Journal of Biomedical Optics

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Alexey P. Popov, Alexander V. Bykov, Igor V. Meglinski, "Influence of probe pressure on diffuse reflectance spectra of human skin measured *in vivo*," *J. Biomed. Opt.* **22**(11), 110504 (2017), doi: 10.1117/1.JBO.22.11.110504.

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-like 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. [DOI: 10.1117/1.JBO.22.11.110504]

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

Paper 170582LR received Sep. 1, 2017; accepted for publication Oct. 27, 2017; published online Nov. 20, 2017.

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;[1,2] 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*.[2] 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.[3,4] 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/cm^2 (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.[7,8]

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.[9] Significant increase in the fluorescence intensity was observed from a mouse liver and heart upon pressure up to 5 N/cm^2 (50 kPa).[10] 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/cm^2 (25.8 kPa) for the liver and 4.06 N/cm^2 (40.6 kPa) for the heart.

A set of probe pressures of 4, 9, 13, 17, and 20 N/cm^2 (40, 90, 130, 170, and 200 kPa) were applied continuously on a mouse thigh muscle *in vivo*.[11] 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

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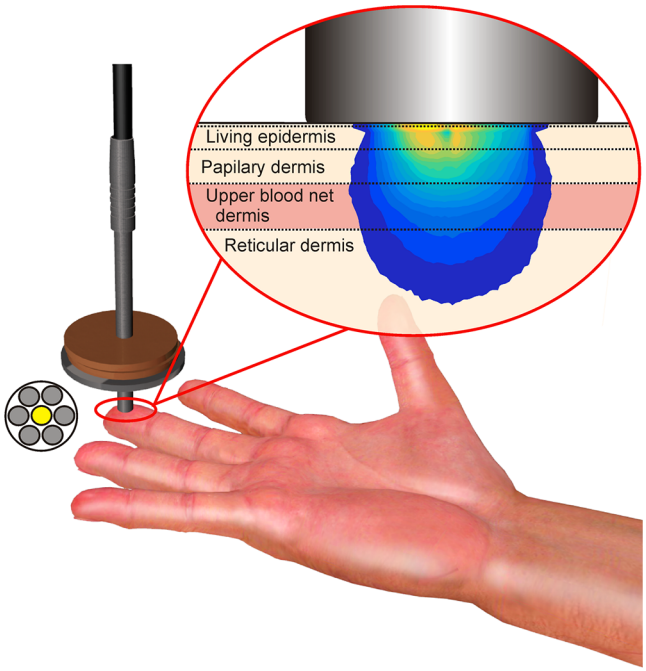

Fig. 1 Schematics of the setup used for pressure-affected skin reflectance studies.

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 0.933 N/cm^2 (2.02, 3.88, 5.76, 7.87, and 9.33 kPa) were achieved for male and female volunteers with dark, light dark, and light skin.[12] 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.[13] 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 subtle 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.

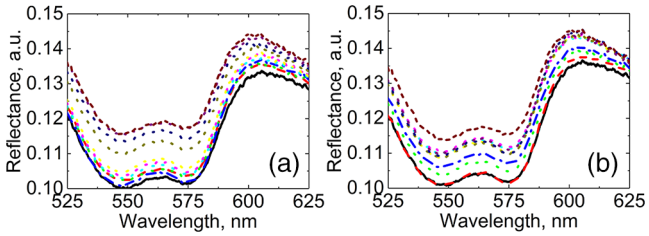

Fig. 2 Reflectance spectra of the human skin (right middle-finger pad) measured upon (a) loading from 0 up to 35 kPa and (b) unloading from 35 to 0 kPa with 10-g metal plates (pressure step equals 3.125 kPa). The lowermost and the uppermost curve correspond, respectively, to the lowest/no load (0 kPa) and the highest load (35 kPa).

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[6]

$$S_{\text{final}}(\lambda) = \frac{S(\lambda) - S_{\text{dark}}(\lambda)}{S_{\text{lamp}}(\lambda) - S_{\text{dark}}(\lambda)}. \quad (1)$$

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

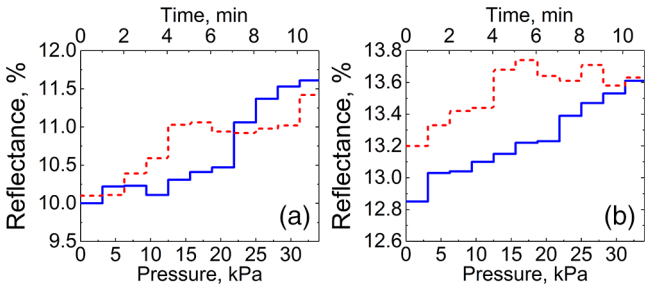

Fig. 3 Relative intensity at (a) 550-nm and (b) 625-nm wavelengths upon increasing (solid line) and decreasing (dashed line) probe pressure; corresponding values are taken from Fig. 2.

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 trend, two wavelengths from the "green" ($\lambda = 550$ nm) and "red" ($\lambda = 625$ nm) spectral ranges were chosen and were followed 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. 3(a)] than for the red [see Fig. 3(b)].

Qualitatively similar changes in the baseline signal were observed by photoplethysmography (PPG), upon $2-40$ kPa load.[14] 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 ($\lambda = 525$ 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 compressing superficial capillaries from the skin interior.[15,16]

4 Discussion

To interpret the experimental results, we performed Monte Carlo simulations of the sampling volume[17] using an online freely available computational platform.[18,19] 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 (eumelanin and pheomelanin), and the background (the "baseline") were taken from the literature.[17,20,21] The effect of pressure was simulated in such a way that the blood content of the three uppermost blood-containing layers is eliminated sequentially: initially, in the papillary dermis, then in the upper blood net dermis and, finally, in the reticular dermis. The latter was

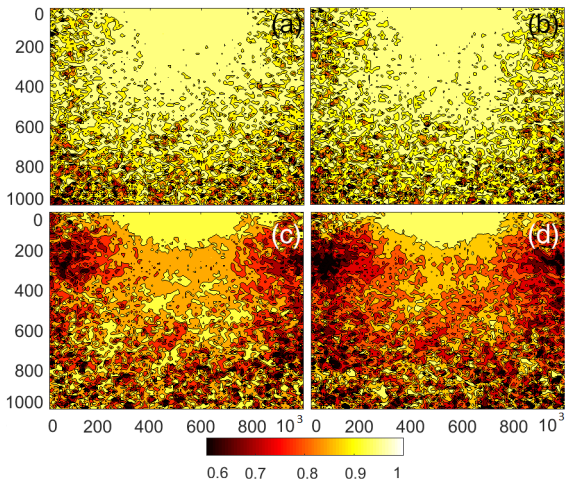

Fig. 4 Simulated in-depth correlation maps between the sampling volumes of the intact and the pressurized skin. Illumination is performed with 550-nm light. The effect of pressure is simulated by eliminating contribution of the blood from (a) the papillary dermis by 20% and (b) entirely, (c) from the upper blood net dermis and (d) upper part of reticular dermis. The white color corresponds to the highest degree of correlation—1, dark red—to 0.6. The numbers indicate depth (vertical) and length (horizontal) in micrometers.

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 corresponding components of the sampling volume matrices were calculated in the following way:

$$y_{i,j} = e^{-x_{i,j}}, \tag{2}$$

where $x_{i,j} = |\frac{a_{i,j}-b_{i,j}}{a_{i,j}}|$; $a_{i,j}$, $b_{i,j}$ 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 spectral response upon application of the increased pressure when illuminated with the green light. The staircase-shaped solid curve [see Fig. 3(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 kP) 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 correlation 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. 3(a), 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. 3(b)], although not significantly—the reflected intensity increases only 6% compared with 15% for the green light [see Fig. 3(a), the 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. 19 and 20. This results in less-contrasted correlation maps than those shown in Fig. 4; 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.

Acknowledgments

The authors are grateful to Dr. Matti Huiku (General Electric Healthcare) for fruitful discussion and valuable comments at the initial stage of the letter preparation. The Academy of Finland (grants 260321 and 290596), the Russian Science Foundation (project 15-14-10008), MEPhI Academic Excellence Project (Contract No. 02.a03.21.0005), and the Government of Russian Federation (Grant No. 074-U01) are acknowledged for financial support.

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