# Determination of VIS- NIR absorption coefficients of mammalian fat, with time- and spatially resolved diffuse reflectance and transmission spectroscopy

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**Abstract**

The objective is derived from the need for a reliable absorption spectrum of lipids for component analysis of in vivo tissue spectra. NIR in vivo spectroscopy enables to derive the concentration of the key tissue constituents absorbing in the 600-1100 nm range, that is oxy- and deoxy-hemoglobin, water and lipids. Yet, although the first three constituents are already well characterized in literature, quite few data are available on mammalian lipids. In the present proceeding we report the absorption spectrum of a clear purified oil obtained from pig lard. Absorption coefficients were measured with time resolved and spatially resolved diffuse reflectance spectroscopy techniques. At temperatures of 37°C and higher it is a clear transparent liquid thus suitable for collimated transmission measurements. In total three independent measurement techniques were employed the determine the absorption coefficients of mammalian lipids.



**OCIS codes:** (160.4760) Optical properties; (170.3660) Light propagation in tissues; (999.9999) spectroscopy; reflectance, lipids

## 1. Introduction

The absorption spectra obtained from NIR spectroscopic techniques are supposed to be a linear combination of the absorption spectra of the chromophores present in the tissue. Decomposition of the measured absorption spectra into its components can be used to quantify absolute tissue chromophore concentrations. Furthermore, tissue oxygenation and total haemoglobin content can be calculated from these quantities. It is therefore essential to have detailed knowledge of the intrinsic absorption spectra of these 4 components. Oxy, de-oxy hemoglobin spectra and water are well-quantified en commonly applied. A basic tissue fat spectrum however has not been determined so far. The fat spectra used for spectral decomposition vary among the different research groups. These spectra all differ from each other with respect to their spectral shape as well as the magnitude of the main absorption peaks and may contain chlorophylls, making absolute comparison and interpretation of results and techniques impossible. The reason for the absence of a basic fat spectrum in the literature is that saturated mammalian fat is not available as a pure clear liquid. In the present paper we report the absorption spectrum of a clear oil obtained from mammalian pig lard. At room temperature this oil is a solid grease that displays strong scattering properties. Absorption and scattering properties of this solid grease were measured using time resolved and spatially resolved diffuse reflectance spectroscopy (TRS and DRS). At temperatures of 37°C and higher it is a clear liquid with only minimal scattering properties. Hence, an independent measurement of the absorption spectrum can be made using collimated transmission measurements.

## 2. Materials and Methods

*2.1 Lipid purification*

5 kg of pig lard was divided in pieces of 1 $cm^3$ and placed in water with a temperature of 90$^o$C. After a while a thin layer of pure oil from the lard formed on top of the water, and was removed by a spoon and placed in a separate container. This process was continued for 6 hours until the oil secretion process had slowed down. The substance obtained still contained water and other visible tissue structures. After cooling the oil had solidified into a pure white grease that could be separated easily from the remaining water and a gelatinous substance. The solid oil was then heated once more to 80$^o$C and filtered twice in liquid state. After that the oil was heated again to 80$^o$C and poured onto a filter containing Sodium sulphate to remove the last traces of water. Finally the oil was placed in a centrifuge for 30 minutes at 1000 rpm at a constant temperature of 70 $^o$C. The bottom of the reaction tubes still contained some remaining sediment and the pure oil was separated by pipette from the tube, resulting into 250 ml of oil that is visually clear at temperatures of 37$^o$C and up.

*2.2 Collimated transmission measurement*

Light from a 100 W quartz tungsten halogen lamp is coupled into an optical fiber leading to a cuvette holder and collimated to a beam of approximately 2mm diameter. Three different cuvettes were used (10, 20 and 50 mm). The collection of the transmitted light was performed by an integrating sphere with a collection port much larger that the beam diameter to compensate for differences in beam diameter caused by the divergence of the light beam and the different cuvette path lengths. The collected transmittance was spectrally projected onto a 16-bit 256-1024 pixel CCD camera cooled to –30$^o$C Cuvette holder plus integrating sphere were placed in an oven. Measurements were performed at constant temperatures of 37, 60 and 80$^o$C. For each cuvette path length three sequential transmission and background measurements were performed.

The transmission data was averaged over the 3 sequential measurement and the background was subtracted. The absorption coefficients were calculated for 3 path length differences (50-10, 50-20 and 20-10) according to equation 1.

$$\mu_{a,meas}(\lambda) = \frac{\left[ LN\left(\frac{I_{10}/I_{50}}{d_{50}-d_{10}}\right) + LN\left(\frac{I_{20}/I_{50}}{d_{50}-d_{20}}\right) + LN\left(\frac{I_{10}/I_{20}}{d_{20}-d_{10}}\right)\right]}{3} \qquad \text{Eq(1)}$$

Absorption coefficients were calculated for all 3 temperatures (37. 60, and 80 $^o$C), to investigate possible influence of temperature on the absorption coefficient.

*2.3 Spatially resolved Diffuse Reflectance Spectroscopy (DRS)*

Source light originated from a 100 W quartz tungsten lamp and was fibre- optically coupled into the sample. Diffuse reflectance was measured at 9 different source detector fibre distances, and was coupled into a cooled (-30$^o$C) imaging CCD spectrograph. A standard solution of the diffusion approximation to the transport equation was fitted on the experimental reflectance data. The 9-point fit for all wavelength entries suggested that scattering could be approximated with a simple wavelength dependent function ($a\lambda^{-b}$) and was incorporated as constraint. Further details on the fit method can be found in [1,2].

*2.4 Time Resolved diffuse reflectance spectroscopy (TRS)*

The measurement set-up consists in a fully automated system for time resolved reflectance spectroscopy continuously tunable in the 610-1050 nm range [3,4]. A synchronously-pumped mode-locked dye (DCM) laser was used as the excitation source from 610 to 700 nm, while an actively mode-locked Titanium:Sapphire laser provided light in the wavelength range of 705 to 1050 nm. A couple of 1 mm plastic-glass fibres delivered light into the tissue and collected the reflected photons at a relative distance of 1.5 cm. A double microchannel plate photomultiplier and a PC board for time-correlated single-photon counting were used for detection. The reduced scattering and absorption spectra were obtained from fitting the experimental data with a standard solution of the diffusion approximation to the transport equation. The best fit was reached with a Levenberg-Marquardt algorithm by varying both $\mu_s$' and $\mu_a$ in order to minimise the reduced $\chi^2$.

**Results**

Transmission measurement of visually clear lard oil still contained some scattering, the result at 80 $^{o}$C clearly shows a much lower scattering contribution, suggesting that the scattering observed is related to microscopic traces of solidification at the lower temperatures rather than impurities. In order to eliminate the Mie-like scattering contribution from the absorption coefficient we've fitted 11 gaussian shaped absorption peaks plus a Mie like scattering function through the transmission result as in shown equation 3.

$$\mu_{a.model} = A \cdot \exp\left[-B \cdot LN\left(\frac{\lambda}{\lambda_o}\right)\right] + \sum_i C_i \cdot \exp\left[\left(\frac{(\lambda_i - \lambda)}{\sigma_i}\right)^2\right] \qquad (3)$$

Where scattering is defined by A and B , $C_i$, $\lambda_i$, $\sigma_i$ the absorption magnitude, centre wavelength and band width of absorption band indicated with *i*. Fit constraints were non-negative fit parameter and absorption coefficient results. The scattering result of the fit was the subtracted from the transmission results to obtain the absolute absorption coefficients. The average absorption coefficients at 37 and 60$^{o}$C are shown in figure 1. The relative error (($\mu_{amodel}$-$\mu_{ameas}$)/$\mu_{amodel}$)) is within 5% accurate over the entire wavelength interval (600 up to 1100nm). Results for TRS and DRS are depict in figure 2.

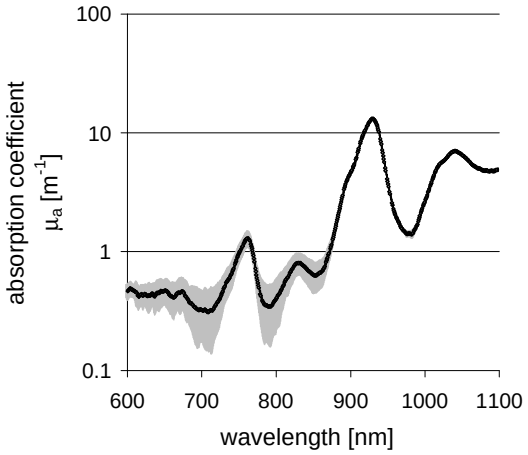


Fig. 1. Absorption coefficient corrected for scattering contribution versus wavelength. The error bars represent the standard deviation over the 2 temperatures i.e. 37, 60 $^{o}$C.

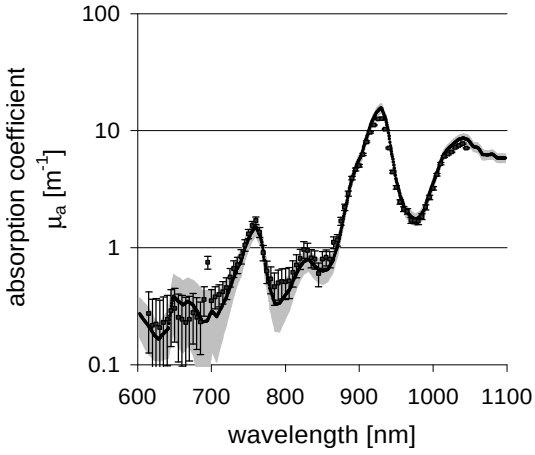


Fig. 2. Absorption coefficient versus wavelength of pig oil at 15 $^{o}$C in solid state resulting from the DRS (solid line grey error bars) and TRS (black dotes) measurements. The error bars represent the standard deviation over the 3 sequential repositioning measurements.

**4. Discussion and conclusions:**

In this proceeding we've presented the absorption spectrum of pure mammalian fat measured by three independent methods. A maximal amount Mie-like scattering was subtracted from the attenuation data as seen in figure 2 and 3 due the employed fit constraints. Despite the good fit results still some uncertainty remains regarding the absolute amount of scattering subtracted. The absorption coefficients determined at 80$^{o}$C become smaller at wavelengths higher then 980 nm and may reflect temperature dependence. The results of the TRS and DRS measurement reveal less spectral information at lower wavelengths, this may indicate insufficient capacity to uncouple scattering from absorption especially for low absorption. The results of all three techniques coincide within standard deviation. Incorporation of standardized intrinsic absorption spectra enables for a more reliable comparison between techniques and methods used in NIR spectroscopy for the analysis of absolute tissue chromophore content.

**5. References**


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