

## Effect of temperature on the optical properties of *ex vivo* human dermis and subdermis

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**Abstract.** The effect of temperature on the optical properties of human dermis and subdermis as a function of near-infrared wavelength has been studied between 25 °C and 40 °C. Measurements were performed *ex vivo* on a total of nine skin samples taken from the abdomen of three individuals.

The results show a reproducible effect of temperature on the transport scattering coefficient of dermis and subdermis. The relative change of the transport scattering coefficient showed an increase for dermis ( $(4.7 \pm 0.5) \times 10^{-3} \text{ }^\circ\text{C}^{-1}$ ) and a decrease for subdermis ( $(-1.4 \pm 0.28) \times 10^{-3} \text{ }^\circ\text{C}^{-1}$ ). Note that the magnitude of the temperature coefficient of scattering was greater for dermis than subdermis. A reproducible effect of temperature on the absorption coefficient could not be found within experimental errors. System reproducibility in transport scattering coefficient with repeated removal and repositioning of the same tissue sample at the same temperature was excellent at  $\pm 0.35\%$  for all measurements. This reproducibility enabled such small changes in scattering coefficient to be detected.

### 1. Introduction

The influence of temperature on the optical properties of human tissues is of general interest in the field of biomedical optics. Variations in tissue temperature are at their most extreme during hypothermia associated with cardiopulmonary bypass procedures and in skin under normal environmental conditions. Skin is particularly relevant as most optical measurements of brain and muscle are non-invasive, requiring transmission of light through the skin in both directions. Additionally, recent publications have suggested that blood glucose can be determined through its effect on the tissue scattering coefficient (Kohl *et al* 1995, Bruulsema *et al* 1997). The effect of glucose on scattering is small and it is important to compare its magnitude with the effect of temperature on the scattering coefficient.

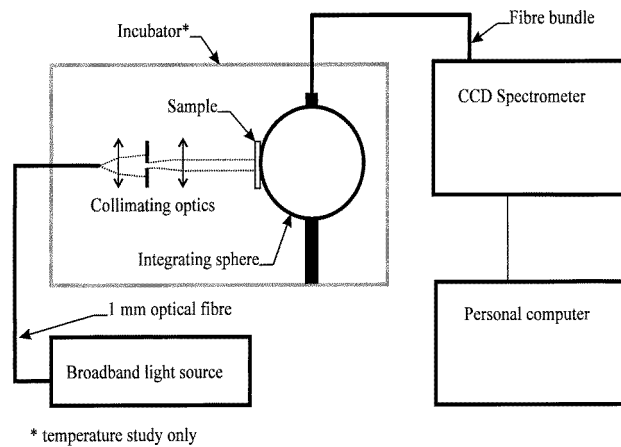
Diffuse reflectance and transmission measurements were carried out on thin dermis and subdermis samples at four different temperatures (25, 30, 35, 40 °C) using an integrating sphere which was placed in a temperature controlled environment.

The optical properties, absorption coefficient and transport scattering coefficient, were determined by using an inverse Monte Carlo method in which the measurements of diffuse

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reflectance and transmittance of skin samples are compared with the results of a Monte Carlo model of light transport in a medium of similar geometry.



**Figure 1.** Experimental set-up.

## 2. Methods

### 2.1. Experimental set-up and technique

An integrating sphere (Labsphere RT-060-SF) was placed inside a modified neonatal incubator in which the temperatures could be set from ambient temperature, usually 25 °C, to a maximum of 45 °C (figure 1). Diffuse reflection measurements of tissue samples were compared with a 50% reflection standard (Labsphere SRS-50). The so-called 'comparison method' (Jacquez and Kuppenheim 1950) was applied to determine the diffuse reflectance and transmittance of the sample compared with a standard. A more detailed description of the set-up and experimental technique can be found in the accompanying paper (Simpson *et al* 1998).

The temperature of the samples was monitored indirectly by a thermocouple placed inside a separate sample holder which was filled with gelled water and positioned inside the temperature controlled chamber. The thermocouple was calibrated in a water bath with a mercury thermometer.

### 2.2. Sample preparation

Specimens were obtained from the abdomen of three patients undergoing plastic surgery. All samples were measured within 24 h and were stored at 4 °C. The whole skin specimens were washed with saline before the samples were dissected for measurements. Three samples of dermis (including epidermis) and subdermis were taken from each patient. All samples had a thickness of 2 mm.

Circular samples of 16 mm diameter were cut from dissected dermis and placed in glass covered black PVC mounts. The tissue samples were sealed with glass coverslips on either side of the mount which were fixed with cyano-acrylate adhesive. A more detailed description of the sample holder is reported in Simpson *et al* (1998).

Samples of subdermal tissue layers adjacent to the dermis were obtained and placed in identical glass covered mounts. Care was taken to achieve a watertight seal to avoid possible evaporation of fluids especially at higher temperatures.

### 2.3. Measurement methods

At the beginning of each set of experiments, the CCD spectrophotometer was wavelength-calibrated against a neon lamp to an accuracy better than 1 nm. The samples were placed in the appropriate ports of the integrating sphere and the diffuse reflection and transmission intensities measured three times for both the front and back of the sample in turn, removing and replacing the sample holder between measurements. This yielded a total of six measurements per sample and temperature. These sets of measurements will be referred to as 'reproducibility data sets'. The total exposure time per single measurement was approximately 1 s. Illumination of both sides of the sample was necessary as the samples of the dermis have different reflectivities from opposite sides. The temperature inside the incubator was increased in steps of 5 °C, starting from 25 °C, to 40 °C. The tissue samples were allowed to equilibrate for 30 min at each temperature before each measurement.

The fitting of a light trap to the integrating sphere removed the specular reflection from the glass coverslip during the reflectance measurements. In previous measurements, a coverslip was placed in front of the reflectance standard to achieve a more accurate comparison with the sample. The reflectance standards are hydrophobic and it was found that the drying of the thin layer of water between the reflectance standard and glass introduced a large error. Therefore, in this study all measurements were carried out using a bare reflectance standard since the emphasis of this study was on reflectance reproducibility as a function of temperature instead of the measurement of absolute optical properties.

The reflectance and transmittance measurements of the dermis samples showed different spectra for the transport scattering coefficient,  $\mu'_s$ , when front and back illumination were compared. For this reason the analysis of the effects of temperature on the changes in the optical properties was done separately for the front and the back illuminations of all samples.

A Monte Carlo model of light/tissue interaction, described in Simpson *et al* (1998), was implemented for 4000 combinations of scattering and absorption coefficients. The model produces the integrated intensities of reflectance and transmittance for a particular pair of coefficients over the entire surface of the simulated sample. These values are tabulated for a range of coefficients (in this case  $\mu_s = 1\text{--}75 \text{ mm}^{-1}$ ,  $\mu_a = 0.001\text{--}0.1 \text{ mm}^{-1}$ , with fixed  $g$  factor of 0.9) and the results of the measurements can then be compared with the model results. The values are linearly interpolated between data points to produce more accurate results.

### 2.4. Temperature sensitivity of the reflectance standard

The possibility of measuring the temperature coefficient of the measuring apparatus and not the sample was carefully considered. The comparative measurement of reflectance and transmittance against standards are always made at the same temperature so this in itself does not lead to a temperature-dependent measurement error. However, the presence of a temperature coefficient in the reflectance standard would lead to an error as the reflectance measurement is determining the temperature coefficient of the sample minus that of the reflectance standard. For this reason, the reflectance standard was heated in an oven to a temperature of 95 °C, placed onto the illuminated port of the integrating sphere and

allowed to cool down. The temperature of the standard was monitored with a thermocouple during cooling, and the intensities measured at appropriate temperature steps. There was no observable change in reflectance ( $< 0.3\%$ ) over the temperature range from  $65^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ .

### 3. Results

The optical coefficients were calculated for the front and back illumination of each sample. The stratum corneum side of the dermis is classified as being the front of the dermis. Similarly, the subdermal tissue section normally in contact with the dermis was classified as being the front of the subdermis. Normalized transport scattering coefficient spectra ( $\mu'_s = \mu_s(1-g)$ ) were calculated to illustrate the fractional change in the optical coefficients for the front and back illuminations respectively. This was done by dividing the mean of the reproducibility data sets of higher temperatures by the mean of the appropriate reproducibility sets at  $25^{\circ}\text{C}$  for each sample.

#### 3.1. Dermis

An increase in  $\mu'_s$  with increasing temperature could be observed in all dermis samples. Although the absolute values of the individual spectra vary, a qualitative increase in the transport scattering coefficient could be observed. The relative changes in the transport scattering coefficient for back and front illumination are of similar magnitude. Figure 2 shows the relative changes in  $\mu'_s$  with temperature after being normalized to the spectrum at  $25^{\circ}\text{C}$ . The increase in scattering seems to be larger from  $25^{\circ}\text{C}$  to  $30^{\circ}\text{C}$  compared with the other temperature steps. The data are an average of all patients and the error bars represent the standard deviation in normalized transport scattering coefficient of nine samples ( $n = 9$ ). The normalized sets, used in the calculation of the standard deviation, are similar for the front and the back of the samples. Features which would suggest a small wavelength dependence could be observed.

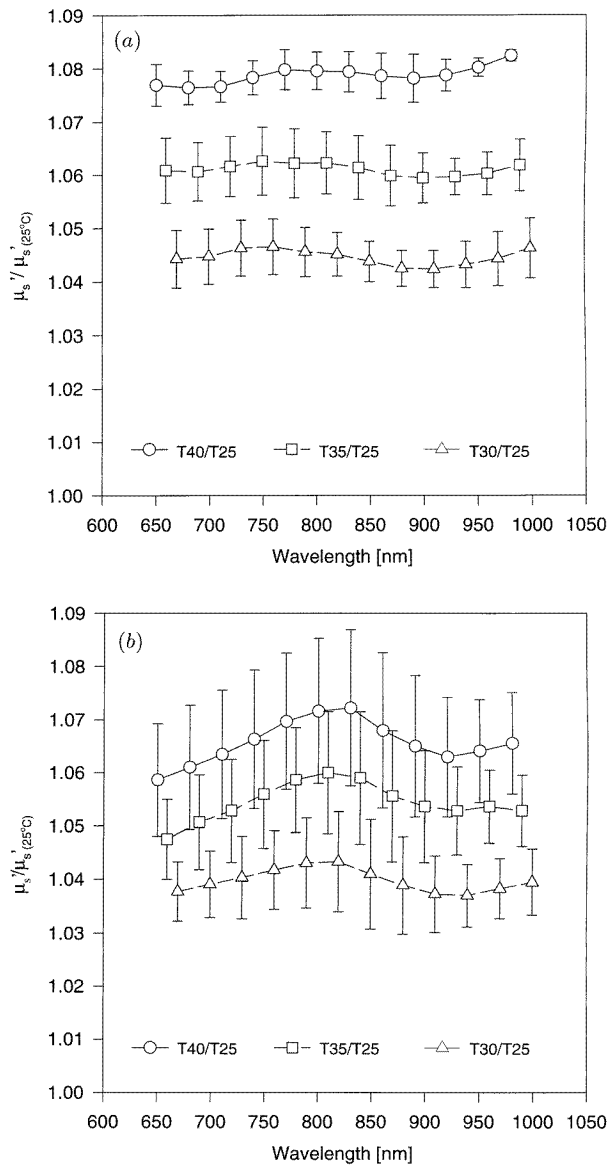
For the absorption coefficient,  $\mu_a$ , no significant change with increasing temperature could be observed in any direction. This is depicted in figure 3 for illumination of the back of the sample.

#### 3.2. Subdermis

As with the dermis samples, the optical coefficients were calculated for front and back illumination of each sample. The results of  $\mu_a$  and  $\mu'_s$  spectra of front and back measurements were similar due to a higher homogeneity of subdermal tissue when compared with dermal tissue.

Here a decrease in the transport scattering coefficient with increasing temperature could be seen. Figure 4 illustrates the relative changes in  $\mu'_s$  with temperature and the error bars are the standard deviations for the nine samples. The relative changes in scattering coefficient show similar values for front and back illumination. A relatively small change in scattering occurs from  $25^{\circ}\text{C}$  to  $30^{\circ}\text{C}$  but greater changes occur for the other temperature steps.

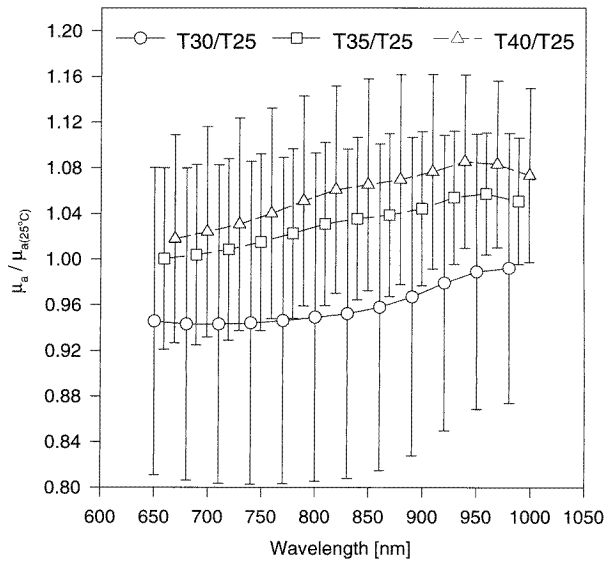
As with dermal tissue, no significant change in the absorption coefficient of subdermis was observable with temperature (figure 5).



**Figure 2.** Normalized scattering spectra,  $\mu_s'/\mu_s'(25^\circ\text{C})$ , of the dermis: (a) for back illumination, (b) for front illumination.

### 3.3. Average temperature coefficient of the transport scattering coefficient

Although there may be a nonlinear response of the transport scattering coefficient with temperature (and more speculatively a wavelength-dependent response), the most useful characteristic obtained from this study is an average temperature coefficient. The wavelength dependence is not statistically significant in this study and a more extensive study with smaller temperature steps is necessary to confirm the extent of nonlinearity which is suggested by the change between 25 and 30 °C compared with the other temperature steps.

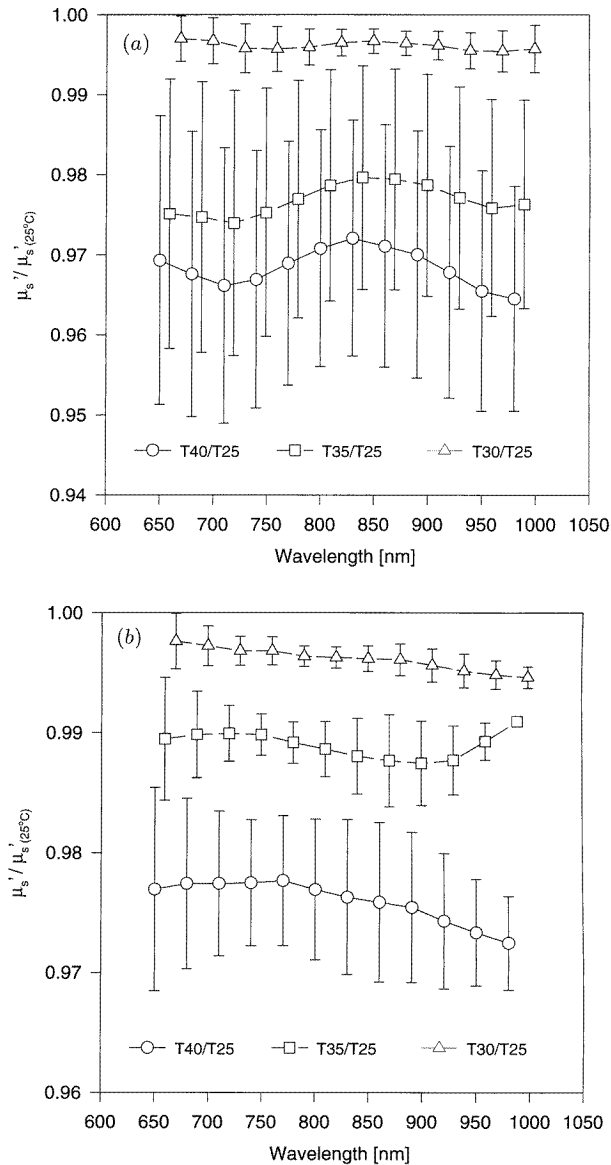


**Figure 3.** Normalized absorption spectra,  $\mu_a/\mu_a(25^\circ\text{C})$ , of the dermis (back illumination).

The normalized transport scattering coefficient spectra were averaged over the 650–1000 nm range at each temperature for all nine samples (three samples from each of three patients, front and back illumination). Data for front and back illumination were also averaged, since the temperature coefficient of transport scattering coefficient calculated separately for front and back illumination did not show significant differences. Individual average data points can be seen in figure 6 for dermis and in figure 7 for subdermis joined by fainter grey lines. Figures 6 and 7 also show the linear regression lines through the data points together with the 95% confidence intervals of the regression lines as full black lines. Clearly the temperature coefficients of scattering for dermis and subdermis are significantly different as indicated by the confidence intervals. The actual temperature coefficient is  $(4.7 \pm 0.5) \times 10^{-3} \text{ }^\circ\text{C}^{-1}$  for dermis and  $(-1.4 \pm 0.28) \times 10^{-3} \text{ }^\circ\text{C}^{-1}$  for subdermis within a 95% confidence interval for the temperature range from  $25^\circ\text{C}$  to  $40^\circ\text{C}$ . Two points were ignored in the calculation of the temperature coefficient for the subdermis because they were deemed as outliers, being greater than five standard deviations from the mean.

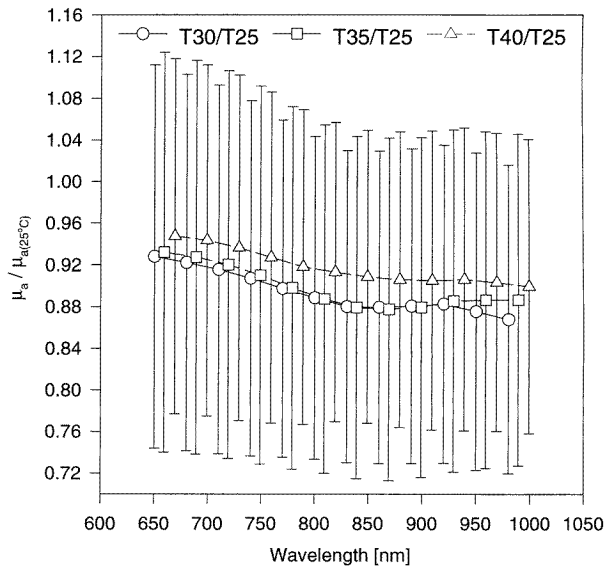
#### 4. Discussion

Although the normalized data show generally good results with clear changes in tissue scattering with temperature over a  $15^\circ\text{C}$  range, the spectra of the individual patients show that these optical measurements are at the limit of the accuracy attainable with this method. Figure 7 shows a few data points away from the general cluster. The most likely explanation relates to sample preparation. Subdermis samples were generally more difficult to prepare and sometimes contained small air bubbles under the coverslip. The increased temperatures may have caused the trapped air to expand or possibly dissolved air to de-gas, which would result in larger errors. Subdermal tissue also has a weaker structure, which could have allowed movements of the tissue inside the sample holder which was not possible with dermal samples.

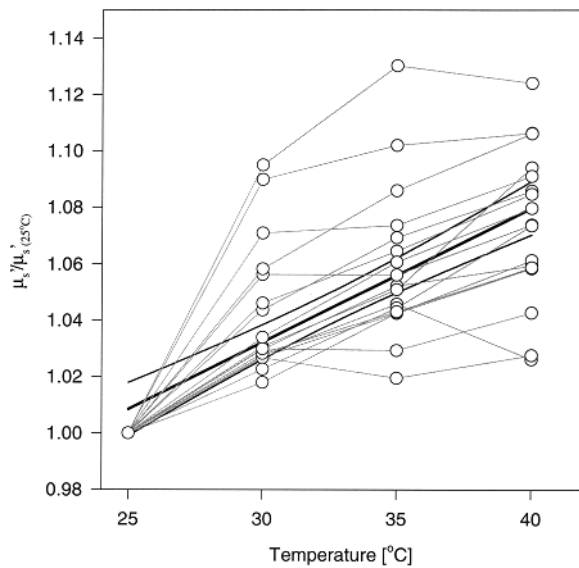


**Figure 4.** Normalized scattering spectra,  $\mu_s' / \mu_s'(25^\circ\text{C})$ , of the subdermis: (a) for back illumination, (b) for front illumination.

However, this study has shown that the sensitivity of this technique is very good and that significant temperature induced changes in  $\mu_s'$  of tissue samples could be detected. The accuracy and repeatability of the measurements was excellent, being better than 0.35% reproducibility in the transport scattering coefficient and 3% in absorption coefficient. The reproducibility of the method was determined by measuring diffuse reflectance and transmittance of the same sample three times, the sample being removed and replaced between the measurements. The poorer reproducibility in absorption coefficient is simply a feature of error propagation in the reflectance and transmittance



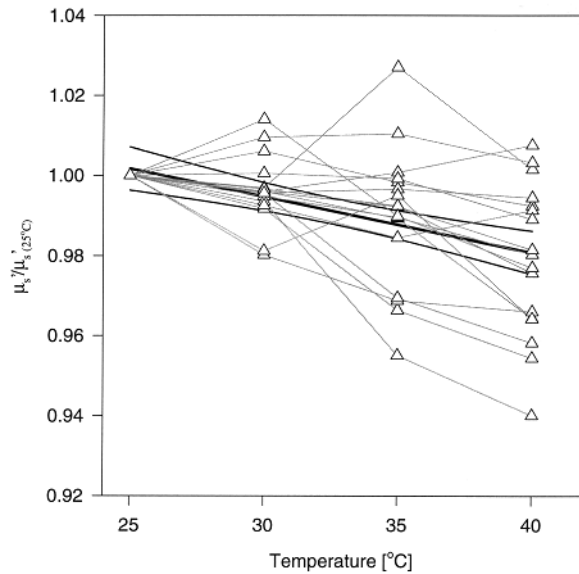
**Figure 5.** Normalized absorption spectra,  $\mu_a / \mu_a(25^\circ\text{C})$ , for subdermis (back illumination).



**Figure 6.** Temperature coefficient of dermis, normalized to  $25^\circ\text{C}$ . Symbols represent the values for nine samples for front and back illumination joined by fainter grey lines. Also indicated is the first-order regression line (full black line) with its 95% confidence interval (thinner full line).

measurements through the Monte Carlo look-up table. Small errors in the reflectance and transmittance measurements are magnified significantly more in the absorption coefficient than the transport scattering coefficient. This means that we were unable to determine whether subtle temperature-dependent changes in absorption coefficient were present.





**Figure 7.** Temperature coefficient of subdermis, normalized to 25°C. Symbols represent the values for nine samples for front and back illumination joined by fainter grey lines. Also indicated is the first-order regression line (full black line) with its 95% confidence interval (thinner full line).

Temperature effects of the major tissue absorbers, haemoglobin and water, have been studied elsewhere, reporting small changes (< 3%) in the absorption bands of water and haemoglobin in the temperature range of 20°C to 40°C (Sfameni *et al* 1997) and larger changes (30%) in the temperature range of 20 to 300 K (Cordone *et al* 1986).

There is little existing literature on temperature effects on tissue optical properties in this temperature range. A positive temperature coefficient was found in a study by Troy *et al* (1996) on canine prostate tissue. The measurements were carried out over temperatures ranging from 25°C to 65°C. The temperature coefficient of the transport scattering coefficient for these tissues was  $23 \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$  over the temperature range 25°C to 40°C. The different tissue type or possibly the measurement technique that was employed could explain the far greater change in the transport scattering coefficient compared with the one observed in this study. Troy *et al* (1996) suggest that the degradation of cellular components was responsible for the dramatic increase in the scattering coefficient, although this should not have been the case below the tissue coagulation threshold. Jaywant *et al* (1993) also report an increase in the transport scattering coefficient (5–10%  $^\circ\text{C}^{-1}$ ) at temperatures between 40°C and 80°C in bovine muscle and liver tissues. They see little change in bovine white matter  $\mu'_s$  in this temperature range. Between 30°C and 40°C, the change in the transport scattering coefficient is only significant for muscle tissue (1–2%  $^\circ\text{C}^{-1}$ ). The change in  $\mu'_s$  at temperatures above 45°C is largely attributed to a change in the size and number of scattering particles due to coagulation and is unlikely to be reversible. The temperature range as well as the tissue types were different from the ones investigated in this study, making a direct comparison of results very difficult. In this study, the scattering changes were an order of magnitude smaller over a physiological temperature range and reversible.

An explanation of the observed negative temperature coefficient of scattering for subdermis is not too difficult to find. The main scattering components of subdermis are assumed to be lipids in membranes and vacuoles. Studies have shown that lipids undergo phase changes at certain temperatures which alter their orientation, mobility and packing order. Glyco-lipids found in human cell membranes undergo phase changes in the temperature range from 25 °C to 45 °C, namely the transition from a gel phase through a stable crystalline phase to a liquid-crystalline phase with increasing temperature. Transformations from an ordered to a conformationally disordered state have been observed at the gel/liquid-crystalline phase transitions of all lipids. The decrease in scattering coefficient seen experimentally with increasing temperature is therefore consistent with an increase in fluidity known to occur in lipids with increasing temperature (Lewis *et al* 1990, Mantsch *et al* 1987).

Explanations for a positive temperature coefficient of scattering of the dermis are more difficult to identify. Collagen fibres are the main scatterers in the dermis according to Jacques (1996) and Saidi *et al* (1995), and as such become the main candidate to explain the temperature effect. Modifications of collagen fibre structure are the most plausible in our opinion, possibly through changes in hydration.

It is important to remember that these structural changes in the tissues with increasing temperature might have had an influence on the angular dependence of light scattering as well as scattering coefficient. The method in this paper gives the transport scattering coefficient (Simpson *et al* 1998). Hence, the change in transport scattering coefficient could arise from either a change in true scattering coefficient or the angular dependence of light scattering.

Lastly, it is interesting to try and extrapolate the results of this paper to other tissues. Dermis is an example of high-protein tissue and subdermis is an example of high lipid concentration tissue (Woodward and White 1986). It is interesting to speculate that scattering caused by all proteins in tissue might have a positive temperature coefficient while lipids have a negative temperature coefficient, though we do not have evidence to support this speculation at the moment. Tissues such as grey matter, which have equal lipid and protein content, could be neutral.

## 5. Conclusions

In this study, temperature induced changes in the optical properties of human dermis and subdermis have been detected over a wavelength range of 650–1000 nm. Nine samples of human skin, obtained from individuals during plastic surgery, were prepared and measured. The skin was taken from the abdomen.

Only the scattering coefficient showed statistically significant changes with temperature. The direction of the change in scattering was different for dermis and subdermis, with a decrease in the scattering coefficient for the subdermis and an increase for the dermis with temperature. The absolute temperature coefficient of scattering was also different, showing a larger change for dermis ( $(4.7 \pm 0.5) \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$ ) than subdermis ( $(-1.4 \pm 0.28) \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$ ). The absorption spectra showed no significant change with temperature in any direction.

The finding that a tissue which is largely protein has a positive temperature coefficient and a tissue which is largely lipid has a negative temperature coefficient leads to interesting possibilities in tissues where the protein/lipid ratio is intermediate, such as brain tissue.

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