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Abstract. This work reports that the laser fluence rate inside porcine skin varied notably with the change of tissue water content under the same laser irradiation conditions. The laser fluence rate inside skin tissue samples with varying water content was measured using an optical fiber sensor, while the target was irradiated either by a low-level 635 or 830 nm laser (50 mW/cm²). It was demonstrated that the distribution of laser fluence rate inside the target is strongly affected by tissue water content and its profile is determined by the water content dependency of optical properties at the laser wavelength. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE)[DOI: 10.1117/1.JBO.20.5.051027]

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1 Introduction

The use of low-level lasers for therapeutic purpose [low-level laser therapy (LLLT)] has been widely investigated over the past decades and is acknowledged as being effective in certain areas of treatment, such as wound healing,1 pain reduction,2 anti-inflammation,3 nerve regeneration,4 etc. In LLLT, therapeutic effects are known to depend on the absorption of laser energy by a therapeutic target, which is typically assumed to be influenced by laser irradiation parameters, such as irradiance, irradiation time, and wavelength. In general, when laser energy absorption was insufficient, little therapeutic effects were observed, whereas too much laser energy caused negative suppressing effects.5–11 For example, Bjordal et al.5 reported that there existed an optimal irradiance range for pain reduction in chronic joint disorders, which depended on the therapeutic target (finger, toe, knee, etc.) and laser wavelength. For wound healing, Demidova-Rice et al.7 determined a biphasic dose response curve of 635 nm light, which had a maximum positive effect at the energy density of 2 J/cm². In terms of laser wavelength, they reported that 820 nm showed better results than 635, 670, and 720 nm for the same energy density of 1 J/cm².

Although the propagation and absorption of laser light in a biological tissue is primarily governed by the laser irradiation parameters, it can also be affected by tissue condition changes even under the same laser irradiation conditions. One of the crucial tissue conditions is tissue water content. Earlier studies showed that the optical properties (absorption and scattering coefficients) of biological tissue varied substantially with the change of tissue water content.12–16 For example, Cilesiz and Welch15 reported that the absorption coefficient of the human aorta increased by ∼35% for 490 and 515 nm wavelengths and ∼42% for 630 nm wavelength when native human aorta was dehydrated by 50 to 90%. The authors also reported that the reduced scattering coefficient of the same sample increased by ∼9% due to dehydration for all three wavelengths. However, from the study of dorsal sections of full-thickness rat skin, Rylander et al.16 reported that dehydration could reduce light scattering in collagenous and cellular tissue. Noting that the optical properties of biological tissue were affected by dehydration, dehydration was also adopted as a method to induce variation in tissue optical property. For example, dehydration by air-immersion16 or agent-immersion17 was reported to enhance optical clearing by reducing light scattering in tissue.

On the other hand, the water content of human skin is known to change significantly between individuals, between body parts, or even with time for the same body part. For instance, Nakagawa et al.15 reported that the average water content of forearm skin of the elderly (mean age: 64) was 5.8% higher than that of young people (mean age: 21) and also that the average water content in forearm skin of healthy male increased by 2.3% in the afternoon from that in the morning. Similarly, the water fraction of women breast skin was reported to vary up to 60% among individuals.19 It was also reported that the relative water content of cheek was about twice higher than that of forearm and knee for the same person, which was attributed to the difference in stratum corneum thickness.20

Therefore, as the patient or target skin for LLLT is changed, it is likely that the optical properties of the target also change due to water content change as reported in the above studies, which in turn will result in a difference in laser light propagation and absorption even under the same laser irradiation conditions. While the administration of an optimum laser dose to a target skin is known to be critical in achieving desired therapeutic effects during LLLT, little information is available in the literature on how the variation of absorption and scattering coefficients due to water content change of the target affects the propagation of laser light.

In this work, the effects of water content on the distribution of laser light within biological tissue during low-level laser irradiation are investigated using visible (635 nm) and infrared (830 nm) lasers. It is demonstrated that the distribution of laser fluence rate measured along the laser beam propagation
direction differs with respect to tissue water content as well as laser wavelength. Based on numerical calculation results, it is discussed how the induced variations in absorption and scattering coefficients as a result of tissue water content change affect the fluence rate distribution inside tissue.

2 Materials and Methods

2.1 Sample Preparation

For experiments, porcine dorsal skin was used as the sample because of its similarity in structural characteristics to human skin. The porcine skin sample was obtained from a local abattoir immediately after postmortem and stored in a saline solution at 4°C to minimize dehydration and structural change until measurement. All measurements using the porcine skin sample (thickness ≈ 4 mm) were conducted at room temperature within 12 h from the collection.

The water content of a porcine skin sample, \( w_t \) (wt.%), was estimated using the following equation:

\[
w_t = \frac{M_{\text{wet}} - M_{\text{dry}}}{M_{\text{wet}}} \times 100,
\]

where \( M_{\text{wet}} \) is the mass of either a native or partially dehydrated sample (g) and \( M_{\text{dry}} \) is the mass of a dehydrated sample (g). The mass of dehydrated porcine skin (\( M_{\text{dry}} \)) was measured after dehydrating the native porcine skin at 50°C for 30 min using a moisture analyzer (MB45, Ohaus Corp.). Based on Eq. (1), the water content of native porcine skin was estimated to be ∼37 wt.%. The measured water content of 37 wt.% for native porcine skin appeared lower than the values reported in other studies (60 to 75 wt.%) for human and porcine skins. The reason for the relatively low water content in this study may be attributed to the difference in drying conditions. The water in human skin is customarily classified into three types: tightly bound water (0 to 7 wt.%), bound water (7 to 35 wt.%), and free water (above 35 wt.%). While free water is known to be readily removed in natural or air-dry conditions, earlier studies showed that the bound water could be removed only after significant dehydration, such as drying at 147°C for 50 h, 23 to 40°C for 140 h, or 100°C for 2 h, which significantly exceeded our drying condition (50°C for 30 min). Thus, it is considered that a significant portion of bound water remained after dehydration in the porcine skin samples in this study. Provided that the bound water content of ∼30 wt.% is accounted for the samples in this study, the actual water content may rise to ∼57 to 67 wt.%.

2.2 Laser Irradiation Unit

Two different continuous wave diode lasers with wavelengths of 635 nm (SIFC635, Thorlabs Inc.) and 830 nm (WSLP-830-050m-M-PD, Wavespectrum Laser Inc.) were used for the irradiation of samples because these wavelengths are frequently employed in LLLT. Both laser beams were collimated using a collimating lens (C230TM-B, Thorlabs Inc.) as shown in Fig. 1. The radii of collimated beams \( (w_0) \) of the 635 and 830 nm lasers were 1 and 1.1 mm, respectively. To match the irradiances of both lasers on the sample surface at ∼50 mW/cm², the irradiance level typically used in LLLT for pain reduction and anti-inflammation, the powers of the 635 and 830 nm lasers were set to 1.6 and 1.9 mW, respectively. The irradiation time was controlled with an electronic shutter and set to 20 s for all measurements.

2.3 Fluence Rate Measurement

While the sample surface was irradiated by the collimated laser beam (635 or 830 nm), the fluence rate inside the porcine skin along the laser beam propagation direction was measured at varying depths using an optical fiber sensor. The optical fiber sensor was fabricated as shown in Fig. 1 by inserting a commercial multimode optical fiber (M42L02, Thorlabs Inc.) into a hollow metal needle (outer diameter = 311 μm, inner diameter = 159 μm, length = 1.2 cm, tip angle = 20 deg) and gluing the surface of fiber cladding and the inner surface of the needle with epoxy resin. The fluence rate was measured over the depth range of 0.5 to 3 mm with a 0.5 mm interval by advancing the optical fiber sensor toward the sample surface. To prevent bulging of the sample surface while advancing the optical fiber sensor, a quartz window (thickness = 1 mm) was placed onto the sample surface and then the sample was clamped at both sides using two hollow acrylic plates (see Fig. 1). The depth of the optical fiber sensor from the sample surface was precisely calibrated with respect to the bottom surface of the quartz using translation stages. The light collected by the optical fiber sensor was measured with a photodiode (DET36A, Thorlabs Inc.), whose output voltage signal, \( S \) (V), is expressed by

\[
S = A \cdot P_1 \cdot P_2 \cdot \Phi,
\]

where \( A (= \pi r_e^2, \text{cm}^2) \) is the cross-sectional area of a fiber core with a radius \( r_e \) (cm), \( P_1 = (1 - \cos \theta_1)/2 \) is the ratio of fiber acceptance angle \( \theta_1 \) to the entire solid angle \( (4\pi) \), \( P_2 \) is photodiode responsivity (V/W), which varies with laser wavelength, and \( \Phi \) is the fluence rate (W/cm²). By using Eq. (2), the fluence rate at each depth was estimated from the measured voltage signal.
2.4 Optical Property Measurement

The optical properties (absorption and reduced scattering coefficients) of porcine skin at varying water content conditions were measured for both laser wavelengths as follows. First, the total reflectance and transmittance of each water content sample were measured using a double-integrating sphere (AvaSphere-30, Avantes, port diameter = 6 mm). Then, from the measured total reflectance and transmittance data, the optical properties were estimated using an inverse adding-doubling program. In a double-integrating sphere measurement, the sample thickness was recommended to be $\sim 1/10$ of the port diameter or smaller to reduce edge loss. The thickness of native sample for optical property measurement was $\sim 700 \, \mu\text{m}$ as measured with digital vernier calipers (CD-20CP, Mitutoyo Corp.), which, however, decreased with dehydration to $\sim 680, 660, 630,$ and $600 \, \mu\text{m}$ at the water content of 34, 31, 28, and 25 wt.%, respectively. Below 25 wt.%, the sample thickness showed little change. In addition, the edge loss compensation function of the inverse adding-doubling program was applied for the optical property calculation.

The measurement of optical properties was carried out for samples with the water content range of 19 to 37 wt.% with a 3 wt.% interval. For each water content condition, three samples were tested and each sample was measured three times. The results from three test samples are presented by their average and relative standard deviation.

2.5 Calculation of Fluence Rate

The fluence rate of incident laser beam inside the porcine skin samples was also calculated using the diffusion equation for comparison with experimental data. For samples like skin tissue for which scattering dominates over absorption, the propagation of laser light can be reasonably described by the diffusion equation, a simplified form of the radiative transfer equation. Under the conditions of time-independent state, cylindrical symmetry, and normally incident collimated beam, the diffusion equation is expressed by

$$\mu_s \Phi_d(r, z) - D \nabla^2 \Phi_d(r, z) = (3D\mu_g + 1)\mu_s \Phi_i(r, z),$$

where $\Phi_d(r, z)$ is the diffuse fluence rate ($\text{W/m}^2$), $g$ is the anisotropy factor incorporating the effects of directionally dependent scattering, $\mu_s$ and $\mu_a$ are the absorption and scattering coefficients ($\text{m}^{-1}$), $D = [3(\mu_s + \mu_a)]^{-1}$ is the diffusion coefficient, where the reduced scattering coefficient ($\text{m}^{-1}$) is defined by $\mu_s \equiv \mu_s / (1 - g)$. For a laser beam with nearly uniform spatial profile, the reduced incident fluence rate, $\Phi_i(r, z)$ ($\text{W/m}^2$), is expressed by

$$\Phi_i(r, z) = (1 - R) \frac{P}{\pi w_0^2} \exp(-\mu_s z) \quad \text{at} \quad r \leq w_0,$$

$$\Phi_i(r, z) = 0 \quad \text{at} \quad r > w_0,$$

where $R$ is the specular reflectance off the air/tissue surface at normal incidence and $P$ and $w_0$ are the laser power ($\text{W}$) and beam radius (m) at the tissue surface, respectively.

At the tissue surface where the diffusion approximation does not hold, the following refractive index mismatched boundary condition was applied:

$$\Phi_d(r, z) - 2C_R D \frac{\partial \Phi_d(r, z)}{\partial z} + 6C_R D \mu_s g \Phi_n(r, z) = 0,$$

where $C_R$ relates to the internal reflection at the interface and can be approximated to be.

$$C_R = 1.6896 - 4.90466n + 4.33904n^2 - 0.13755n^3,$$

where $n$ is the relative refractive index representing the ratio of tissue refractive index to that of the ambient medium. In this study, $g = 0.86, R = 0.023,$ and $n = 1.36$ were utilized for calculation.

3 Results and Discussion

Figure 2 shows the absorption ($\mu_a$) and reduced scattering ($\mu_s$) coefficients of the porcine skin measured at varying water content conditions for the 635 and 830 nm wavelengths. It is shown that the $\mu_s$ of native porcine skin sample (37 wt.%), which was measured to be $0.39$ and $0.68 \, \text{cm}^{-1}$ for 635 and 830 nm, respectively. $\mu_s$ values of porcine skin reported in earlier studies were $\sim 0.5 \, \text{cm}^{-1}$ in $600$ to $850 \, \text{nm}$ regime or $0.7$ and $1.6 \, \text{cm}^{-1}$ at $633$ and $850 \, \text{nm}$, respectively. Compared to these results, the absorption coefficients measured in this study are considered to lie in an acceptable range. Contrary to that the absorption of biological tissue at $830 \, \text{nm}$ is typically lower than at $635 \, \text{nm}$ in the optical window. The $\mu_s$ at $830 \, \text{nm}$ wavelength is higher than that at $635 \, \text{nm}$ wavelength, which is understood to be attributed to the absence of blood in the in vitro samples.
Sample because light absorption by blood (specifically hemoglobin) is stronger at a 635 nm wavelength. Earlier study for the absorption of in vitro porcine skin dermis sample also reported that \( \mu_s \) at a 850 nm wavelength (1.6 cm\(^{-1}\)) was higher than that at a 633 nm wavelength (0.7 cm\(^{-1}\)). Note that \( \mu_s \) of the 635 nm wavelength in Fig. 2(a) shows a clear tendency of increase for decreasing tissue water content. For the 830 nm wavelength, although the data points show much greater relative standard deviation and poorer consistency than the 635 nm wavelength case, a tendency of slight increase is still observable as the tissue deviation and poorer consistency than the 635 nm wavelength although the data points show much greater relative standard deviation and poorer consistency than the 635 nm wavelength case, a tendency of slight increase is still observable as the tissue water content decreases. The observed increase of \( \mu_s \) for decreasing water content may be attributed to denser packing of collagen fibers during shrinkage of the tissue sample by dehydration. On the other hand, \( \mu_s \) of the porcine skin in Fig. 2(b) shows nearly the same pattern for both wavelengths. \( \mu_s \) decreased gradually as the water content decreased from the native condition of 37 wt.% to \( \sim 22 \) wt.% but dropped rather rapidly as the water content decreased from 22 to 19 wt.%. The observed decrease of \( \mu_s \) as a result of dehydration of skin tissue agrees with an earlier report.

Figure 3 shows the measured and calculated fluence rate distribution along the depth of porcine skin samples for different water content conditions. Each experimental data point represents the average and relative standard deviation from nine measurements on the same sample. The average difference between measured and calculated fluence rates at 635 nm wavelength was \( \sim 4.5\% \) (maximum = 13.5\% at 1.5 mm depth for 25 wt.% sample) and that at 830 nm wavelength was \( \sim 5.5\% \) (maximum = 14.5\% at 2 mm depth for 31 wt.% sample). First of all, the results show that the fluence rate and its distribution changed notably as tissue water content changed under the same laser irradiation conditions. Note that the irradiance at the sample surface was fixed at 50 mW/cm\(^2\) for all measurements. For biological tissue in which \( \mu_s, \mu_t, \mu_s, \mu_s ' \) is satisfied, it is known that (1) if \( \mu_t ' \) remained constant, an increase of \( \mu_s \) results in an overall decrease of fluence rate at all depths and (2) if \( \mu_s \) remained constant, a decrease of \( \mu_s ' \) leads to a decrease of fluence rate at and near the surface but an increase in deeper locations. According to this information, the fluence rate at and near the surface should have decreased while that in deeper locations increased as the water contents dropped from 37 to 19 wt.% because \( \mu_s \) was also decreased as shown in Fig. 2(b). This expected change in fluence rate distribution was in fact observed in the data for the 830 nm wavelength in Fig. 3(b), where the fluence rate at 0.5 mm depth decreased by maximum 32\% while that at 3 mm depth increased by a maximum 25\%. The calculated fluence rate also agreed closely with the measured data for this wavelength. Unlike the 830 nm wavelength case, however, the measured and calculated data for the 635 nm wavelength in Fig. 3(a) do not show exactly the same trend. While the fluence rate change at and near the surface showed a trend similar to the 830 nm wavelength case (maximum 31\% decrease), that in the deeper locations revealed an unclear pattern.

The observed difference in fluence rate distribution between the 635 and 830 nm wavelengths can be better understood by examining how \( \mu_s \) and \( \mu_s ' \) affect the fluence rate of both laser wavelengths as the water content of the target tissue changes from 37 to 19 wt.%. For this purpose, taking \( \mu_s \) and \( \mu_s ' \) of the 37 wt.% sample as the reference values, the influence of \( \mu_s \) and \( \mu_s ' \) change on the fluence rate distribution was investigated for the following three cases by numerical simulation: (1) \( \mu_t ' \) was changed to that of the 19 wt.% sample while \( \mu_s \) remained the same (at the 37 wt.% value), (2) \( \mu_s ' \) was changed to that of the 19 wt.% sample, while \( \mu_s \) remained the same, and (3) both \( \mu_s \) and \( \mu_s ' \) were changed to those of the 19 wt.% sample, as summarized in Table 1 with the corresponding \( \mu_s \) and \( \mu_s ' \) values. Figure 4(a) shows the calculation results of \( \Phi_{case1}/\Phi_{37wt.\%} \) for the 635 nm wavelength, where \( \Phi_{casej} \) represents the fluence rate calculated with one of the three combinations of \( \mu_s \) and \( \mu_s ' \). When \( \mu_t ' \) only was changed to that of the 19 wt.% sample as described in the first case (for this case, \( \mu_s ' \) decreased by 47\%), \( \Phi_{case1}/\Phi_{37wt.\%,635nm} \) became <1.0 at and near the surface but increased gradually >1.0 as the depth.

![Figure 3](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/figure3.png)

**Figure 3** Fluence rate profiles of the (a) 635-nm and (b) 830-nm lasers along the laser beam propagation direction in different water content samples.

<table>
<thead>
<tr>
<th>Case</th>
<th>( \mu_s ) (cm(^{-1}))</th>
<th>( \mu_s ' ) (cm(^{-1}))</th>
<th>( \mu_s ) (cm(^{-1}))</th>
<th>( \mu_s ' ) (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 wt.% (Reference)</td>
<td>0.39</td>
<td>27.33</td>
<td>0.68</td>
<td>19.62</td>
</tr>
<tr>
<td>Case 1 (( \mu_s ' ) of 19 wt.%)</td>
<td>0.66</td>
<td>14.67</td>
<td>0.76</td>
<td>10.31</td>
</tr>
<tr>
<td>Case 2 (( \mu_s ) of 19 wt.%)</td>
<td>0.66</td>
<td>27.33</td>
<td>0.76</td>
<td>19.62</td>
</tr>
</tbody>
</table>

Note: Bold values represent the values changed from the reference values at 37 wt.%.
increased. Next, when μs only was changed to that of the 19 wt.% sample (μs increased by 68%), Φ_{case 2}/Φ_{37 wt.%} dropped to below that of the 37 wt.% sample and the ratio was almost constant over the entire depth in the experiments (0.5 to 3 mm). For the 830 nm wavelength, the fluence rate ratio kept increasing almost linearly with respect to depth, which represents that the fluence rate of the 19 wt.% sample in this deeper region became greater than that of the 37 wt.% sample.

In the LLLT point of view, the above results may be interpreted as follows. Assuming that the optimal laser irradiance was determined at the water content condition of the native sample (37 wt.%), (1) provided that the therapeutic target region lies within ~1 mm depth from the surface, the laser irradiance should be increased to achieve the same therapeutic effects regardless of激光波長 if the water content of target tissue is lower than the native sample and (2) provided that the therapeutic target region lies beyond ~1 mm depth from the surface, laser irradiance should be changed in consideration of the laser wavelength. That is, for the 635 nm wavelength, the laser irradiance should be kept nearly the same or slightly increased if the water content of target tissue is lower than the native sample, whereas for the 830 nm wavelength, the laser irradiance needs to be reduced to achieve the same therapeutic effects in this deeper region.

4 Conclusion
From the experimental and numerical study of porcine skin irradiated by low-level 635 and 830 nm lasers, it was shown that the fluence rate and its distribution along the laser propagation direction changed notably as the water content of porcine skin was reduced from that of a native sample (37 wt.%) to 19 wt.%.

The above-explained difference in fluence rate distribution between the 635 and 830 nm wavelengths suggests that the fluence rate ratio of both wavelengths had almost the same distribution in the region close to the sample surface (for depth < ∼1 mm, region 1), whereas for the 830 nm wavelength, the laser irradiance needs to be reduced to achieve the same therapeutic effects in this deeper region.

The above-explained difference in fluence rate distribution between the 635 and 830 nm wavelengths suggests that the fluence rate ratio of both wavelengths had almost the same distribution in the region close to the sample surface (for depth < ∼1 mm, region 1), whereas for the 830 nm wavelength, the laser irradiance needs to be reduced to achieve the same therapeutic effects in this deeper region.

References


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