squares fits to heating and cooling data. The error bars and confidence interval show ±1 standard error. (Color online only.)

Kakuta et al.[2] and McGlone et al.[5] also observed different behavior in their measurements during heating and cooling. Kakuta et al. observed discrepancy in their absorbance measurement and postulated that it may be due to the permanent structural changes in the micelles during heating. McGlone et al. also observed different scattering behavior during heating and cooling. Because of this hysteresis, the temperature coefficient, β, for the scattering coefficient was estimated using only data from the heating period, with a linear least-squares fit.

4 Discussion

A positive temperature coefficient of absorption (α) of $(2.0 \pm 0.02) \times 10^{-4} \, \text{cm}^{-1} \, \text{K}^{-1}$ at 740 nm and $(2.3 \pm 0.3) \times 10^{-4} \, \text{cm}^{-1} \, \text{K}^{-1}$ at 840 nm was calculated from the data plotted in Fig. 4. At 800 nm (data not shown), we calculated a temperature coefficient of $(-7 \pm 1) \times 10^{-5} \, \text{cm}^{-1} \, \text{K}^{-1}$. The near-infrared absorption features arise from overtones and combination bands of the vibrations of the water molecule.[23] At 837 nm, the main contribution is a combination of symmetric and asymmetric stretch and bending vibrations. The 739-nm absorption peak is caused by symmetric and asymmetric stretch vibrations. The sensitivity of water absorption to temperature arises from changes to the strength of the hydrogen bonds and microscopic changes in the structure of the water.[7,24]

There is some disagreement in the literature over the precise value of water's near-infrared temperature coefficient. Our results at 740 and 840 nm are in good agreement with those reported by Hollis[25,26] for water but are 40% higher than the temperature coefficient of water reported by Langford et al. (Fig. 6).[7] The temperature coefficient of absorption for Intralipid measured by McGlone et al.[5] are about 50% lower than our results. At 800 nm, our result is more consistent with the data reported by Langford et al.[7] and McGlone et al.,[5] underestimating Hollis' observation by 75%.

The temperature coefficient of Intralipid absorption was estimated in 10-nm steps between 710 and 850 nm using the data plotted in Fig. 3. At most wavelengths, the temperature coefficient was estimated from absorption measured at 22, 30, 35, and 40°C (squares). At 740, 800, and 840 nm, the results, obtained from high-temperature resolution measurements, discussed earlier are plotted (triangles). The error bars indicate 68% confidence interval of the regression values. Large errors—at 820 nm, for example—occur when there is a significant variation in laser power during a measurement. Overall, our results agree in magnitude and spectral shape with values reported in the literature.

The prediction intervals in Fig. 4 give an indication of the temperature sensitivity of our setup. The minimum tempera-
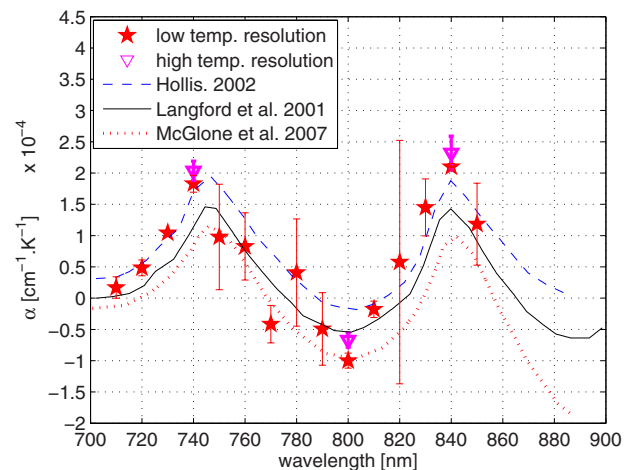
Fig. 6 Temperature coefficient of absorption for 1.8% solution of Intralipid plotted against wavelength. The temperature coefficient for pure water reported by Hollis and Langford et al.[7,25,26] and the temperature coefficient for Intralipid (1%) reported by McGlone et al.[5] are also included. Error bars indicate 68% confidence interval.
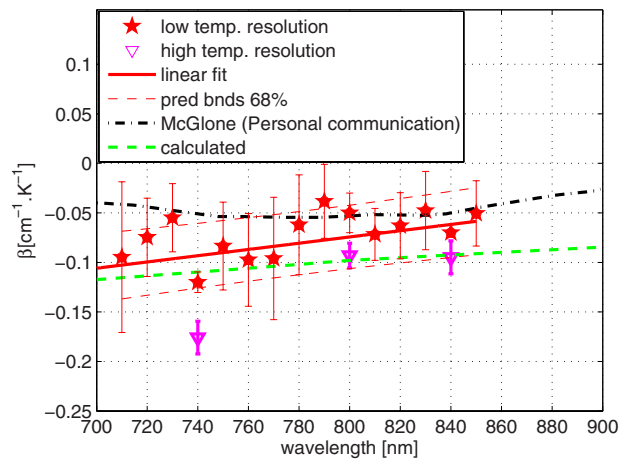
Fig. 7 Temperature coefficient for reduced scattering coefficient (±1 standard error) plotted against wavelength. The red line shows a linear fit to the temperature coefficient, and the dotted lines indicate 68% confidence intervals. The dashed (green) line shows the calculated temperature coefficient. (Color online only.)

ture change observable within the error limits is $4°C$ and $6°C$ at 740 and 840 nm, respectively. This is probably insufficient for applications requiring precision measurement. Moreover, the presence of other absorbing species, such as hemoglobin, in biological samples will likely demand even greater accuracy. However, we believe that the largest source of error lies in the precision of the phase measurements and the short-term stability of the source. We believe that more precise temperature measurements can be achieved by including active feedback to control the amplitude stability of our laser source.

The temperature coefficient of the reduced scattering coefficient (β) was estimated, as for absorption, using linear regression. However, as we found a significant difference in scattering behavior during heating and cooling, only heating data was used. The results are plotted in Fig. 7. At most wavelengths, the temperature coefficient was estimated from reduced scattering measured at 22, 30, 35, and $40°C$ (squares); at 740, 800, and 840 nm, approximately 10 measurements between 30 and $40°C$, as well as $22°C$ were used (triangles). A linear regression line through the reduced scattering temperature coefficient shows a general decrease in temperature sensitivity as wavelength increases.

The scattering coefficient of a turbid media is affected by temperature in two ways. First, as temperature increases, the volume of the medium expands, diluting the scattering effect.[5] However, more significantly, the refractive index of water also decreases with temperature,[27] leading to a greater mismatch between the scattering particles and the absorbing medium. We have modeled the temperature coefficient of the reduced scattering coefficient of Intralipid based on Mie scattering and changes of the refractive index with temperature. We used published particle-size distribution data for Intralipid[9] and assumed a refractive index temperature sensitivity of 3.54×10^{-4} K^{-1}, as that value is typical of the major fatty acids components of soya bean oil and similar derivatives.[28] The result is included in Fig. 7 as a green dashed line for comparison. McGlone et al. measured temperature coefficients for

scattering in a 1.2% Intralipid solution by continuous wave measurements. Their data, scaled to 1.8%, are illustrated as well. Within the large uncertainty, our measurements agree with theory and other measurements.

5 Conclusion

We have used frequency-domain, photon-migration spectroscopy to separately measure absorption and reduced scattering temperature coefficients in a turbid medium for the first time. The optical temperature coefficients of the liquid, tissue-simulating phantom 1.8% Intralipid were estimated from 710 to 850 nm in the physiologically relevant 30 to $40°C$ temperature range. At 740, 800, and 840 nm (key wavelengths in the water absorption temperature coefficient), we measured absorption temperature coefficients of $(2.0 \pm 0.02) \times 10^{-4}$, $(-7 \pm 1) \times 10^{-5}$, and $(2.32 \pm 0.03) \times 10^{-4}$ cm^{-1} K^{-1} and reduced scattering temperature coefficients of -0.18 ± 0.02, -0.09 ± 0.01, and -0.09 ± 0.02 cm^{-1} K^{-1}, respectively. The temperature coefficients observed for absorption closely follow the published results for water, as expected. The temperature coefficients observed for reduced scattering exhibited a general decrease as wavelength increased, consistent with predictions made by Mie theory and literature.

We observed hysteresis behavior in the reduced scattering measurements made at 740 nm that did not appear to occur at higher wavelengths. The reduced scattering coefficient decreased with temperature but did not increase again as the solution was cooled until sometime later. This hysteresis did not affect the absorption measurements. This result suggests that similar observations of this behavior reported in extinction measurements are caused by changes in the scattering micelles.

These results suggest that temperature measurement, with a precision of $\pm 4°C$, in turbid media is feasible based on the absorption coefficient's temperature coefficient. However, an improvement in precision will be required for many applications.

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