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Influence of probe pressure on diffuse reflectance spectra of human skin measured in vivo

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Abstract. Mechanical pressure superficially applied on the human skin surface by a fiber-optic probe influences the spatial distribution of blood within the cutaneous tissues. Upon gradual load of weight on the probe, a stepwise increase in the skin reflectance spectra is observed. The decrease in the load follows the similar inverse staircase tendency. The observed stepwise reflectance spectra changes are due to, respectively, sequential extrusion of blood from the topical cutaneous vascular beds and their filling afterward. The obtained results are confirmed by Monte Carlo modeling. This implies that pressure-induced influence during the human skin diffuse reflectance spectra measurements in vivo should be taken into consideration, in particular, in the rapidly developing area of wearable gadgets for real-time monitoring of various human body parameters.

Keywords: human skin; diffuse reflectance spectra; probe pressure; cutaneous vascular beds.

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1 Introduction

Since recently, in addition to practical routine biomedical diagnostic applications, noninvasive measurements and accurate quantitative analysis of the reflectance spectra of human skin have an exponentially growing interest from the industries associated with the sportswear design and smart well-being sensors, e.g., for an assessment of physical load during sports activities. Understanding of the impaired oxygen delivery in the skin is vital for explaining the etiology of a variety of diseases, including venous ulceration and diabetic neuropathy, whose origin lies in a disturbed skin blood microcirculation,

However, the absolute measurements of the blood oxygen saturation of the skin as well as of most other biological tissues are not trivial. A combination of the intense elastic light scattering and the heterogeneous distribution of blood within the tissues (both the spatial confinement of hemoglobin within microvessels and the organization of microvessels into physiologically distinct plexi) render the extraction of an accurate and clinically interpretable blood oxygen saturation value from a measured reflectance spectrum that is extremely difficult. In the last decade, a number of theoretical and experimental techniques have become available and potentially capable to elucidate the issues surrounding the use of optical reflectance spectroscopy for noninvasive measurements of skin blood oxygenation in vivo. There is also an intensively growing interest in the skin blood flow and skin blood microcirculation quantitative measurements in the frame of various clinical applications.

Obtaining reliable results and their further standardization require well-controlled probing conditions of the experiment. In Ref. 5, an automated system was developed and used to measure reflectance in the visible (450 to 850 nm) and near-infrared (950 to 1600 nm) spectral ranges upon loading/unloading. The objects were pig feet ex vivo and human palms above the abductor pollicis brevis muscle in vivo. The linearly increasing pressure introduced by the system was up to 70 or 150 kPa, but no indication of the pressure step was given. In the visible range, an increase in the diffuse reflectance was detected for both types of samples upon increased pressure despite the absence of blood in the porcine tissue. The dependence of the reflected intensity on pressure was obtained, although no specific attention was paid to the features of the spectrum at subtle pressure (up to 35 kPa). In Ref. 6, 950- to 1600-nm spectral range was used for diffuse reflectance measurements due to its higher sensitivity to the probe pressure than that in “the optical window” (650 to 900 nm) mainly due to difference in water absorption. Certain recommendations were proposed for three scenarios (fully automated application, probes with integrated springs, and manual operation) to limit the effect of contact pressure on classification performance of diffuse reflectance setups with fiber-optic probes. In the latter scenario, a mean contact pressure is recommended to be about 35 kPa for the best soft-tissue classification.

No detectable effects of the external pressure of 7, 14, and 21 N/cm² (that is, 70, 140, and 210 kPa) posed by a probe were found on the cervix fluorescence in vivo measurements with the excitation at 320 to 470 nm and detection at 330 to 700 nm due to insufficient pressure to alter blood flow.

The autofluorescence of reticular dermis of the human skin with a reduced blood volume was measured in vivo by application of the external mechanical pressure on the probe within 0 to 140 kPa. Significant increase in the fluorescence intensity was observed from a mouse liver and heart upon pressure up to 5 N/cm² (50 kPa). Here, the probe pressure-induced alteration of the local hemodynamics and resulted in the local ischemia. The difference in elastic properties of the two biotissues caused different thresholds for pressure detection: 2.58 N/cm² (25.8 kPa) for the liver and 4.06 N/cm² (40.6 kPa) for the heart.

A set of probe pressures of 4, 9, 13, 17, and 20 N/cm² (40, 90, 130, 170, and 200 kPa) were applied continuously on a mouse thigh muscle in vivo. In contrast to in vitro studies, the reflectance increased. The reflectance spectra within 350 to 700 nm were fitted with a model using parameters such as blood
volume fraction, hemoglobin oxygen saturation, blood vessel radius, and reduced scattering coefficient described by the exponent of the power-law or the Mie theory. This resulted in an increase in the reduced scattering coefficient at the 700-nm wavelength. The applied pressure induced compression of blood vessels, thus reducing the blood vessels radii and blood oxygen saturation. However, the blood volume fraction was varied >20% for the pressure applied and does not seem to follow the trend. This led to the conclusion that the blood volume fraction did not depend on the probe pressure.

The light reflectance measurements for a probe placed on a forearm with the pressure of 0.202, 0.388, 0.576, 0.787, and 9.33 kPa were achieved for male and female volunteers with dark, light dark, and light skin. The obtained results were analyzed in the framework of the diffusion theory in the case of reduction in the blood content and constant blood content. An increase in the scattering was observed in both cases.

When the probe pressure was applied onto the neck and forehead, the obtained results suggest that in both cases, extruding blood from the biotissue decreases the absorption. However, the mismatch between the refractive indices of the scatterers and the surrounding intracellular liquid reduces scattering in the case of the neck, whereas in the case of the forehead the scattering increases due to probing of collagen fibers.

Thus, the majority of previous studies suggest that the probe pressure significantly influences the reflectance of light and optical properties of biological tissues in vivo and confirm the importance of this mechanical parameter in the framework of routine optical measurements.

In the current study, we consider changes of reflectance spectra of human skin upon local application of subatmospheric pressure (up to 35 kPa) on the fiber-optic probe and discuss the observed peculiarities in the framework of blood content changes with Monte Carlo simulations used to support the findings.

2 Materials and Methods

A fiber-optic probe RP25 (Thorlabs) coupled with an Illuminator EK-1 Fiber Optic Light Source LE.5210-230 (EUROMEX, The Netherlands) with an integrated near-infrared blocking filter (a cut-off wavelength 1000 nm) and a portable spectrophotometer CCS200 (Thorlabs) operating within the 200- to 1000-nm wavelength range were used. The probe with a surface area of 32 mm$^2$ (diameter 6.4 mm) housed one illuminating fiber (200-μm core diameter, 220-μm cladding diameter, and NA = 0.22) surrounded by six collecting fibers (with the same parameters as the illuminating fiber). The probe was sequentially loaded with the round copper plates (10 g each). A waiting time of 20 s was used before recording the skin reflectance spectra. According to the University of Oulu Ethics Committee regulations, informed consent was obtained from all tested subjects. The measurements were performed on five volunteers (Caucasians, age: 27 to 37, Fitzpatrick skin types 2 and 3) on the right middle-finger pad. Two measurements were done on every person. The experimental arrangement is schematically shown in Fig. 1.

The recorded reflectance spectrum $S(\lambda)$ at each load was averaged over 20 spectra collected during 500-ms exposure time. The final spectrum $S_{\text{final}}(\lambda)$ was obtained by normalization on the lamp spectrum $S_{\text{lamp}}(\lambda)$ taking into account the dark spectrum $S_{\text{dark}}(\lambda)$, according to

$$S_{\text{final}}(\lambda) = \frac{S(\lambda) - S_{\text{dark}}(\lambda)}{S_{\text{lamp}}(\lambda) - S_{\text{dark}}(\lambda)}.$$  

3 Results

The reflectance spectra were recorded for the loading and unloading of the skin. Typical measured spectra are shown in Fig. 2. As one can see, the reflectance pits associated with the characteristic absorption peaks of the oxyhemoglobin (around 550- and 575-nm wavelengths) are well visible initially, whereas upon the increase in the load and, therefore, suppression of capillary loops and the upper blood net dermis, their amplitudes become reduced (see Fig. 2 upper curves).

Upon loading the probe, the pressure applied onto the skin and the blood capillaries increases. The pressure sequentially pushes the blood out of the capillaries in the blood-containing layers located at different depths, thus decreasing the blood content. This results in the reduced absorption of the blood in the green–yellow spectral range (525 to 575 nm), with the subsequent increase in the reflectance. The decrease in the blood content vanishes the oxyhemoglobin concentration within the
probing skin volume. This is illustrated by the blurring of
the representative two-pit pattern upon increase in the load
[Fig. 2(a)]. In the “red” spectral range (600 to 650 nm) the
reflectance increases, although no specific manner is detected.
Interestingly, we have also observed grouping of the reflectance
spectra upon the uniform pressure changes. This effect has never
been described in the literature, to our knowledge.

To elucidate the peculiarities of the reflectance spectra
broad range, two wavelengths from the “green” (λ = 550 nm) and
“red” (λ = 625 nm) spectral ranges were chosen and were fol-
lowed with the loading/unloading (Fig. 3). The values are drawn
in a stepwise (“staircase”) manner to highlight the features and
associate them with the changes in the physiological state of
the skin. It is distinctly seen that despite the equally changing load,
the reflected intensity changes nonuniformly: there are regions
both of moderate increase and jumps of the reflected intensity.
They are explained by the sequential clamping of the blood
vessels located at different depths within the skin. Additionally,
the relative change in the reflected intensity corresponding to
the highest and lowest pressure is twice larger for the green
light [see Fig. 4(a)] than for the red [see Fig. 4(b)].

Qualitatively similar changes in the baseline signal were
observed by photoplethysmography (PPG), upon 2–40 kPa
load. Although a suggestion about pressure-induced occlusion
of the superficial skin blood vessels was made, no quantitative
analysis was performed to reveal the reasons behind. Origins of
the more distinct PPG signal from human palms at green
(λ = 525 nm) than that at near-infrared (810 nm) illumination
upon external large area pressure (3.6 to 7.2 kPa) were explained
in frames of a new model accounting for pulsating arteries
pressing superficial capillaries from the skin interior.

4 Discussion
To interpret the experimental results, we performed Monte
Carlo simulations of the sampling volume using an online
freely available computational platform. We used a seven-
layer model of the skin accounting for stratum corneum
(50-μm-thick), living epidermis (80 μm), papillary dermis
(100 μm), upper blood net dermis (80 μm), reticular dermis
(1620 μm), deep blood net dermis (200 μm), and subcutaneous
fat (5900 μm). Optical parameters of skin layers caused by
the presence of water, oxy- and deoxyhemoglobin, melanin (eume-
lanin and pheomelanin), and the background (the “baseline”)
were taken from the literature. The effect of pressure was
simulated in such a way that the blood content of the three
uppermost blood-containing layers is eliminated sequen-
tially: initially, in the papillary dermis, then in the upper blood
net dermis and, finally, in the reticular dermis. The latter was
represented by two sublayers—with the thickness of 180 and
1440 μm (the blood is extruded from the thinner sublayer
only). To reveal subtle changes in the sampling volume during
the application of pressure, correlation maps between corre-
sponding components of the sampling volume matrices were
calculated in the following way:

\[ y_{ij} = e^{-x_{ij}}, \]

where \( x_{ij} = \frac{a_{ij} - b_{ij}}{\sigma_1} \); \( a_{ij} \) and \( b_{ij} \) are the elements of the sampling
volume matrices in the case of no pressure (initial state) and
upon loading, respectively. Defined in such a way, values of
the correlation matrix elements are within \([0, 1]\).

The correlation maps (Fig. 4) show the changes in the spec-
tral response upon application of the increased pressure when
illuminated with the green light. The staircase-shaped solid
curve [see Fig. 4(a)] can conventionally be divided into three
intervals: (1) 0 to 3 kPa, (2) 3 to 22 kPa, and (3) 22 to
34 kPa. The presented results are similar for all test subjects, in
terms of the stepwise reflectance response and of the location of
the three indicated regions (0 to 3 kPa, 3 to 22 kPa, and 22 to
34 kPa). The deviations of the reflectance intensity values within
those three bands are up to 5% for the green light and 2% for
the red light.

The initial level of pressure (0 to 3 kPa) corresponds to the
smallest introduced pressure [see Fig. 4(a)]. The white color
indicates that such a pressure causes no significant differences
compared with the intact skin. The intermediate level of pressure
(3 to 22 kPa) also does not significantly affect the skin
[see Fig. 4(b)]. The situation changes substantially, however,
when the pressure increases further (22 to 34 kPa), the correla-
tion values decrease in certain areas to 0.8 and even 0.6 [see
Figs. 4(c) and 4(d)]. The decrease in the load follows the similar
staircase-like trend [see Fig. 4(d), the dashed curve]. The longer
preserved relatively high reflected intensity can be explained by
ischemia and gradual filling of capillaries with blood.
The skin spectral response to the pressure at red (625 nm) light illumination also changes in a stepwise manner [see Fig.iliation also changes in a stepwise manner [see Fig. a solid curve] and the steps are less steep. Both phenomena are explained by one-order higher oxy- and deoxy-hemoglobin absorption at 550-nm wavelength compared to that at 625-nm wavelength see e.g. Refs. and 20. This results in less-contrasted correlation maps than those shown in Fig. B, with this in mind, Monte Carlo simulations for the latter case are not presented.

5 Conclusions

We showed experimentally that the application of even subtle pressure onto the skin surface in vivo alters the intensity of optical reflectance spectra in a stepwise manner. Despite the weights of the applied loads are equal, the steps of the corresponding reflected intensity are not. These stepwise changes are associated with the sequential extrusion of blood from the topical cutaneous vascular beds (such as papillary dermis, upper blood net dermis and reticular dermis), well supported by the results of Monte Carlo modeling. This implies that pressure-induced effects during skin optical measurements in vivo should be taken into account, in particular, in such rapidly developing area as application of wearable gadgets for monitoring human body parameters in real time.

Disclosures

The authors declare no conflicts of interest.

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