Effect of probe pressure on skin tissue optical properties measurement using multi-diameter single fiber reflectance spectroscopy

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Abstract

Multi-Diameter Single Fiber Reflectance spectroscopy (MDSFR) allows rapid and noninvasive extraction of tissue optical properties that requires the fiberoptic to be placed in contact with the tissue. To ensure a good optical contact, the application of probe pressure is inevitable and usually not well controlled. In this study, we investigated the effect of probe pressure by performing in vivo MDSFR measurements (400–1600 nm) on human inner forearm skin with controlled probe indentation and continuous pressure monitoring. For the probe pressures up to 28.4 s mm$^{-2}$ (213 mmHg), the reduced scattering coefficient $\mu_s$ pivoted around 800 nm (increasing scattering slope) while the phase function related parameter $\gamma$ decreased (up to 20%, depending on the wavelength). A significant decrease of hemoglobin concentration ($\sim 81\%$), oxygen saturation ($\sim 86\%$), apparent vessel diameter ($\sim 100\%$) as well as the displacement of extracellular water ($\sim 24\%$) was observed with increasing probe pressure. A correlation was observed between the changes in water volume fraction and phase function related parameter gamma $\gamma$. We theorize that the latter changes in phase function may be due to the induced deformation of the tissue structure and the displacement of extracellular water. The extensive analysis of the pressure induced changes in the measured MDSFR spectra and the related changes in optical properties demonstrate that the effects of probe pressure must be minimized to avoid bias in the results.

1. Introduction

Elastic scattering spectroscopy has demonstrated considerable value in medical diagnosis and therapeutic planning due to the ability to noninvasively extract tissue optical properties and physiological parameters [1–4]. Many forms of spectroscopic techniques utilize a hand-held fiberoptic probe design, which has many practical advantages compared to fixed imaging geometries [5]. On easily accessible tissue such as skin, handheld probes allow easy and rapid measurements from different angles. Fiberoptic probes can also be easily integrated with endoscopic procedures so that difficult-to-reach places such as the esophagus can also be accessed. In all cases, a direct optical contact between the probe and the tissue is required when performing measurements. Therefore, operators usually gently push the probe onto the tissue surface, thereby applying pressure. Unfortunately, the application of the pressure is usually not well controlled [6] and may deform the measured tissue and thus induce tissue structure and composition changes. These changes may lead to changes in tissue optical properties. To study the underlying tissue physiological changes caused by the application of probe pressure, various studies have investigated the magnitude of these effects. Cerussi et al investigated the effect of operator-applied force on diffuse optical spectroscopy (DRS) by integrating a force transducer into a handheld fiberoptic probe and concluded that the force (the product of probe pressure and contact area) applied is not a significant source of variations of the extracted tissue.
optical properties and physiological parameters [5]. Reif et al found that the reduced scattering coefficient significantly increased along with a decrease of vessel diameter and oxygen saturation induced by probe pressure using a source-detection separation geometry [7]. Jiang et al, however, revealed a pressure-induced increase of total hemoglobin and oxygen saturation when using DRS [8]. These different observations make it difficult to draw general conclusions.

Single Fiber Reflectance spectroscopy (SFR) [9] is a promising technique for medical diagnosis based on extraction of optical properties such as reduced scattering coefficient and physiologically relevant parameters such as blood oxygen saturation. SFR has a smaller detection volume (\(<\text{mm}^3\)) compared to DRS (\(\text{mm}^3—\text{cm}^3\)). However, for quantitative extraction of scattering properties, two or more SFR measurements with different diameters are required, a technique that has been called Multi-diameter Single Fiber Reflectance spectroscopy (MDSFR). Brooks et al reported variations of in vivo MDSFR measurements of optical properties and vascular parameters (blood oxygen saturation, blood volume fraction and apparent blood vessel diameter) of human skin and attributed the variations in their measurements to the physiological changes induced by the contact force [6]. To study the effect of probe pressure on skin optical properties using MDSFR in a systematic manner, we performed in vivo visible to near-infrared MDSFR measurements on one volunteer’s inner forearm while varying the one-dimensional indentation onto the skin surface. The aim of measurements in the wavelength range 400–1000 nm was to extract tissue scattering optical properties, melanin, and vascular-related parameters. Measurements in the NIR wavelength range (1000–1600 nm) aimed at obtaining information of water and fat content. Tissue deformation is induced by indentation of the MDSFR probe. The probe pressure was measured simultaneously using a force transducer integrated. The MDSFR spectra are analyzed with a previously described semi-empirical model to extract tissue optical properties and understand the effect and mechanism of the pressure-induced changes [10].

2. Methodology

2.1. Experimental setup

The in-house made MDSFR spectroscopy system collects light in the visible (VIS) and near-infrared (NIR) wavelength region (figure 1). It utilizes two multimode low-OH measurement fibers of 400 (Polymicro) and 1000 \(\mu\text{m}\) diameter (Edmund). Broadband light (360–2500 nm) was emitted by two separate halogen light sources (Avantes HAL-S mini) and guided to the SMA connectors connecting the two measurement fibers. A fraction of the incident light was back-scattered and collected by the measurement fibers. The collected light was then delivered to the spectrometers. The VIS spectrometer (working spectral range: 200–1160 nm) was connected with both fibers via 2 shutters (Ocean Optics Inline Shutter) while the NIR spectrometer (working spectral range: 900–1750 nm) was connected only with the 1000 \(\mu\text{m}\) fiber without a shutter. The 400 and 1000 \(\mu\text{m}\) fiber were positioned and glued together in the center channel of a cylindrical aluminum tube of 5 mm diameter. The fiber and tube surfaces were aligned and polished at 15° angle to eliminate the internal reflection induced by the refractive index mismatch between the fiber and sample in contact. The whole system was operated electronically and controlled with a LabVIEW program (National Instruments, Austin Texas).

MDSFR measurements were done by switching on the light sources one by one and activating the appropriate shutter in the fibers leading to the VIS and NIR spectrograph. A measurement sequence generated a 400–1000 nm MDSFR spectrum for 400 and 1000 \(\mu\text{m}\) fibers and a 1000–1600 nm SFR spectrum in the NIR for the 1000 \(\mu\text{m}\) fiber only:

- source 1 is on, shutter 1 and 3 are open, the 1000 um fiber delivers and collects light. Both the VIS and NIR spectra are saved
- source 2 is on, shutter 2 and 4 are open, the 400 um fiber delivers and collects light. Only the VIS spectrum is saved.

The probe was inserted and tightly fitted in the channel of a cylindrical probe holder (25 mm diameter), which was attached to a motorized linear stage (Thorlabs, 25 mm linear range motorized translation stage). The stage controlled the one-dimensional indentation of the probe onto the surface of the skin. A force sensor (Futek, LSB200, 10 lb) was integrated to monitor the application of probe pressure continuously during the measurements.

2.2. MDSFR reflectance model

The MDSFR reflectance model used to analyze the VIS MDSFR spectra (wavelength range 400–1000 nm) in this study was described previously by Kanick et al [9]. In the following equations, it is assumed that all optical properties are wavelength dependent (and therefore, the spectroscopic signal is naturally wavelength...
Figure 1. Schematic of VIS-NIR MDSFR system with computer-controlled shutters, probe indentation, integrated force transducer, and data acquisition. The VIS spectrometer was connected with both the 400 \( \mu \text{m} \) and 1000 \( \mu \text{m} \) diameter fibers, while the NIR spectrometer was connected only with the 1000 \( \mu \text{m} \) fiber without a shutter. Both measurement fibers were integrated into a single measurement probe and mounted into a probe holder.

The single fiber reflectance measured with a fiber of diameter \( d \), \( R_{SF,d} \), is expressed by the product of the reflectance in absence of absorption \( R_{0, SF,d} \) and the effect of absorption \( A \) (equation (1)).

\[
R_{SF,d} = R_{0, SF,d} \cdot A.
\] (1)

The reflectance in absence of absorption measured by the fiber of diameter \( d \), \( R_{0, SF,d} \), can be expressed as:

\[
R_{0, SF,d} = \eta_{\text{limit}} \left( 1 + \rho_3 e^{-\rho_1 \mu'_s d} \right) \cdot \left[ \frac{(\mu'_s d)^{\rho_2}}{\rho_1 + (\mu'_s d)^{\rho_3}} \right]
\] (2)

where \( \eta_{\text{limit}} \) is the lower limit of the single fiber collection efficiency (equation (3)).

\[
\eta_{\text{limit}} = \left( \frac{NA}{n_{\text{medium}}} \right)^2
\] (3)

in which \( NA \) is the numerical aperture of the measurement fiber and \( n_{\text{medium}} \) is the refractive index of the medium in contact with the surface of the measurement fiber. \( \eta_{\text{limit}} \) is approximately 2.7% for an \( NA = 0.22 \) immersed in water (\( n_{\text{medium}} \approx 1.33 \)).

The reduced scattering coefficient \( \mu'_s \) is predefined using equation (4),

\[
\mu'_s = \mu'_s(\lambda_0) \cdot \left( \frac{\lambda}{\lambda_0} \right)^{-b}
\] (4)

in which \( \mu'_s(\lambda_0) \) is the reduced scattering coefficient at 800 nm and \( b \) is the scattering slope (\( b > 0 \)). We assume that the reduced scattering coefficient \( \mu'_s \) is fiber diameter independent.

Also appearing in equation (2), \( [\rho_1, \rho_2, \rho_3] \) are parameters [11] that are \( \gamma \) dependent \([2.31 \gamma^2, 0.57 \gamma, 0.63 \gamma^2]\), where \( \gamma \) is a wavelength-dependent parameter that characterizes the phase function which describes the angular dependence of scattered light (equation (5)) [12].

\[
\gamma = \frac{1 - g_2}{1 - g_1}
\] (5)

The parameters \( g_1 \) and \( g_2 \) are the first and second Legendre moments of the scattering phase function. \( g_1 \) is usually referred to as the scattering anisotropy factor. It is assumed that the scattering properties of the tissues sampled by different fibers are identical (i.e. homogeneous distribution of scatterers).
The MDSFR model was derived based on Monte Carlo simulations in which a Modified Henyey Greenstein phase function (MHG PF) was assumed (equation (6)) [9].

\[ P_{MHG} = \alpha P_{HG} + (1 - \alpha) \frac{3}{4\pi} \cos^2 \theta \]  

(6)

where \( \alpha \in [0, 1] \) was a factor that weights the contribution of Henyey Greenstein phase function (\( P_{HG} \)) and the added Rayleigh like scattering part. In this study, \( \alpha \) was fitted as a wavelength independent parameter. The first and second Legendre moments of an MHG can be expressed as [12], \( g_0 \) is the first Legendre moment (scattering anisotropy) of the Henyey Greenstein PF. \( \gamma \) is wavelength dependent and dimensionless.

\[ g_1 = \alpha g_{HG} \]  

(7)

\[ g_2 = \alpha g_{HG}^2 + \frac{2}{5} (1 - \alpha) \]  

(8)

Combining equations (7), (8) and (5), the parameter \( \gamma \) for the MHG PF is expressed by equation (9),

\[ \gamma = \frac{1 - \alpha g_{HG}^2 - \frac{2}{5} (1 - \alpha)}{1 - \alpha g_{HG}}. \]  

(9)

In previous MDSFR studies, \( \gamma \) was fitted as a free parameter at each wavelength [6, 13]. This approach significantly increased the number of fit parameters during the spectral fit, which might lead to over-fitting. A predefined model of \( \gamma \) as function of wavelength can avoid the potential overfit by reducing the number of fit parameters. Therefore, in this current study, \( \gamma \) is predefined with the model shown in equation (9) combined with a predefined model of \( g_{HG} \) suggested by Zhang et al [14], to reduce the risk of overfit. The predefined model of \( g_{HG} \) is derived from a mathematical fit on the results of Mie calculation of a phantom to mimic a MHG PF [15], where \( \lambda \) is the wavelength [400 to 1600 nm] and \( c \) is one of the fit parameter determining the exact shape of \( g_{HG} \).

\[ g_{HG} = 1 - c \lambda^2. \]  

(10)

In the case of human skin tissue, the effect of absorption \( A \) on the measured reflectance \( R_{SF,d} \) can be expressed using equation (11):

\[ A = e^{-\mu_a.d \cdot T_{layer}^2} \]  

(11)

in which the Beer Lambert term accounts for the absorption of light by blood [16], while the absorption of melanin pigments was expressed by the \( T_{layer}^2 \) term as in equation (11). \( T_{layer} \) is the transmission of a assumed superficially localized melanin layer described by Zhang et al [14].

\( \langle L_d \rangle \) in the Beer–lambert term in equation (11) is the effective path length of the detected photons by the fiber of diameter \( d \), which was previously derived by Kanick et al from Monte Carlo simulations (equation (12)) [9, 17].

\[ \frac{\langle L_d \rangle}{d} = \frac{C_{PF} \cdot 1.54}{(\mu_d^{0.18}) (0.64 + \mu_a^{0.64})} \]  

(12)

where \( C_{PF} \) is a phase function dependent parameter which equals to 0.68\( \gamma^{0.6} \). Please note that the effective pathlength and thus the sampling volume of the detected photons increases with increasing fiber diameter. Therefore, different fibers sample different tissue volumes. In case of an inhomogeneous distribution of absorbers, this may lead to a different average absorption coefficient due to possible inhomogeneous concentration of the absorbers on the scale of the measurement volumes. For this reason, the absorption coefficient is treated here as a fiber diameter dependent parameter, \( \mu_{a,d} \). In this study, \( \mu_{a,d} \) is defined as equation (13).

\[ \mu_{a,d} = F_{cor,d} \cdot \mu_{a,d} = F_{cor,d} \cdot C_{hemoglobin,d} \cdot [\text{StO}_2,d \cdot \mu_{a,HbO} + (1 - \text{StO}_2,d) \cdot \mu_{a,Hb}] \]  

(13)

In which \( \mu_{a,d} \) is the absorption coefficient of whole blood. \( \text{StO}_2,d \) is the blood oxygen saturation averaged over the measurement volume, determined by the fiber diameter. \( \mu_{a,HbO} \) and \( \mu_{a,Hb} \) are the micro-molar absorption coefficients of oxygenated and deoxygenated hemoglobin [mm\(^{-1}\) M\(^{-1}\)]. \( C_{hemoglobin,d} \) is the total concentration [\( \mu \text{M} \)] of hemoglobin and \( F_{cor,d} \) is a correction factor that accounts for the influence of the
inhomogeneous distribution of the blood which is determined by $\mu_{a,d}$ and effective blood vessel diameter
$D_{v,d}$ [$\mu m$] using equation (4) [16].

$$F_{\text{car},d} = \frac{1 - \exp[-\mu_{a,d} D_{v,d}]}{[\mu_{a,d} D_{v,d}]}.$$  (14)

The absorption of melanin pigments in skin tissue was described using a melanin layer model by Zhang
et al [14]. $T_{\text{layer}}$ in equation (11) is the transmission of an assumed superficially positioned melanin layer,
where only a surface fraction $f$ has melanin present; the incident photons can pass the other $1-f$ fraction
without encountering any melanin. The absorption of the layer has the same effect on all detected photons
by all fibers in the MDSFR measurements and is thus independent of the photon pathlength. When the
photons propagate through the fraction of the layer containing melanin pigments, the transmission can be
calculated using equation (15).

$$T_{\text{melanin}} = e^{-\mu_{a,mel} d_{\text{mel}}}$$  (15)

where $\mu_{a,mel}$ stands for the absorption coefficient of the melanin-containing fraction of the layer and $d_{\text{mel}}$
stands for the thickness of the melanin layer. When light was propagating through the fraction of the layer
not covered by melanin pigments, there is no attenuation of light due to melanin, thus $T_{\text{melanin,free}} = 1$.

The effective transmission of the melanin layer can be calculated using equation (16):

$$T_{\text{layer}} = f \cdot T_{\text{melanin}} + (1-f) \cdot T_{\text{melanin,free}} = 1 - f \cdot (1 - e^{-\mu_{a,mel} d_{\text{mel}}})$$  (16)

$\mu_{a,mel}$ is taken as the sum of the contribution of the absorption from two main melanin pigments:
eumelanin (black and brown) and pheomelanin (red) [18]:

$$\mu_{a,mel} = (\varepsilon_{\text{eumelanin}} C_{\text{eumelanin}} + \varepsilon_{\text{pheomelanin}} C_{\text{pheomelanin}}) \ln (10)$$  (17)

in which $C_{\text{eumelanin}}$ and $C_{\text{pheomelanin}}$ are the concentration of eumelanin and pheomelanin [mg · mm$^{-3}$];
$\varepsilon_{\text{eumelanin}}$ and $\varepsilon_{\text{pheomelanin}}$ are the corresponding extinction coefficients [mm$^{-1}$ · mg$^{-1}$ · mm$^3$] [19].

The transmission of the melanin layer is then re-written in equation (18) where the surface density of eumelanin
and pheomelanin $\kappa_{\text{eumelanin}}$ and $\kappa_{\text{pheomelanin}}$ [mg · mm$^{-2}$] are the product of the melanin layer thickness $d_{\text{mel}}$
[mm] and the concentration $C_{\text{eumelanin}}$ and $C_{\text{pheomelanin}}$ [mg · mm$^{-3}$] respectively.

$$T_{\text{layer}} = 1 - f \cdot \left(1 - e^{-\ln(10)} \left(\varepsilon_{\text{eumelanin}} \kappa_{\text{eumelanin}} + \varepsilon_{\text{pheomelanin}} \kappa_{\text{pheomelanin}}\right)\right).$$  (18)

The surface density of melanin pigments is used as the fit parameter rather than thickness and concentration
because fitting both the layer thickness and the concentrations individually would lead to a
dependency between these two parameters.

2.3. Quantitate absorption coefficient in NIR wavelength range from single 1000 µm fiber reflectance
spectra
The NIR spectra of the 1000 µm fiber were analyzed with a model described previously by Kanick
et al which allowed quantification of the absorption coefficient without the prior knowledge of scattering properties
using an optimized parameter set [$\rho_1$, $\rho_2$, $\rho_3$, $C_{PF}$], i.e. $\rho_1 = 6.82$, $\rho_2 = 0.969$, $\rho_3 = 1.55$ and $C_{PF} = 0.944$.
When analyzing the NIR spectra, the background scattering model was defined with a 4th order polynomial
as shown in equation (19) ($\lambda_0 = 800 nm$) [20].

$$\rho_{\text{S,NIR,eff}} = a_1 \cdot \left(\frac{\lambda}{\lambda_0}\right)^{-1} + a_2 \cdot \left(\frac{\lambda}{\lambda_0}\right)^{-2} + a_3 \cdot \left(\frac{\lambda}{\lambda_0}\right)^{-3} + a_4 \cdot \left(\frac{\lambda}{\lambda_0}\right)^{-4}.$$  (19)

$a_1$, $a_2$, $a_3$, and $a_4$ are the fit parameters determining the exact shape of $\rho_{\text{S,NIR,eff}}$. This approach allows
sufficient freedom to correct for the physically incorrect assumption that [$\rho_1$, $\rho_2$, $\rho_3$, $C_{PF}$] were independent
of the wavelength and $\gamma \cdot \rho_{\text{S,NIR,eff}}$ is no longer the reduced scattering coefficient, but the effective reduced
scattering coefficient to account for the background scattering.

Melanin related parameters were not fitted again from the NIR spectra. The absorption of the melanin
pigments of the NIR spectra was described using melanin related parameters fitted from the VIS MDSFR
spectra obtained with the same indentation and the layer model described in section 2.2. The absorption coefficient was expressed in equation (20) as the sum of the contribution of blood (equation (13)), water and fat [21].

\[
\mu_{a,nir} = \mu_{a,d}^{t} + \mu_{a,water} + \mu_{a,fat}. \tag{20}
\]

The absorption spectrum of water changes subtly with temperature. To get an accurate fit we corrected the water absorption for temperature changes using equation (21).

\[
\mu_{a,water} = \mu_{a,water}(34 \text{ °C}) + \left(34 - T_{body}\right) \cdot \text{Corr}_{T,water} \tag{21}
\]

where \(\mu_{a,water}(34 \text{ °C})\) is the absorption coefficient of pure water at 34 °C measured by Nachabé et al [21]; \(T_{body}\) is the temperature of the body water within the sampling volume; \(\text{Corr}_{T,water}\) is the temperature correction coefficient for light absorption by water [\(\text{m}^{-1} \text{°C}^{-1}\)] [22].

The absorption of melanin pigments and blood were not fitted as free parameters in the analysis of the NIR spectra, but the fitted vascular parameters generated from the analysis of the VIS MDSFR spectra were used for the NIR wavelength range.

### 2.4. Data acquisition and analysis

The probe was warmed up to skin surface temperature (~32 °C) by immersion in warm water before being positioned on the skin of the volunteer with gentle contact. Water was applied between the probe and the skin to ensure a good optical contact prior to the measurements. The absolute reflectance of the skin was calculated using equation (22):

\[
R_{skin,d} = R_{IL,d} \times \frac{(I_{skin,d} - I_{b,d})}{(I_{IL,d} - I_{b,d})} \tag{22}
\]

where \(I_{skin,d}, I_{b,d}, I_{IL,d}\) stand for the signal intensity of skin tissue, dark current and undiluted Intralipid 20% measured with the fiber of diameter \(d\). \(R_{IL,d}\) is the absolute reflectance of an undiluted Intralipid 20% sample, determined using the Fresnel reflection method with flat polished 400 and 1000 \(\mu m\) fibers from the same batch of the fibers used in the probe [23]. The reflected intensity of undiluted Intralipid 20% \(I_{IL,d}\) and background intensity \(I_{b,d}\) were measured prior to the measurements on each volunteer to account for the fluctuation of the output of the light source and the ambient light. \(I_{b,d}\) was measured by immersing the probe in deionized water in a black container with light on.

The probe pressure was recorded continuously by the integrated force transducer throughout the whole measurement process. The first in vivo MDSFR measurement was performed after the probe was positioned in gentle contact (zero indentation) with the inner forearm skin of the volunteers in sitting position. The measurement site was located at the level of the heart. An in vivo MDSFR measurement was then performed after each new indentation of the probe. The step of the indentation was 0.5 mm. The maximum indentation depth is 13 mm. Ten spectra were obtained at each indentation and the average spectrum and standard deviation were calculated. A non-linear least-squares fit of the MDSFR model (equation (1)–(18)) was performed simultaneously on the spectra obtained from the 400 and 1000 \(\mu m\) fibers from 400 to 1000 nm. Subsequently, a second non-linear least-squares fit based on the SFR model (equation (1)–(3), (11)–(21)) was performed on the spectra obtained from the 1000 \(\mu m\) fiber from 1000 to 1600 nm. The following parameters were fitted from the measured VIS MDSFR spectra:

- the reduced scattering coefficient at 800 nm: \(\mu_{s}'(\lambda_0)\) [\(\text{mm}^{-1}\)]
- the scattering slope: \(b\) [\(\text{--}\)]
- a weighing factor of the contribution of Henyey Greenstein phase function (HG PF) and added Rayleigh like scattering part: \(\alpha\) [\(\text{--}\)]
- a parameter determining the exact shape of \(g_{HG}\): \(c\) [\(\text{--}\)]
- the surface density of eumelanin and pheomelanin: \(\kappa_{eumelanin}\) and \(\kappa_{pheomelanin}\) [\(\text{mg} \cdot \text{mm}^{-2}\)]
- the surface fraction of the melanin layer: \(f\) [\(\text{--}\)].

The vascular parameters are considered to depend on fiber diameter and were fitted separately for the spectra of the 400 and 1000 \(\mu m\) fibers:

- the concentration of hemoglobin: \(C_{hemoglobin,d}\) [\(\mu M\)]
- the oxygen saturation: \(StO_2,d\) [%]
- the vessel diameter: \(D_{v,d}\) [\(\mu m\)].
The following parameters were fitted from the measured NIR SFR spectra of the 1000 μm fiber:

- parameters determining the exact shape of $\mu'_\text{NIR, \text{off}}$: $a_1, a_2, a_3$ and $a_4$ [mm$^{-1}$]
- the volume fraction of water: $V_{\text{water}}$ [-]
- the volume fraction of fat: $V_{\text{fat}}$ [-]
- the temperature of body water: $T_{\text{body}}$ [°C]

The measurement error of the spectra at each wavelength obtained from each fiber was used as a weighing factor in the fit so that spectral regions with large standard deviations due to instrument noise had less influence on the fit. The fit algorithm minimized the reduced Chi-square, $\chi^2_{\text{red}}$, the difference between measurement and model, weighed by the measurement error:

$$
\chi^2_{\text{red}} = \frac{\sum_{i=0}^{\text{max}, \text{sec}} \left( \frac{R_{i,n} - M_{i,n}}{\sigma_{i,n}} \right)^2}{\nu}
$$

and $R_{i,j}$, $\sigma_{i,j}$ and $M_{i,j}$ stand for the reflection measurement, the measurement error and the model value at wavelength $i$ of the spectrograph with fiber number $j$, $\nu$ for the number of degrees of freedom calculated from $\nu = n - m - 1$, with $n$ the total number of measurements (i.e. wavelengths in all spectra of all fibers) and $m$ for the total number of model parameters in the fit. The model was used to fit the measurement spectra by varying the model parameters until the lowest possible value for $\chi^2_{\text{red}}$ was obtained. The confidence interval of each fitted parameters was estimated using the approach described previously by Amelink et al [24]. The 95% confidence intervals were calculated from the square root of the diagonal elements of the covariance matrix multiplied by 1.96, where the covariance matrix was obtained by multiplying the inverse of the second derivative matrix of $\chi^2$ with respect to its free parameters by $\chi^2/\nu$.

3. Results

The force applied during the probe indentation was measured continuously by a force transducer integrated between the motorized stage and the fiberoptic probe. The applied pressure calculated based on the readout of the force transducer and the size of the contact area between the probe and the skin are plotted in figure 2. The relationship between probe pressure and indentation is nonlinear and demonstrated a sawtooth wave shape. Every peak corresponds to one step in probe indentation, after which a gradual decrease of the probe pressure is observed until another probe indentation step is executed. The peak probe pressure corresponding to each probe indentation is plotted in figure 3, ranging roughly from 3 to 28.4 mN mm$^{-2}$ (23–213 mmHg) while the normal blood pressure range of adults is 10.7 to 16.0 mN mm$^{-2}$ (80–120 mmHg). There is no pressure build-up when the probe indentation increased from 0 to 1 mm; the probe pressure remains 3 mN mm$^{-2}$ (23 mmHg), which indicates the probe was still in gentle contact. Thus, the reflectance spectra and respective tissue optical properties of ‘gentle contact’ are redefined as the average of first three measurements.

The reflectance spectra of the 1000 μm fiber acquired under increasing probe pressure (figure 4) depict that both the reflectance amplitude and the absorption features change. The increase of the reflectance amplitude in the visible wavelength range is obvious and the blood absorption feature between 400 and 650 nm gradually disappears as the pressure increases. The change in the reflectance amplitude becomes less visible with the increase of the wavelength. It also shows that the absorption of water is influenced by the probe pressure since the dip around the water absorption peak at around 1450 nm gets smaller with the increase of the wavelength.

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The reduced scattering coefficient at 800 nm $\mu'_i (\lambda_0)$ and the scattering slope $b$ were fitted from each pair of VIS SFR measurement spectra of each indentation. The fitted $\mu'_i (\lambda_0)$ and scattering slope $b$ are plotted in figure 6 together with the respective probe pressure. The errorbars represents the 95% confidence intervals of the fit. The fitted $\mu'_i (\lambda_0)$ ranges from 1.03 to 1.09 mm$^{-1}$ without significant change with the increase of the pressure, while the fitted scattering slope $b$ increases from 1.8 to 2.3 as the pressure increases.

The reduced scattering coefficient was predefined as equation (4). The spectra of the reduced scattering coefficient, calculated based on the average $\mu'_i (\lambda_0)$ and $b$ shown in figure 6, are depicted in figure 7 (left). The reduced scattering coefficient ranges from 3.6 to 5.2 mm$^{-1}$ at 400 nm and it ranges 0.63 to 0.71 mm$^{-1}$.
at 1000 nm. The reduced scattering coefficient spectra pivot around roughly 800 nm: the reduced scattering coefficient increases with the increase of the indentation from 400 nm to the regime around 800 nm. The magnitude of the increase decreases with increasing wavelength. No significant changes are observed around 800 nm while the reduced scattering coefficient decreases as the wavelength increases further from 800 nm, while the magnitude of the decrease increases with the increase of the wavelength.

Combining the fitted weighing factor of the contribution of Henyey Greenstein phase function (HG PF) and added Rayleigh like scattering part: $\alpha$ and the fitted $c$ determining the exact shape of $g_{HG}$, the spectra of $\gamma$ were calculated using equations (9) and (10) and plotted in figure 7 (right). The spectra of gamma demonstrate an ascending trend as the wavelength increases. The value of gamma decreases with the increase of the probe pressure. The amplitude of the decrease is also wavelength dependent. Gamma values range from 1.0 to 1.1 at 400 nm and from 1.54 to 1.6 at 1000 nm.

The melanin pigments related parameters: the surface density of eumelanin $\kappa_{eumelanin}$ and pheomelanin $\kappa_{pheomelanin}$ [mg · mm$^{-2}$] and surface fraction $f$ [-] were generated from the fit of the measured VIS MDSFR.
Figure 4. The reflectance spectra of the 1000 µm fiber measured under different probe indentations, showing a decrease in light absorption by blood, an increase in the scattering slope (pivoting around 800 nm) and a decrease in light absorption by water.

Figure 5. The measured VIS MDSFR (400 µm fiber: blue dot; 1000 µm fiber: red dot) and NIR SFR spectra (1000 µm fiber: black dot) measured with the probe in gentle contact with skin tissue (zero indentation) and the respective best fit (400 µm fiber VIS: blue solid line; 1000 µm fiber VIS: red line; 1000 µm fiber NIR: black line). The error bars indicate the 95% confidence interval of 10 sequential measurements.

spectra. The surface density of eumelanin $\kappa_{\text{eumelanin}}$ (left) and surface fraction $f$ (right) are plotted in figure 8. The errorbar represents the 95% confidence intervals of the fit. The surface density of pheomelanin $\kappa_{\text{pheomelanin}}$ is not plotted because the fitted values are extremely low ($10^{-12}$ mg · mm$^{-2}$). The surface density of eumelanin $\kappa_{\text{eumelanin}}$ increases gradually from 1.01 to 1.63 [$10^{-3}$ mg · mm$^{-2}$] with increasing indentation. The surface fraction $f$ of the melanin layer varies between 0.25 and 0.28 and does not show obvious trend of changes as the probe pressure changes.

The fitted vascular parameters: oxygen saturation [−], hemoglobin concentration [$\mu$M] and vessel diameter [$\mu$m], are fiber diameter dependent. The vascular parameters fitted based on the VIS spectra acquired from the 1000 µm fiber and the respective 95% confidence intervals are shown in figure 9. The
fitted blood oxygen saturation ranges from 16% to 0.54%. The oxygen saturation increases in the beginning with increasing probe pressure. The oxygen saturation starts to drop after the probe pressure exceeds 12.6 mN mm\(^{-2}\) (95 mmHg). The fitted hemoglobin concentration demonstrates a gradual descending trend (from 8.6 to 2.5 \(\mu\)M) with the increase of the probe pressure until the probe pressure reaches 15.7 mN mm\(^{-2}\) (118 mmHg), after which, the hemoglobin concentration remains roughly constant even the pressure goes up further. The fitted vessel diameter demonstrates a similar descending trend (from 5 to 0 \(\mu\)m) to the fitted hemoglobin concentration until 15.7 mN mm\(^{-2}\) (118 mmHg) as well, after which, no further changes are observed with the increase of the pressure. The fitted hemoglobin concentration based on the spectra of the 400 \(\mu\)m fiber is extremely low (\(\sim10^{-13}\) \(\mu\)M), thus not shown here.

Water, fat volume fraction [-] and the body water temperature [\(^\circ\)C] are fitted based on the NIR spectra acquired from the 1000 \(\mu\)m fiber and plotted in figure 10 together with the respective 95% confidence intervals. The fitted water volume fraction gradually decreases from 0.47 to 0.36, while the fitted fat volume

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**Figure 6.** The fitted scattering amplitude \(\mu_s(\lambda_0)\) (left) and scattering slope \(b\) (right) as a function of probe pressure. The scattering amplitude remained constant, while the scattering slope increased with increasing probe pressure.

**Figure 7.** Left: The reduced scattering coefficient as a function of wavelength acquired for different probe pressures, showing pivoting of the spectrum around 800 nm, with the scattering slope increases with increasing pressure; right: Gamma \(\gamma\) as a function of wavelength as determined for different probe pressures. The fitted \(\gamma\) decreased with increasing probe pressure.
Figure 8. Effect of the probe pressure on the eumelanin surface density (left panel) and melanin surface fraction (right panel). The eumelanin surface density increased with increasing probe pressure while melanin surface fraction remained constant.

Figure 9. The effect of probe pressure on blood oxygen saturation, hemoglobin concentration and vessel diameter. The oxygen saturation increased for small increasing probe pressures but dropped after the probe pressure exceeds 12.6 mN mm$^{-2}$ (95 mmHg). Both hemoglobin concentration and vessel diameter demonstrated a gradual descending trend with increasing probe pressure till 15.7 mN mm$^{-2}$ (118 mmHg), after which both parameters remained constant.

fraction increases slightly as the probe pressure increases. The fit results show that the body water temperature did not change significantly during the measurements and remained rather stable between 31 to 32 °C.

4. Discussion

4.1. Probe indentation, pressure application, and tissue deformation
During the process of the one-dimensional probe indentation perpendicular onto the skin surface, external pressure is applied on skin tissue, as a result, tissue in contact with the probe is deformed. This deformation leads to the changes in the underlying structure and compositions, such as the collagen fiber alignment and the concentration or the volume of various body liquids. These changes alter the measurement of
Figure 10. The effect of probe pressure on fitted water (blue), fat volume fraction (black) and body water temperature (red). The water volume fraction decreased while fat volume fraction demonstrated a slight increase with increasing probe pressure. The body water temperature remained roughly constant.

MDSFR/SFR spectra; thus, the extracted tissue optical properties and the physiological information do no longer describe the natural status (without deformation). Previous studies focused on the effects of probe pressure \[25\text{–}27\], while the term 'tissue deformation' remained untouched. The mechanical properties of different tissues, or even of the same tissue of different individuals vary, thus the same probe pressure might generate different degrees of tissue deformation \[28\], which further lead to different amounts of changes in tissue structure and composition. An instant increase of the probe pressure was observed every time when the probe indented further as shown in figure 2, which was followed by a gradual decrease of the probe pressure. The decrease is due to the stress-relaxation of skin tissue as skin tissue is viscoelastic \[29\]. In this study, we, therefore, decided to perform MDSFR/SFR measurements right after each probe indentation. Thus, we correlated the probe pressure around each peak (labeled in figure 2) to each set of MDSFR/SFR measurements. The increase of probe pressure per indentation increases, while the speed of the stress decay also increases because of the increase of the level of the probe pressure \[30\text{,}31\]. It is observed from figure 2 that the build-up of probe pressure only happened after the 3rd indentation, where probe pressure can be interpreted as a sign of firm physical contact between the probe and the measured skin tissue.

4.2. Probe pressure effects on MDSFR/SFR spectra
The effects of the probe pressure on both the reflectance amplitude and the absorption features are shown in figure 4, which indicate the changes in tissue scattering and absorption properties respectively. A reflectance ratio defined as the ratio of the reflectance spectrum of the 1000 \(\mu m\) fiber measured with the spectrum measured when the fiberoptic probe was in gentle contact (zero indentation) is depicted in figure 11. An obvious increase of the reflectance ratio was observed accompanied by the gradual disappearance of the blood absorption feature between 400 and 650 nm with the increase of the indentation. The increase of the reflectance was up to 20% in the wavelength range where the blood absorption is strong while the reflectance ratio reduces to below 2% at higher wavelengths. In the NIR wavelength range, a similar increase of the reflectance was observed around the water absorption peak at around 1450 nm, which is an indication of changes in water content within the optical sampling volume. The reflectance in the wavelength range of 1150 to 1350 nm and 1550 to 1600 nm decreased with increasing pressure. The difference in the trend of the reflectance changes in different wavelength regime suggests changes in the tissue scattering phase function induced by the probe indentation. Lim \textit{et al} reported a similar wavelength-dependent reflectance changes during \textit{in vivo} DRS measurements on human neck and forehead skin tissue under probe pressure of 124 and 42 mN mm\(^{-2}\) respectively. Lim \textit{et al} also reported the time duration effect of probe pressure on the reflectance \[26\]. In our study, however, the probe pressure is much lower compared to the probe pressure used by Lim \textit{et al}. Besides, the increase of the pressure was gentle and slow. Each set of measurements at each indentation took several seconds, which also give the physiological changes of tissue time to occur. Therefore, we believe that, in our experiment design, the time duration of probe pressure did not have a significant
influence. Cugmas et al performed in vivo NIR DRS measurements on three proximate skin sites of the human hand (Above muscle, bone, and vein) under dynamic pressure control. He found similar phenomena to what we observed in our NIR SFR spectra, e.g. the applied contact pressure induced a decrease in the reflectance in the wavelength range where there was no significant water absorption and an increase the reflectance where water absorption was strong [32]. Cerussi et al did not observe any significant changes (within several %) induced by contact force in either the scattering nor the absorption, however, the size of the contact area of the probe used was significantly larger than for our probe, while their contact pressure was more than one order of magnitude smaller than the pressure range in our study.

4.3. Probe indentation/pressure effects on skin tissue optical properties

4.3.1. Probe indentation/pressure effects on skin tissue absorption properties

As shown in figure 9, the hemoglobin concentration and vessel diameter decreased with increasing probe indentation and pressure until the probe indentation reached 9.5 mm with respective probe pressure of 15.7 mN mm$^{-2}$ (118 mmHg), where the vessel diameter reduced to almost 0 µm and the hemoglobin concentration also decreased to a minimal value of approximately 2.5 µM. The probe indentation increased further to 12 mm with a respective probe pressure of 28.4 mN mm$^{-2}$ (213 mmHg), however, neither the vessel diameter nor hemoglobin concentration changed further. The decrease of vessel diameter and hemoglobin concentration was intuitively expected since the probe indentation would compress the skin tissue and gradually would block blood vessels. Blood vessels seemed to be completely blocked when probe pressure reached 15.7 mN mm$^{-2}$ (118 mmHg) which was approximately the upper limit of the ideal blood pressure of an adult (120 mmHg, ∼16 mN mm$^{-2}$). Previous studies have reported the decrease of hemoglobin concentration and vessel diameter as a result of tissue compression frequently in various situations such as in vivo skin measurements [7, 26] and mammography procedures [33, 34]. Cugmas et al [32], and Jiang et al [8], however, stated the opposite based on their research findings that the applied contact pressure increased the concentration of hemoglobin. Jiang et al attributed the increases of hemoglobin concentration to the vasodilation that might be induced by the prolonged pressure application, which is supported by research findings on pressure-induced vasodilation (PIV). PIV was found to increase the blood flow or delay the decrease in cutaneous blood flow produced by local application of low pressure to the skin [35, 36]. Blood oxygen saturation demonstrated a surprising increase from 37% to above 50% in the first half of the probe indentation process. We expected, however, a drop in blood oxygen saturation due to the blockage of the blood flow, which was expected to hinder the oxygen supply. Jiang et al reported a similar increase of blood oxygen saturation in a similar study on the pressure effects where they applied continuously increasing pressure on human breast tissue, which they attributed to vasodilation [8]. However, we did not observe an increase of the blood vessel diameter in our study.
As shown in figure 10, the water volume fraction decreased by more than 30% when probe pressure reached 28.4 mN mm$^{-2}$ (213 mmHg). No bruise or visual signs of tissue damage were shown post measurements, which indicated body water displacement did not comprise intracellular water but only extracellular water, including both intravascular (blood plasma) and interstitial fluid. It was observed that the decrease of hemoglobin concentration stopped when the probe indentation reached 9.5 mm with the respective probe pressure of 15.7 mN mm$^{-2}$ (118 mmHg), while the water volume fraction continued to decrease for the whole process of probe indentation. The slope of the water volume fraction—probe pressure curve decreased as the probe pressure increased. The pressure-induced decrease of water volume fraction was also observed previously [8, 33], however, Cugnas et al observed an slight increase of the water volume fraction (∼3%) as a result of the probe pressure, while the underlying mechanism was, unfortunately, not explained. In our study, the fat volume fraction increased slightly from 0.08 to above 0.11, which might be due to the sampling depth change caused by the displacement of extracellular water.

The melanin layer surface fraction did not change while the surface density of eumelanin increased as the probe indentation and pressure went up, which might be caused by the stretching of skin tissue and melanin layer.

4.3.2. Probe indentation/pressure effects on skin tissue scattering properties

It was expected that the water loss caused by probe pressure might increase the density of scatterers within the sampling volume and consequently induce an increase of the reduced scattering coefficient, however, the scattering slope $b$ did change significantly, besides the changes of gamma $\gamma$ shown in figure 7 (right), all indicate the changes in tissue scattering phase function.

One explanation of the changes in the phase function is the deformation of tissue structures induced by the probe pressure. The scattering slope $b$ is usually related to the mean size of the scattering particles, which further links to the anisotropy factor of the scattering media [18]. The increase of the scattering slope $b$ indicates that the mean size of the scattering particles decreases within the optical sampling volume, which maybe due to the deformation of submicron tissue structures such as mitochondria and lysosomes [18]. Furthermore, as the mean size of scattering particles decreases, the anisotropy factor decreases and the contribution of the isotropic scattering increases [18]. The increasing contribution of isotropic scattering also correlates with the decrease of $\gamma$ shown in figure 7 (right), which indicates the increase of back-scattering. Reif et al, however, reported an decrease of the scattering slope after pressure application during in vivo DRS measurements on mouse thigh muscle, which they attribute to an increase of the density of large organelles per unit volume [7]. Marquez et al found that both the scattering amplitude and slope of chicken breast tissue varied with different probe orientations during oblique incidence reflectometry measurements which they attributed to different alignments muscle fibers [37]. Nickell et al also reported a directional dependence of light propagation through skin. This anisotropy is believed to be caused by the preferential orientation of collagen fibers [38]. In skin tissue, collagen fibers are expected to contribute to scattering and have a dominant orientation which is parallel to the skin surface [39]. In our measurement, the alignment of collagen fibers was very likely to be changed by the probe pressure, which would lead to phase function changes in the underlying skin tissue. Another explanation for the changes of scattering properties could be that light sampled deeper into the dense connective tissue layer of the dermis, which is rich in high scattering collagen [26].

Besides the deformation of tissue structures, the displacement of extracellular water could be another reason for the changes in tissue scattering phase function. Abundant scattering particles exist in extracellular water such as vesicles [40–42]. The displacement of extracellular water carries away scattering particles and changes the refractive index mismatch between scattering particles and the surrounding medium (The refractive index of water $\approx$1.33; the refractive index of solid structures of skin tissue $\approx$1.43 − 1.53), which lead to changes in the contribution of scattering from scattering particles in extracellular water. Drew et al found that the light penetration depth increased subjected to compression, which they attributed to the changes in scattering phase function as a result of the decrease of tissue refractive index mismatch caused by the displacement of water, which made the skin tissue more forward scattering (anisotropy factor of skin tissue went up) [43]. This hypothesis is further supported by a clear correlation between gamma $\gamma$ at 800 nm and the water volume fraction in figure 12.

4.4. How to mitigate and make use of effects of probe pressure?

MDSFR/SFR has invaluable potentials in disease diagnosis for the ability of noninvasive and accurate extraction of tissue optical properties. It is demonstrated in this study that probe pressure had significant influence on MDSFR/SFR spectra, which can further lead to biased tissue optical properties and physiological parameters. The amplitude of changes induced by probe pressure also depend on the
Figure 12. The change in $\gamma$ at 800 nm correlated with the change of water volume fraction indicating a tissue phase function change as a result of body water displacement.

mechanical properties of the tissue measured. The softer the tissue is, the bigger deformation will be generated with the same level of probe pressure, the more uncertainty will be introduced to the fitted optical properties. For accurate determination of tissue optical properties using MDSFR/SFR, the effects of probe pressure should be minimized in order to avoid the pressure-induced bias in the results. However, the application of probe pressure is also essential to ensure a good optical contact during the measurements. Given what is shown in Figure 2, it is optimal to maintain the probe pressure between 3 and 3.7 mN mm$^{-2}$ (23–28 mmHg) on the measurement subject in this study to minimize the pressure effects and ensure a good optical contact. For general MDSFR/SFR measurements, it is useful to integrate a force transducer to fiberoptic probe to monitor the pressure continuously, although this might be challenging during endoscopy procedures and the optimal pressure might differ due to the variations in tissue mechanical properties. We do recognize the difference in our experiment design and real clinical measurements, where the probe is pressed against the skin surface and measurements are taken right away. In real clinical measurements, we expect to observe the same probe pressure effects reported in our study, but the amplitude of the changes might be smaller because less time was given for physiological changes to occur. However, it also depends on the time needed to perform the measurements (integration time, number of measurements to perform, time consumption for optical shutters to open and close, system communication time etc). The less it takes to complete a set of measurements, the less changes probe pressure induces.

It would be ideal to be able to track tissue deformation together with the probe pressure, which, however, remains challenging in practice. It is also recommended to design a relatively big contact area between the probe and tissue in order to reduce the pressure when using the same force and to lower the difficulty of probe orientation. In the situations where there is no pressure monitor, the reduced scattering coefficient at 800 nm might be an excellent optical marker for disease diagnosis, since $\mu_s$ around 800 nm was least influenced by probe pressure (within 5% change).

We do address the need to involve more subjects of various conditions in this study. Jiang et al found strong correlation between the body mass index (BMI [kg · m$^{-2}$]) of the volunteers and pressure-induced tissue optical properties. Lim et al reported different optical responses to probe pressure at different body sites (neck, forehead, and finger). We expect to expand this study by including more subjects of different age, gender, BMI and body sites.

Although probe pressure can cause many troubles in measurements using hand-held probes, its dependence on body sites has potential to be used for tissue classification. Cugmas et al claimed that the pressure-induced changes in the tissue optical properties were found to be site-specific and were modeled as a polynomial function of the applied contact pressure, resulting in a high (90%) average classification sensitivity and specificity [32].
5. Conclusion

We found that probe pressure had significant influence on the in vivo acquisition of MDSFR/SFR skin spectra, which further affected the extracted skin tissue optical properties. The changes in the optical properties can be explained by changes in the physiological parameters of tissue. For probe pressures below 15.7 mN mm$^{-2}$ (118 mmHg), which roughly equals to the upper limit of blood pressure of healthy adults, a significant decrease of hemoglobin concentration (from 8.7 to 2.8 µM) and blood vessel diameter (from 9.9 to $\sim$0.5 µm) was observed. For higher probe pressures, no further changes in hemoglobin concentration and blood vessel diameter were observed. Blood oxygen saturation, however, increased first (from 0.30 to 0.54) as the probe pressure went up to 12.6 mN mm$^{-2}$ (95 mmHg), and gradually decreased to 0.19 as the probe pressure reached 28.4 mN mm$^{-2}$ (213 mmHg). The water content decreased from 0.47 to 0.36 while a slight increase of the fat content (roughly from 0.08 to 0.11) was observed. The changes in scattering properties: $\mu_s$ and $\gamma$, were wavelength-dependent; $\mu_s$ pivoted with the increase of probe pressure around roughly 800 nm; $\gamma$ decreased as the probe pressure increased and the magnitude of the decrease decreased with the increase of the wavelength. We theorize that the wavelength dependence of changes in scattering properties relate to changes in tissue scattering phase function/anisotropy induced by the deformation of submicron tissue structures and the displacement of extracellular water. Clearly, the effects of probe pressure must be minimized during MDSFR/SFR measurements to avoid bias in the results.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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