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Abstract. Contact pressure induced by manually operated fiber optic probes can significantly affect the optical properties of the studied tissue. If the contact pressure and the changes in optical properties are measured properly, then the complementary information can be used to obtain additional insight into the tissue physiology. However, as reliable assessment of the contact pressure in the existing diffuse reflectance setups is difficult, the impact of contact pressure is usually neglected or considered as a source of errors. We introduce a measurement system for controlled application of contact pressure and for the acquisition of diffuse reflectance spectra, which is suitable for *in vivo* studies and for overcoming the limitations of the existing measurement setups. A spectral-contact-pressure plane is proposed to present the combined information, highlighting the unique tissue response to the applied pressure. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.19.2.020501]

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1 Introduction

Diffuse reflectance spectroscopy (DRS) in the visible (VIS) and near-infrared (NIR) spectral ranges is a noninvasive spectroscopic technique frequently used for the assessment of soft tissue properties. A portable fiber optic probe, convenient for deployment in a clinical environment, is typically used to acquire the spectra. To improve the light coupling and the overall measurement repeatability,¹ the probe is usually pressed against the skin surface. The applied contact pressure leads to structural changes in the skin and the underlying soft tissue, which affect the optical properties of the observed tissue and thereby the acquired spectra.

The majority of existing studies^{1–8} considered the contact pressure-induced spectral changes as distortions. However, an *ex vivo* study has shown that the contact pressure–induced decrease in tissue scattering can be used as an optical clearing technique.⁹ Consequently, deeper penetration depth can be

achieved. Furthermore, the unique contact pressure–induced spectral changes present an additional valuable source of information for *in vivo* tissue classification.¹⁰ Finally, contact pressure can emphasize the differences between various tissues. It was shown that the number of misclassifications between two measurement sites on a human hand decreased with higher contact pressure.¹¹

Existing studies on the contact pressure–induced spectral changes in DRS used different methods for applying and controlling the level of contact pressure.¹¹ However, many of these systems are useful only for *ex vivo* applications.^{2,4,9} Several measurement systems are limited to a few discrete quasistatic pressure levels^{3,5,6} or applicable only to a specific measurement site.^{10,12} Furthermore, the contact pressure is not always measured at the position of the diffuse reflectance probe,⁷ depends on the probe operator,⁸ or is not acquired simultaneously with the spectral information.¹

It is evident that existing measurement systems cannot provide the accuracy and precision required for effective use of the contact pressure information in *in vivo* diffuse reflectance studies of soft tissue. In order to overcome these limitations, we propose a novel fully automated system for controlled application of contact pressure and acquisition of diffuse reflectance spectra. Finally, a spectral-contact-pressure plane for effective visualization of joint information is introduced.

2 Materials and Methods

Ex vivo VIS/NIR spectra and the corresponding contact pressure data were collected from left back feet of four pigs (hybrids between Piétrain and German Landrace, age: 9 months) on adipose pad between second and fifth digits, plantar from metatarsophalangeal joint (average thickness of skin and adipose pad was 1.9 mm and 1.8 cm, respectively). *In vivo* spectra were acquired from the skin (average thickness was 2 mm) on palm above the abductor pollicis brevis muscle (average thickness was 1.7 cm) of four volunteers (Caucasians, age: 27 to 34, Fitzpatrick skin types 2 and 3). Significant effort was made to apply the contact pressure perpendicularly to the sample surface and to minimize bulk sample movement during the measurements by employing a custom sample holder and individualized clay molds.

The employed measurement system shown in Fig. 1 comprises a commercial NIR spectrometer (NIR-512L-1.7T1, 901 to 1685 nm, Control Development, South Bend, Indiana), a commercial VIS spectrometer (AvaSpec-2048-TEC-FT, 177 to 1098 nm, Avantes, Apeldoornseweg, the Netherlands), and a fiber optic probe (Avantes, FCR-191R200-2-ME, 2 readouts and 17 illumination fibers, 200- μ m core diameter, NA = 0.22, pairwise source–detector separations: 6 \times 278.0, 4 \times 481.5, 2 \times 556.0, 1 \times 834.0 μ m), delivering 8.5 mW of broadband optical power (Avantes, Avalight-HAL, tungsten halogen source). Outer diameter of the probe was 6.35 mm with an effective pressure area of 31.7 mm². The probe was attached to a stainless steel arm equipped with a temperature-compensated silicon piezoresistive force sensor fabricated in UL-FE, LMSE (Ljubljana, Slovenia). The acquisition of contact pressure and spectra was synchronized by a custom logic. Spectrometer exposure time was set to 3 ms, while the force was measured at a rate of 100 kHz. For each acquired spectrum, the contact pressure was calculated as the quotient between the mean force and the probe surface, providing submilligram-force resolution. The spectrometer readout time was about 15 ms,

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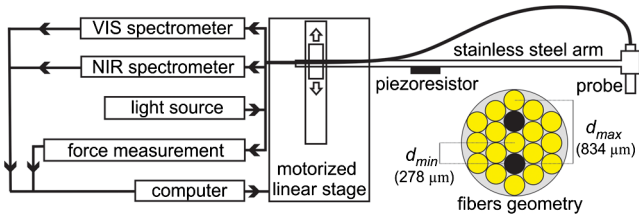


Fig. 1 System for controlled application of contact pressure and acquisition of diffuse reflectance spectra with the layout of probe fibers.

limiting the acquisition rate to 55 Hz. Precise and accurate control of the applied contact pressure in the range from 0 to 150 kPa was ensured by a motorized linear stage and the custom control logic.

The acquired raw spectra underwent two-point intensity calibrations based on the dark response of the sensor array and reflectance of a standard diffuse tile (Spectralon, Labsphere, North Sutton, New Hampshire). The contact pressure (p)-induced changes in the diffuse reflectance spectra $R_p(\lambda)$ were normalized by the zero contact pressure diffuse reflectance spectrum $R_o(\lambda)$:

$$r_p(\lambda) = \frac{R_p(\lambda) - R_o(\lambda)}{R_o(\lambda)}. \quad (1)$$

The calculated normalized spectra were used to effectively visualize the spectral changes as a function of the applied contact pressure in a spectral-contact-pressure plane $P[r_p(\lambda), p]$.

3 Experiments

A comparison of the load application repeatability for the automated system and the two manually operated DRS probes, one aided by a calibrated spring,³ was conducted on the skin surface of one *ex vivo* porcine foot sample using the exact same location. In this way, the influence of external parameters on the comparison was minimized. Twenty measurements were taken by each probe, and the tissue was given 30 s to recover from the previous measurement. The stainless steel arm of the automated system was used to accurately assess the contact pressure of the two manual probes handled by an experienced operator. The operator was instructed to apply and maintain a light contact pressure for 2 s. The target contact pressure of the calibrated spring system, aiding the operation of the second manual probe, was set to 70 kPa and the operator was again instructed to apply and maintain the target contact pressure for 2 s. Likewise, the automated system was programmed to apply 70 kPa at a rate of 5 mm/s, starting 8 mm above the skin surface, stopping for 2 s after reaching the target contact pressure, and finally reversing the described operation. A 5-mm tissue displacement was observed at the target contact pressure of 70 kPa. The measured variances of the applied contact pressure were compared within a 1-s time window, as illustrated in Fig. 2.

A similar procedure was followed in the second experiment, where the spectral and contact pressure information was acquired from the four *ex vivo* and four *in vivo* samples using only the automated system. However, this time, the target contact pressure was set to 150 kPa, while the load application rate was kept at 5 mm/s. Likewise, each measurement was repeated 20 times and the tissue was given 30 s to recover from the previous measurement. The results were used to assess

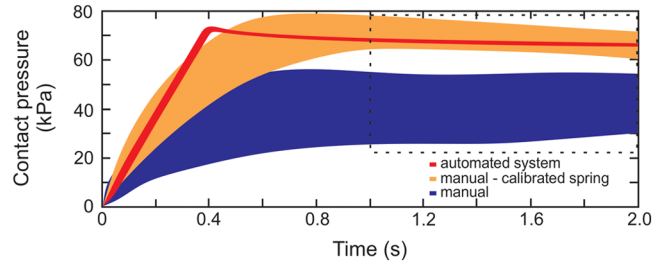


Fig. 2 Load repeatability comparison of the automated and two manually operated probes, in which one was aided by a calibrated spring, estimated from a time window of 1 s.

the repeatability of *in vivo* measurements and introduction of the spectral-contact-pressure plane.

4 Results and Discussion

One of the main reasons for the introduction of the proposed automated system is to ensure precise contact pressure control and measurements independent of the probe operator. Precise and accurate pressure control is essential when studying the influence of low-contact pressure, e.g., near or under the normal arterial blood pressure (13 to 18 kPa). At these levels, the variability of contact pressure applied by a manually operated probe is much too high, which was confirmed by the results of the load application repeatability experiment presented in Fig. 2. As expected, the highest variability of contact pressure was observed for the manually operated probe (7.58 kPa), slightly lower for the manually operated probe aided by a calibrated spring (2.90 kPa), and about 30-fold lower for the automated system (0.22 kPa).

The automated measurement system provides control over the probe displacement, contact pressure scale, and the rate of its application. In order to assess the repeatability of load application and reflectance measurements *in vivo*, 20 measurements acquired from the palm skin of a single volunteer were analyzed. The acquired data can be divided into five main regions (Fig. 3). Region I was acquired prior to the first contact of the probe with the skin surface. The standard deviation (STD) of the measurements obtained for a stationary probe was well

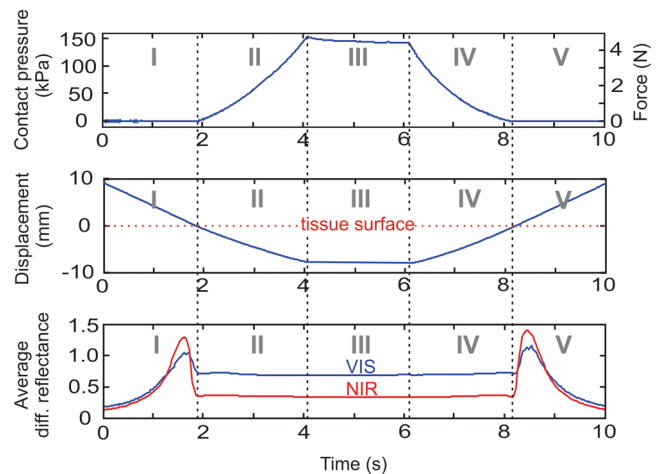


Fig. 3 A typical *in vivo* measurement recorded by the automated system: (top) contact pressure and force, (middle) probe displacement, and (bottom) average diffuse reflectance in the visible (VIS) (450 to 850 nm) and near-infrared (NIR) (950 to 1600 nm) spectral ranges.

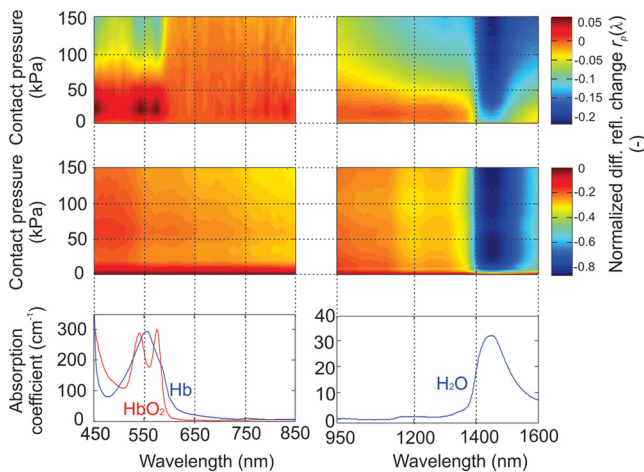


Fig. 4 Spectral-contact-pressure plane of (top) a human palm *in vivo* (left, VIS and right, NIR) and (middle) porcine foot *ex vivo* (left, VIS and right, NIR). (Bottom) Absorption coefficient of the main chromophores^{14,15} in VIS (left) and NIR (right) spectral ranges.

below 0.1 kPa. Once the probe motion was activated, STD shortly increased to about 0.3 kPa (Regions I and V), which can be attributed to the mechanical vibrations of the system. The observed rapid increase and decrease in the average reflectance are governed by the geometry of the fibers within the probe and light coupling. Region II describes the changes in the average diffuse reflectance during the application of increasing contact pressure. The applied contact pressure leads to structural changes in the skin and the underlying soft tissue, which reflect in the optical properties and thereby in the acquired spectra. A slightly higher STD of 0.9 kPa was observed after the probe made initial contact with the skin surface. The additional increase could be explained by the minor displacement of the palm during the measurements. Region III clearly shows the gradual changes in the average reflectance during 2 s after stopping the probe at the maximum contact pressure of 150 kPa. The observed changes can be attributed to the gradual adjustment of the soft tissue to the contact pressure including mechanical and physiological changes. Regions IV and V are inverse of the Regions II and I, respectively. However, the observed changes are not identical because skin is a nonlinear viscoelastic material that exhibits delayed recovery from deformation.

In order to effectively present the acquired spectral and contact pressure information, a spectral-contact-pressure plane was introduced. An example of the proposed visualization for one of the four *in vivo* and *ex vivo* samples obtained for Region II can be observed in Fig. 4. As expected, the *in vivo* VIS spectral response acquired from a human palm exhibits dominant gradual changes from 450 to 600 nm due to the changes in the concentrations of chromophores, in particular of the oxy- and deoxyhemoglobin, while the most prominent gradual changes in the NIR spectral range observed at 1450 nm are related to the water absorption. In contrast to the *in vivo* spectral response, *ex vivo* VIS spectral response of the bled-out porcine foot exhibits no changes related to the hemoglobin, while the corresponding NIR spectral response is limited to the water absorption. Previous studies have shown that the observed

spectral changes provide additional information on the tissue,¹⁰ which can be used to improve tissue classification or pathology characterization.¹³ However, the extent of contact pressure-induced spectral changes can also significantly degrade the performance of manually operated DRS probes.¹¹

5 Conclusion

This study introduces a novel system for capturing the *in vivo* dynamics of the pressure-induced spectral and mechanical responses of soft tissue. In the presented approach, measured results are visualized in a spectral-contact-pressure plane. The substantial improvement in the accuracy and precision of the applied contact pressure by the presented method will inspire studies at low-contact pressure levels, near or under the normal arterial blood pressure of 16 kPa, and studies on the pressure-induced tissue response dynamics.

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