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THE VARIATIONS OF WATER IN HUMAN TISSUE UNDER CERTAIN COMPRESSION: STUDIED WITH DIFFUSE REFLECTANCE SPECTROSCOPY

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The reflectance spectrum has been widely adopted to extract diagnosis information of human tissue because it possesses the advantages of noninvasive and rapidity. The external pressure brought by fiber optic probe may influence the accuracy of measurement. In this paper, a systematic study is focused on the effects of probe pressure on intrinsic changes of water and scattering particles in tissue. According to the biphasic nonlinear mixture model, the pressure modulated reflectance spectrum of both *in vitro* and *in vivo* tissue is measured and processed with second-derivation. The results indicate that the variations of bulk and bonded water in tissue have a nonlinear relationship with the pressure. Differences in tissue structure and morphology contribute to site-specific probe pressure effects. Then the finite element (FEM) and Monte Carlo (MC) method is employed to simulate the deformation and reflectance spectrum variations of tissue before and after compression. The simulation results show that as the pressure of fiber optic probe applied to the detected skin increased to 80 kPa, the effective photon proportion form dermis decreases significantly from 86% to 76%. Future designs might benefit from the research of change of water volume inside the tissue to mitigate the pressure applied to skin.

Keywords: Diffuse reflectance spectroscopy; contact pressure; water transportation; Monte Carlo; finite element method.

1. Introduction

Diffuse reflectance spectroscopy (DRS) has shown great potential in the research of noninvasive detection of human compositions.[1] The transmitting of light within human tissue is governed by the morphological, biochemical and physiological characteristics of tissue. The fiber optic probe is commonly applied for spectral acquisition of DRS measurement. In order to avoid the spectral artifacts induced by roughness and movement of tissue surface, the fiber optic probe is placed in contact to the detected tissue surface with certain

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localized compression, that should affect the intrinsic characteristics of the tissue such as thickness and optical properties.[2–10]

Many groups have reported probe pressure effects on spectroscopic measurement in human and other tissue. Chan *et al.*[2] reported that the transmittance increased up to 30%, and the reflectance decreased by 12%, and both absorption and scattering increased by almost twice the original values on *in vitro* porcine aorta tissue under pressure of $2\,\mathrm{kg/cm^2}$. Ti *et al.*[3] investigated the short- and long-term effects of probe contact pressure on *in vivo* diffuse reflectance and fluorescence spectroscopy. The results indicated that probe pressure-induced spectral changes in DRS may be attributed to decreases in local blood volume, blood oxygenation and tissue metabolism. Chen *et al.*[5] investigated the influence of contact state on diffuse reflectance spectral measurement *in vivo* and the variation trend of diffuse reflectance with contact time under the same contact pressure. From the experimental results, the optimal contact state and optimal measuring time of *in vivo* measurement are investigated.

Although previous results demonstrated the variations of diffuse reflectance of *in vitro* skins under certain pressure, these results could not be related directly to *in vivo* measurement because of the differences in tissue type, probe geometry and pressure. It is important to investigate the mechanism of tissue change with the local compression, especially for human skin *in vivo*, and model the variations of reflectance spectra. In this paper, a systematic study is focused on the effects of probe contact pressure on the intrinsic variations of tissue such as water and scattering particles. Based on the biphasic nonlinear mixture model of skin tissue, the mechanism of tissue deformation is studied. The short- and long-term effects of probe pressure on diffuse reflectance spectra are quantified. Moreover, the deformation of human skin and variation of reflectance spectrum under certain pressure are simulated. Future designs might benefit from the current results about mechanism and modeling method of skin deformation under certain pressure.

2. Materials and Methods

2.1. *The mechanical and optical properties of skin*

The skin-referred as viscoelastic material behaves with time-dependent, incompressible, anisotropic and inhomogeneous properties when it is deformed. The mechanical characteristics of skin are affected by various factors such as hydration, thickness, aging, etc.

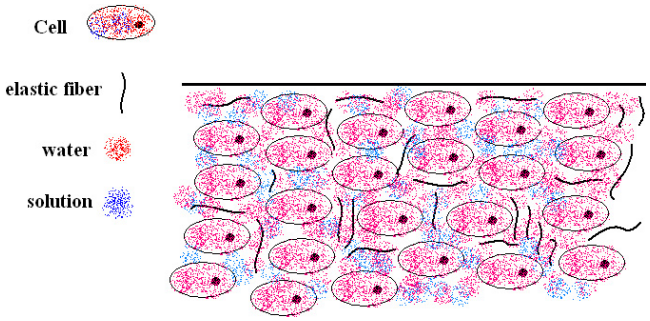


Fig. 1. Schematic of the nonlinear mixture model of skin.

Many models have been developed to characterize the mechanical properties and the deformation mechanism of skin. Oomens *et al.*[11] introduced a biphasic nonlinear mixture theory to discuss the tissue strain with certain stress. With this model, the skin is assumed to be made up of incompressible elastic solid and Newtonian fluid. As shown in Fig. 1, the solid matrix makes up mainly of elastic fiber and cells which plays a major role in the nonlinear mechanical response. The water that filled in the solid matrix will provide a resistance to flow, resulting in time-dependent behavior of the solid material. This model is proved best to represent the composition and mechanical properties of skin.[11,12]

The optical properties of human tissue are assumed to be the mixed model of scattering and absorption particles. Within the near-infrared wavelength range, the main absorption substance within tissue is water, while nucleolus and collagenous fiber bundles provide the scattering characters. Described as the discrete particle model, the scattering coefficient of tissue depends on the scattering cross-section and concentration of the scattering particles. The hybrid hypothesis of Mie and Rayleigh scattering approximation is applied to express the scattering coefficients of human tissue in different wavelengths. According to the measuring result of *in vitro* tissue with integrating spheres, the scattering coefficient of each layer is[13,14]:

$$\mu_s^{\mathrm{epi}} = 1.08 \times 10^8 \lambda^{-2.364} + 135.71\lambda^{-0.258}, \quad (2)$$

$$\mu_s^{\mathrm{dermis}} = 1.19 \times 10^8 \lambda^{-2.427} + 71.476\lambda^{-0.258}, \quad (3)$$

$$\mu_s^{\mathrm{sub}} = 1.08 \times 10^8 \lambda^{-2.525} + 157.494\lambda^{-0.345}. \quad (4)$$

The absorption coefficient of dermis tissue is given as the summation of 70% of the absorption coefficient of water. A total of 20% of the water absorption coefficient is given for the absorption coefficient of the epidermis. Approximately 40% absorption coefficient of lipid and 60% absorption of water are given for the absorption coefficient of subcutaneous tissue.[13,15]

First, the mechanism of tissue deformation under certain pressure is investigated. As the mechanical and optical properties of tissue are defined, the finite element method (FEM) and Monte Carlo (MC) are adopted to simulate the deformation of tissue and the transportation of photons within the tissue. The FEM model utilizes by ABAQUS is adopted to predict the force distribution throughout the tissue resulting from compression and the deformation of the tissue. And then photon transportation and physical quantities before and after the localized pressure are numerically simulated by MC method. The trajectory of a photon is modeled as a persistent random walk. It is easily modified to adjust the measuring system and variable sample geometry.

2.2. *The characteristic water in biomedical tissues*

According to the biphasic nonlinear mixture model, water is a significant contributor to the volume and composition of tissue. It accounts for approximately 60−80% of the total weight of tissue. The mobility of water within this gel meshwork is the deciding factor of the mechanical and optical properties of the tissue.[14]. So it is important to investigate the properties of water within tissue.

With its two hydrogen bond acceptor and donor sites, the H_2O molecule could easily combine with polar molecules of the solid matrix in tissue such as protein and lipid. According to the compactness of the hydrogen bond between the H_2O and protein matrix, the water within skin could be divided into two groups: bulk water and bound water. They possess different mechanical and spectral properties. The bulk water could transport within skin freely. But the bound water is very tightly affiliated and difficult to be removed from the solid matrix.

For the spectral properties, the fundamental vibrations of OH in H_2O are ν_1 (symmetric stretching), ν_2 (bending) and ν_3 (asymmetric stretching). They are also easily broadened and shifted by hydrogen bonding. The results from Dimitri *et al.*[16] proved that the hydrogen bonding makes ν_1 and ν_3 shift toward long wavelength and ν_2 shift toward short wavelength. The absorption bands of H_2O observed within 1100−1300 nm, arise from combination and overtones. The fundamental vibrations and combination absorptions peaks of bulk water and bound water are shown in Table 1.

Table 1. Fundamental vibrations and combination absorption peaks of bulk and bound water.

	ν_1 (cm^{-1})	ν_2 (cm^{-1})	ν_3 (cm^{-1})	Combination absorption peak (nm)
Bulk water	3450	1640	3450	1170
Bound water	3260	1640	3260	1225

2.3. *The deformation process of skin under compression*

When the skin is compressed, the solid matrix is deformed and the highly viscous liquid flowed sideways out of the compressed zone as shown in Fig. 2. This hypothesis matches well with the experimental results. The ratio of deformation and decay time of the skin under pressure is dependent on the elastic properties of the fibers, and the quantity and viscosity of the fluid within the tissue. As the bound water is very tightly combined to the solid matrix within tissue, the measurement of variations of bound and bulk water could reflect the deformation of solid matrix and transportation of bulk water within the tissue under certain pressure.[14]

2.4. *The measurements of water transportation under certain pressure*

The NIR spectroscopy could distinguish and measure different types of water within the human skin.[14] In this paper, we used a commercial clinical spectrometer to measure the reflectance spectra of *in vitro* porcine skin and *in vivo* human skin. The system is described in detail in Fig. 3. Briefly, the spectrometer system consists of the following primary components: (1) a FT-IR spectrometer (Matrix-F, BRUKER Corporation); (2) a fiber optic probe with illumination and detection fibers; and (3) a pressure transducer affiliated to the probe to monitor the contact pressure between the fiber optic probe and tissue.

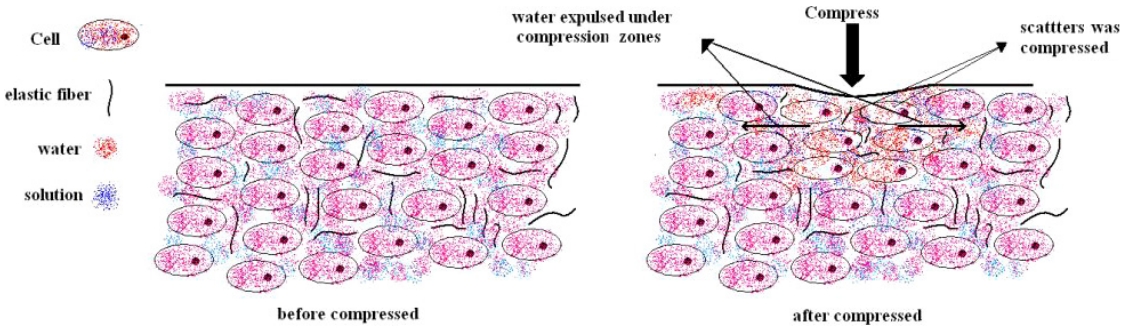


Fig. 2. Skin deformation and structural changes before and after compressed.

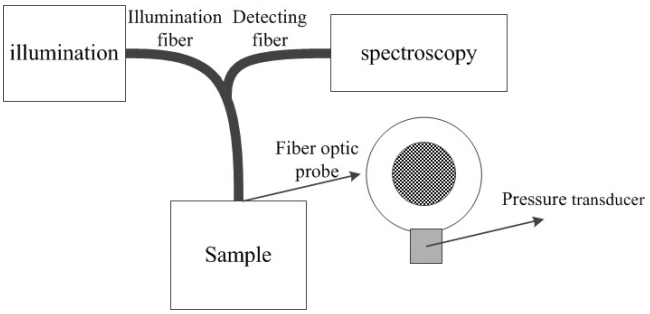


Fig. 3. Schematic of the measurement system and fiber optic probe.

The fiber optic probe is held by a mechanical arm. During a spectral acquisition procedure, the optical probe is placed perpendicularly to the tissue surface and allowed to move freely only in the vertical plane. Due to the movement of the probe, the pressure is applied to the detected area. The levels of probe contact pressure are then monitored by the pressure transducer.

The experiments are implemented by using *in vitro* porcine skin and *in vivo* human skin, respectively. The porcine skin samples are obtained from a local slaughter house and stored at -20°. The epidermis of the porcine skin is carefully removed to minimize its influence. The dermis tissue is immersed in the solution of physiological saline until it shall finally reach the room temperature. For *in vivo* experiments, the forearm skin is measured, because the dermis of forearm skin is fairly thicker and the stratum corneum is thinner than other parts of the body.[17]

When the reflectance spectra are collected, the fiber optic probe is first set to contact with the skin. With the first pressure modulation, the probe moved toward the sample step-by-step. The diffuse reflectance spectra and pressure levels are acquired at each step. The second pressure modulation is performed with the optical fiber probe moving toward the sample and stopped when the sample is pressed by 2 mm. Then the diffuse reflectance spectra were continuously acquired. The influences of contact pressure and time on tissue are then analyzed.

2.5. *The data process and analysis*

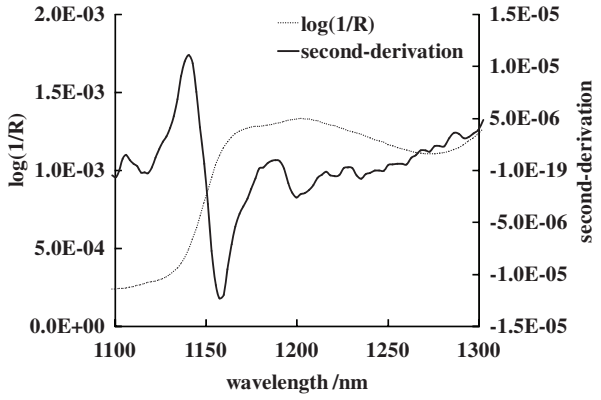
The diffuse reflectance spectra were then measured and transformed into the apparent absorbance as[18]:

$$A = \log\left(\frac{1}{R}\right), \tag{1}$$

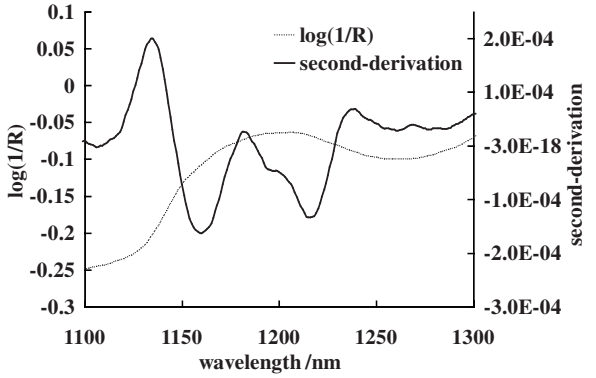
where R is the relative diffuse reflectance of sample to the standard reflector.

Because the biological tissues are highly scattering, the multiple scattering correction (MSC) is applied to the original reflectance spectra in the preprocessing to minimize the effects of scattering variations when the tissue is compressed.

Within the wavelength range of 1100 to 1200 nm, the absorption peaks of bulk and bound water are overlapped. Calculation of the second-derivative of absorbance spectra could enhance the spectral resolution and compensate for baseline shifts with no apparent degradation in the analytical results. The most characteristic feature of the second-order derivative is a negative band with the minimum at the same wavelength as the $\lambda_{\max}$ of the original absorbance spectrum. Derivative spectroscopy is often used to resolve overlapping contributions to broad near-infrared absorption bands. The diffuse reflectance and corresponding second-derivative absorbance spectra of the human skin and pure water are shown in Fig. 4. The results indicate that in the second-derivative spectra the amplitude of 1160 and



(a)



(b)

Fig. 4. Reflectance and second-derivation of (a) water and (b) human skin.

1220 nm are related to the absorption of bulk and bonded water, respectively.

3. Results

3.1. *Change of water within* in vitro *tissue under certain pressure*

Several prominent alterations are observed in the spectral data from the *in vivo* porcine skin. As the *in vitro* porcine skin is compressed, the intensities around 1220 nm were significant increased with the pressure while the intensities around 1160 nm were decreased as shown in Fig. 5. When the pressure applied to the porcine skin increases to 376 kPa, the absorption peak around 1160 nm is almost disappeared. As the porcine skin is compressed, the free water is displaced out of compressed regions and the solid matrix is compressed.[19] Because the bulk water is binding to the protein, it is very difficult to be displaced from the compressed regions.

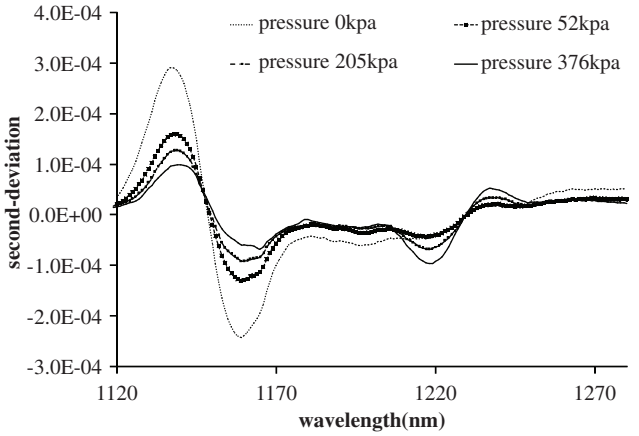
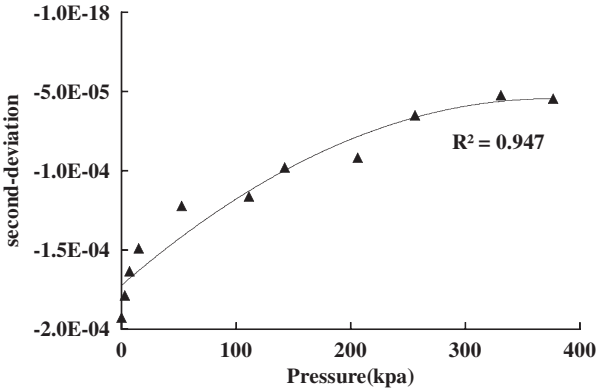


Fig. 5. Variation of second-derivation with probe pressure increased.

The relationship between the pressure and the absorption peak intensities of bulk and bound water is shown in Fig. 6. As the pressure increases to about 400 kPa, the volume of the bulk water



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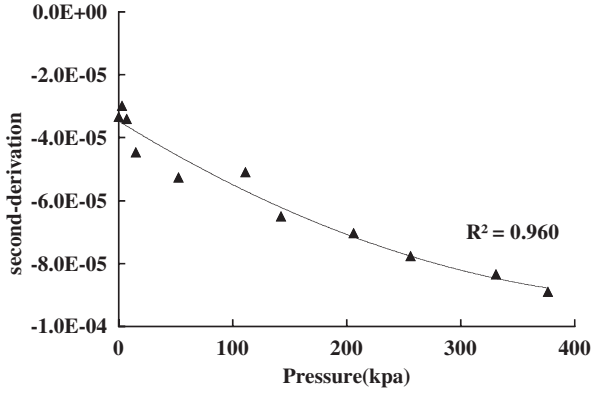


(b)

Fig. 6. Variations of (a) bulk and (b) bound water with probe pressure.

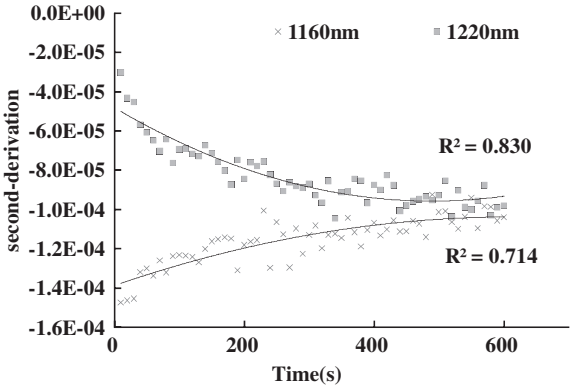


Fig. 7. Variations of bulk and bound water with pressure duration.

reduces to 30% of the original condition. That is the main reason for the tissue deformation.

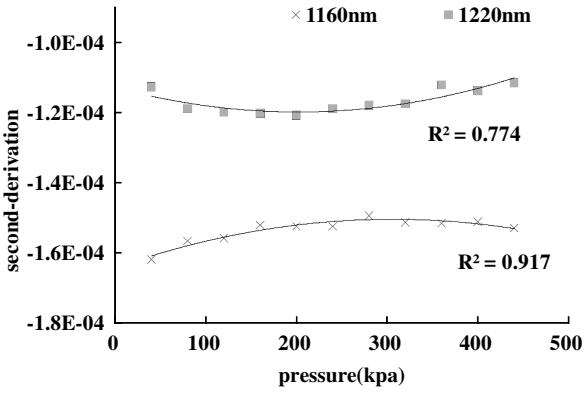
The research on effect of long-term pressure focuses on evaluating the spectral alterations with long-term pressure duration. As the fiber optic probe is fixed on the tissue site, the absorption peak of bulk water within the tissue decreased with pressure duration, while the absorption peak of bound water increased as shown in Fig. 7. The variation ratios of bulk and bound water show a nonlinear relationship with the pressure duration time, and both decreased with time. According to the mechanical properties of biological tissue, the nonlinear relationship between the strain variation ratio and pressure duration time is mainly caused by the migration of bulk water within the tissue. As the bulk water performs Newtonian behavior, its high permeability and low viscosity impede its flow within the tissue. In the early stages of tissue deformation under certain pressure, the bulk water moved out of the compressed region with high migration rate due to the large strain within the tissue. Because of the stress relaxation of biological tissues, the deformation and bulk water migration rate is gradually slowing down. This is commonly referred to as creep, a behavior common to viscoelastic materials. After about 6 min the deformation of tissue and transportation of bulk water are constant. The duration time is also related to the intensity of compression.

3.2. *Variations of water within* in vivo *tissue under certain pressure*

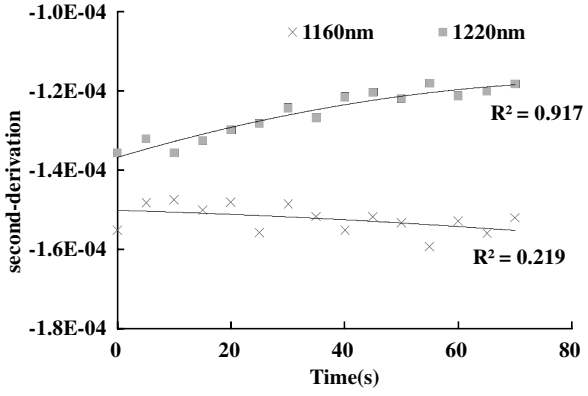
The *in vivo* human skin possesses the similar composition and mechanical properties as the *in vivo* porcine skin, but contains higher proportion of the water especially for the bound water. While the pressure extends to a certain amplitude, the bound water in living tissue could be transformed to bulk water to ensure normal tissue metabolic activity.

As shown in Fig. 8, when the pressure is applied to the *in vivo* skin, the volume of free water within skin is first decreased and then increased as the pressure becomes larger than 250 kPa. The bound water performs in the opposite way. Both the inflection points are basically the same. The experimental results indicated that as the free water is squeezed out of the pressure region, the part of the bound water might change into free water. Compared with the *in vitro* porcine skin, the thikness and bulk water of human skin *in vivo* is less changeable with pressure.

With the pressure applied to *in vivo* skin persisted, the bound water within the tissue decreased continuously as shown in Fig. 8(b). The changes of bulk water volume are relatively small, because the



(a)



(b)

Fig. 8. Variations of bulk and bound water with (a) pressure increased and (b) duration.

bound water continually changes into the free water to protect the normal metabolism of in vivo tissue.

3.3. *Modeling the deformation and reflectance variation of tissue under certain pressure*

In the prior experiment, the skin is considered to be homogeneous, and the variations of different waters within the tissue are measured. As it is measured by reflectance spectra, the variation of water volume within tissue is the primary event as the tissue is deformed under certain pressure. Future application of mechanical effect on optical measurement should be on the basis of the multilayer skin. It requires a comprehensive modeling of the deformation and optical properties variation under certain pressure. The computational FEM model is then adopted to correlate applied mechanical force, tissue water transport, modified optical properties and resulting light transport. This model could serve as a tool for optimizing design parameters of devices and mechanical loading conditions as well as verifying experimental and analytical results.

To correctly model tissue displacement and water transport due to localized compression, both the solid and liquid phases of skin have to be accounted for. The pore fluid diffusion and stress analysis (SOILS analysis) in ABAQUS couples the deformation of a permeable elastic solid with fluid movement. Using the SOILS analysis to represent skin during loading, the tissue was characterized as a fluid permeable medium with linear elastic material properties for the solid. The material properties used in the simplified ABAQUS model are defined below in Table 2. The pressure applied to the skin model in the simulation does not extend to 100 kPa.

The variations of optical properties of human skin under certain pressure depend on the tissue deformation and free water volume variation. As the water volume is changed after the tissue is deformed, the absorption coefficients of tissue under compress of probe is:

$$\mu_a^*(\lambda) = \frac{V_w - \Delta V_w}{V_{\text{skin}} - \Delta V_{\text{skin}}} \mu_a^w(\lambda). \tag{5}$$

On the basis of discrete particle model of soft tissue, a single scattering particle's scattering cross-section is proportional to the square of the scattering particle volume. It has been proposed that skin dehydration might raise light scattering by increasing the volume fraction of scattering particles such as nucleolus and collagen fibrils. Meanwhile the scattering cross-section decreases as the cell structures and fibers are compressed. Counting for these two factors, the scattering coefficients could be expressed as:

$$\begin{aligned}\mu_s^*(\lambda) &= \sum \rho * \sigma * (\lambda) \\ &= \sum \rho \frac{V_{\text{skin}}}{V_{\text{skin}} - \Delta V_{\text{skin}}} \sigma(\lambda) \left(\frac{V_a - \Delta V_a}{V_a}\right)^2 \\ &= \frac{V_{\text{skin}}}{V_{\text{skin}} - \Delta V_{\text{skin}}} \left(\frac{V_a - \Delta V_a}{V_a}\right)^2 \mu_s(\lambda).\end{aligned} \tag{6}$$

In Eq. (6) μ_s^* and μ_s represent the scattering coefficients of tissue before and after compression, respectively. We used a simulation model for a fiber optic probe which consisted of fiber in the center (diameter: 0.2 m, NA = 0.22) as a source of fiber bundle, and surrounding fibers (separation distance: 0.7 mm) for detection of fiber bundle. The number of the injected photons in the MC simulation[20] was 10^8 which were enough to obtain statistically reasonable results. The effective photon proportion is defined as

$$\begin{aligned}&\text{Percentange of effective photons} \\ &= \frac{\text{Photon}_{\text{dermis}}}{\text{Photon}_{\text{total}}} \times 100\%.\end{aligned} \tag{7}$$

Table 2. Material properties of skin in ABAQUS analysis.

	Thickness	Young's modulus	Poisson's ratio	Permeability	Void
Epidermis	0.075 mm	0.18 Mpa	0.45	7.33 * 10^{-8}cm/s	0.67
Dermis	1 mm	0.018 Mpa	0.35	7.33 * 10^{-8}cm/s	0.67
Subcutaneous	∞	0.006 Mpa	0.5	7.33 * 10^{-8}cm/s	0.67

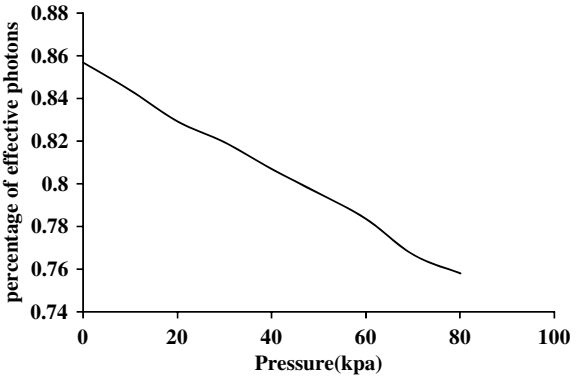


Fig. 9. Variation of effective photons with probe pressure.

As the pressure of fiber optic probe applied to the detected skin becomes larger, the effective photon proportion decreases significantly as shown in Fig. 9. The dermis becomes thinner as the detected skin is deformed by certain pressure, and the number of the photons reflected from the dermis is reduced. The simulation result indicates that when the pressure applied to the skin increases to 80 kPa, the percentage of effective photons decreases from 86% to 76%. It is unfavorable for the detection of compositions within the dermis.

4. Discussion and Conclusion

The results of this study show that probe contact pressure, when exceeding a certain threshold and duration time, could produce a significant impact on reflectance spectrum and detecting efficiency. The previous studies only reported that the compression of tissue could lead to a decrease in the diffuse reflectance of *in vitro* tissue. Since biological tissue with high water content is considered incompressible, the pressure induced by the optical probe is likely to deform the tissue and drive the water out of the compressed skin. The mechanism of the tissue deformation under pressure is comprehensively researched in this paper. Based on the biphasic nonlinear mixture model, the variations of free water and bound water within tissue under certain pressure are measured with second-derivative reflectance spectra. The results indicate that the amount of water displaced in skin due to localized compression is the major factor of reflectance spectra variations. The mixture model seems to be an exceptional method to model these variations. The three-layer skin model is established to quantitatively analyze the influence exerted by fiber-optic probe pressure under any conditions. The results indicated that as the probe pressure applied to the tissue *in situ* increases, the percentage of effective photons from dermis is reduced sharply. It is possible to implement a pressure sensor into conventional optical probe design. With the research, it may be more convenient to use DRS to gauge the pressure level, as the spectral alterations induced by a high level of probe contact pressure seem to originate from changes in local water in tissue. Because the effects of probe contact pressure on *in vivo* optical spectroscopy can be substantial, the level of probe contact pressure should be monitored closely during any *in vivo* spectral acquisition procedure. Future designs might benefit from the research of change of water volume inside the tissue to mitigate the pressure applied to skin.

Acknowledgments

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References

1. V. Karthik, C. Kevin, K. Daniel *et al.*, "Portable, fiber-based, diffuse reflection spectroscopy (DRS) systems for estimating tissue optical properties," *Appl. Spectrosc.* **65**(2), 206−215 (2011).
2. E. K. Chan, D. Protsenko, M. O'Neil *et al.*, "Effects of compression on soft tissue optical properties," *IEEE J. Select. Top. Quantum Electron.* **2**(4), 943−950 (1996).
3. T. Yalin, L. Wei-Chiang, "Effects of probe contact pressure on *in vivo* optical spectroscopy," *Opt. Express* **16**, 4250−4262 (2008).
4. H. Shangguan, S. A. Prahl, S. L. Jacques *et al.*, "Pressure effects on soft tissues monitored by changes in tissue optical properties," in Laser-Tissue Interaction IX SPIE (1998).
5. W. Chen, R. Liu, K. Xu *et al.*, "Influence of contact state on NIR diffuse reflectance spectroscopy *in vivo*," *J. Phys. D Appl. Phys.* **38**, 2691−2695 (2005).
6. K. Rivoirea, A. Nath, D. Cox *et al.*, "The effects of repeated spectroscopic pressure measurements on fluorescence intensity in the cervix," *Amer. J. Obstet. Gynecol.* **191**(5), 1606−1617 (2004).

7. S. Jiang, B. W. Pogue, K. D. Paulsen, "*In vivo* near-infrared spectral detection of pressure-induced changes in breast tissue," *Opt. Lett.* **28**(14), 1212−1214 (2003).
8. R. Reif, M. S. Amorosino, K. W. Calabro, "Analysis of change in reflectance measurements on biological tissues subjected to different probe pressures," *J. Biomed. Opt.* **13**(1), 010502 (2008).
9. V. V. Sapozhnikova, R. V. Kuranov, I. Cicenaite *et al.*, "Effect on blood glucose monitoring of skin pressure exerted by an optical coherence tomography probe," *J. Biomed. Opt.* **13**, 021112 (2008).
10. L. Liang, N. Brandon, R. Narasimhan *et al.*, "Probe pressure effects on human skin diffuse reflectance and fluorescence spectroscopy measurements," *J. Biomed. Opt.* **16**(1), 011012 (2011).
11. C. W. J. Oomens, D. H. Vancampen, H. J. Grootenboer, "A mixture approach to the mechanics of skin," *J. Biomech.* **20**(9), 877−885 (1987).
12. C. G. Rylander, T. E. Milner, S. A. Baranov *et al.*, "Mechanical tissue optical clearing devices, Enhancement of light penetration in *ex vivo* porcine skin and adipose tissue," *Lasers Surg. Med.* **40**, 688−694 (2008).
13. A. N. Bashkatov, E. A. Genina, V. V. Tuchin, "Optical properties of skin, subcutaneous and muscle tissues, a review," *J. Innovative Opt. Health Sci.* **14**(1), 9−38 (2011).
14. A. Hidenobu, E. Mariko, "Non-contact skin moisture measurement based on near-infrared spectroscopy," *Appl. Spectrosc.* **58**, 1439−1446 (2004).
15. T. L. Troy, S. N. Thennadil, "Optical properties of human skin in the NIR wavelength range of 1000−2200 nm," *J. Biomed. Opt.* **6**, 167−176 (2001).
16. E. K. Dimitri, H. Elizabeth, L. Rojana *et al.*, "Probing protein hydration by the difference O-H (O-D) vibrational spectroscopy, Interfacial percolation network involving highly polarizable water-water hydrogen bonds," *J. Molec. Liquid.* **105**(1), 13−36 (2003).
17. Y. Lee, "Skin thickness of Korean adults," *Surg. Radiol. Anat.* **24**, 183−189 (2002).
18. B. C. Wilson, S. L. Jacques, "Optical reflectance and transmittance of tissues: Principles and applications," *IEEE J. Quantum Electron.* **26**(12), 2186−2199 (1990).
19. T. Yu, X. Wen, V. V. Tuchin *et al.*, "Quantitative analysis of dehydration in porcine skin for assessing mechanism of optical clearing," *J. Biomed. Opt.* **16**(9), 095002 (2011).
20. L. Wang, S. L. Jacques, L. Zheng, "MCML — Monte Carlo modeling of light transport in multi-layered tissues," *Com. Met. Pro. Biol.* **47**, 131−146 (1995).