Near-infrared Absorption Property of Biological Soft Tissue Constituents

Cheng-Lun Tsai*    Ji-Chung Chen    Wen-Jwu Wang1

Department of Biomedical Engineering, Chung-Yuan Christian University, Chung-Li, Taiwan 320 ROC
1Department of Chemistry, Tamkang University, Tamsui, Taiwan 251 ROC

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Abstract

Near infrared (NIR) can penetrate relatively deep into biological soft tissues. The NIR absorption property of tissue varies with tissue constituents especially water, fat, collagen, and their combination ratio. Therefore, combination ratio of tissue constituents can be evaluated by decomposing the absorption spectrum to determine the light path length in each constituent. Standardized absorption spectra of tissue constituents are required in order to carry out decomposition. Since water, fat, and protein are the major contributors at NIR spectral region. This study is to measure their absorption spectra from standardized samples as reference for quantifying tissue constituents. Five kinds of major fatty acid found in human fat were mixed in proper ratio as a standard reference. Absorption spectrum of bovine skin gelatin and elastin in hog eye lens were used as references of protein. NIR absorption spectra were measured using a Shimadzu 3101-PC spectrophotometer. The results show that temperature has a strong effect on the absorption property of water but not on fatty acid mixture. Absorption spectrum of elastin is similar to that of dry bovine gelatin. NIR spectroscopy also can be used to characterize or identify different types of soft tissue based on their major chemical composition, such as detecting a fat plaque in a muscular tissue or a tumor in a high fat content tissue.

Keywords: Near infrared spectroscopy, Absorption, Water, Fat, Eye lens

Introduction

Most biological soft tissues have relatively low light absorption property in the visible and NIR spectral regions, especially between 600 nm and 1300 nm. This spectral range is known as a “tissue optical window” or “therapeutic window”.[1] Outside of this region, light is greatly absorbed by tissue pigments (such as hemoglobin and melanin) in the visible spectral region and by tissue water content in the long wavelength NIR spectral region.[2-4] Low absorption also means that less light energy is needed for optical measurement in tissue, and in consequence less thermal damage or other light-tissue interaction will happen. Even though the absorption of NIR by tissue is relatively low, it should not be totally ignored when light has to pass over a few millimeters in tissue. Biological soft tissue also has lower scattering property at NIR region than at visible region. NIR in therapeutic window can penetrate into tissue and reflected back to the surface without being significantly absorbed.[5] But, visible light will be greatly scattered and absorbed within the depth of just a few hundred microns. This makes NIR a good spectral region for performing non-destructive measurements on thick or bulky biological tissue.[6]

Cheong et al. have reviewed optical properties of different kinds of biological tissue published in literature.[7] For most biomedical optics applications, optical properties of tissue determine the light distribution in tissue and the diffusive reflectance intensity. Most biological tissues are not translucent materials whose absorption coefficient can be easily calculated from transmission measurement with the Beer-Lambert law. Many complicated mathematical models have been applied to evaluate the absorption property of a scattering material from measurements. Among them, photon diffusion model and Monte Carlo model are most often used. Since biological soft tissues are non-homogeneous, volume fractions and distribution of tissue constituents will affect the absorption property. Therefore, large variation in tissue absorption property is generally found among different samples, and it is very difficult to accurately measure the absorption properties of biological tissues. By assuming the intrinsic absorption properties of each tissue constituent will remain the same after they are brought together to compose a tissue, the absorption spectra of tissue constituents are measured separately. This assumption is generally true because it is well known that water dominates the

* Corresponding author: Cheng-Lun Tsai
Tel: +886-3-4372152 ; Fax: +886-3-4563171 ext.4599
E-mail : clt@mail.be.cycu.edu.tw
NIR absorption spectra of many high water content intact soft tissues, such as human skin and the aorta.[8] Their NIR absorption spectra all show the absorption peaks that closely match the absorption peaks of water. Although optical measurement can be made in the time, space, or spectral domains to characterize the physical or chemical properties of a material,[5] Spectral measurement is most likely to provide information about the tissue composition. Absorption property of tissue is the combination of different tissue constituents weighted by their volume fractions. With the known absorption spectra of water, fat, and protein, path lengths of light in different tissue constituents can be found. Path lengths change when the volume fractions of tissue constituent change. NIR spectroscopy can be used to detect the change in tissue constituents under certain physiological conditions. This NIR spectroscopic approach also has been applied by Conway et al. to detect tissue fat content by using NIR below 1100 nm.[9] Therefore, the purpose of this study is to set up the standard reference NIR absorption spectra of tissue constituents.

Since the interference of tissue pigments is less significant when longer wavelength is used, this study focused on the absorption property of intact biological tissues and their components in the NIR spectral region to about 1300 nm that is the upper border of “therapeutic window”. Sample materials that were used in this study including distilled water, fatty acid, pork lard, hog eye lens, and bovine gelatin. The transmission spectra of these major tissue components were measured and their absorption peaks in the NIR spectral region were identified based on literature studies.

### Methods and Materials

The NIR transmission spectra in this study were measured using a dual beam spectrophotometer (Shimadzu 3101-PC UV-Vis-NIR) with spectral resolution (the increment step) of 0.1 nm. Spectral bandwidth was 1.2 nm, and wavelength accuracy was 0.6 nm. Average of ten scans was taken on each sample as its absorption spectrum. The specular reflectance at cuvette-sample interface is also wavelength and temperature dependent. To eliminate this effect, the same liquid sample was put in both the reference and the sample cuvettes with different path lengths. Path length of reference cuvette was 1 mm, and the path length of sample cuvette was 2 mm, 5mm, or 10 mm. Transmission through the reference cuvette was treated as the incident light intensity. Based on the Beer-Lambert law, the absorption spectrum, $a(\lambda)$, was calculated from the transmission spectra through two light paths as:

$$a(\lambda) = \frac{1}{x_2-x_1} \ln \frac{I_{x_2}(\lambda)}{I_{x_1}(\lambda)}$$

where $I_{x_2}(\lambda)$ and $I_{x_1}(\lambda)$ are the transmission spectra, $x_1$ is the path length of reference cuvette, and $x_2$ is the path length of sample cuvette. The temperature effect on the absorption spectrum of water was measured by increasing the temperature from 25°C to 43°C with 3°C of increment.

#### 2.2 Fatty acid mixture and pure pork lard

Animal fat contains many kinds of fatty acid. About 95% of human fat are made up of five kinds of major fatty acid: oleic, palmitic, linoleic, stearic, and palmitoleic.[16] These fatty acids have certain similarity in their chemical structures, therefore, their NIR absorption spectra are also similar. Among them, only the oleic acid is in liquid form under room temperature. To obtain the representative absorption spectrum of human fat, a large number of purified human fat samples have to be measured and averaged. A more reproducible result can be obtained by defining a standard sample as a reference. The latter approach was taken in this study. Pure fatty acid purchased from Sigma and R.D.H. were mixed together in a proper ratio as a standard fat sample, as listed in Table 1. The fatty acid mixture was heated up to 40°C to make it melt and transparent. With the standardized mixing ratio, this reference absorption spectrum can be easily reproduced.

To make sure this mixture is an adequate reference, absorption spectra of pure oleic acid, mixed fatty acid, and pure pork lard purchased from local supermarket were compared. Pork lard has been shown to contain fatty acids similar to human fat as shown in Table 1. The rendered pork lard was white and opaque in solid form under room temperature. Pork lard was placed in a beaker and melted by heating up to 60°C. Small air bubble trapped in the melted pure pork fat was filtered using a filter paper. The melted fatty acid was clear, translucent and yellowish in color. Temperature effect on the absorption property of fatty acid was also studied by carrying out the measurement at 40°C, 43°C, and 46°C.

#### 2.3 Hog eye lens and bovine gelatin

Collagen and elastin are the major protein constituents in connect tissue. They are also important contributors to the total tissue NIR absorption. Connective tissues generally have high water content and strong light scattering characteristics. These characteristics make it difficult to acquire the light absorption property from direct measurement. One exception that has a very low light scattering property is eye lens. Its absorption spectrum could be measured with minimal interference from light scattering. Since eye lens would become

<table>
<thead>
<tr>
<th>fatty acids</th>
<th>human depot fat</th>
<th>pork lard</th>
<th>beef tallow</th>
<th>reference sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic</td>
<td>46.9 %</td>
<td>47.5 %</td>
<td>49.6 %</td>
<td>50.0 %</td>
</tr>
<tr>
<td>Palmitic</td>
<td>24.0 %</td>
<td>28.3 %</td>
<td>27.4 %</td>
<td>25.0 %</td>
</tr>
<tr>
<td>Linoleic</td>
<td>10.2 %</td>
<td>6.0 %</td>
<td>2.5 %</td>
<td>10.0 %</td>
</tr>
<tr>
<td>Searic</td>
<td>8.4 %</td>
<td>11.9 %</td>
<td>14.1 %</td>
<td>10.0 %</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>5.0 %</td>
<td>2.7 %</td>
<td>NA</td>
<td>5.0 %</td>
</tr>
</tbody>
</table>

Table 1: Compositions of pure animal fat [16]
Opaque when it starts to dehydrate, sample preparation has to be done with care and the measurement has to be done as soon as possible. Eye lens also has convex surfaces that are not suitable for measuring the absorption coefficient in a spectrometer. To keep light beam in collimation while passing through the center region of eye lens, a plastic sample holder (as shown in Figure 1) was used to clamp the lens to 6 mm in thickness. Two quartz windows compressed eye lens to form flat surfaces on both sides. Two square apertures (4 × 4 mm²) were placed in light paths to block the light that did not pass through the flat regions. Both the reference and sample cells were filled with saline to reduce the specular reflectance and have similar transmission intensity in two light paths. This sample holder was fixed on a Shimadzu film sample holder for carrying out measurement. Samples were measured under room temperature (about 25°C) in an air-conditioned room.

Because there was different amount of water present in two light paths, light absorption contributed by water has to be compensated. The transmission of two light paths was formulated as

\[ I_{\text{water}}(\lambda) = I_0(\lambda) e^{-a_{\text{water}}(\lambda) d} \]  

and

\[ I_{\text{lens}}(\lambda) = I_0(\lambda) e^{-[a_{\text{elastin}}(\lambda)xd + a_{\text{water}}(\lambda)(1-x)d]} \]  

where \( I_{\text{water}}(\lambda) \) and \( I_{\text{lens}}(\lambda) \) are the transmission through two light paths, and \( I_0(\lambda) \) is the incident light intensity. The volume fraction of elastin in eye lens is \( x \), and \( d \) is the thickness of the compressed eye lens. Combining these equations, the absorption spectrum of collagen can be calculated as

\[ a_{\text{elastin}}(\lambda)xd = \ln \frac{I_{\text{water}}(\lambda)}{I_{\text{lens}}(\lambda)} + a_{\text{water}}(\lambda)xd \]  

Gelatin is a kind of protein that might have similar NIR absorption characteristics as that of collagen. A large piece of...
translucent sample of purified gelatin can be made for measuring its absorption spectrum. The dehydrated gelatin was made from bovine skin (Sigma Type B gelatin). Gelatin powder and distilled water were weighed, mixed, and heated to about 60 °C in a beaker until the gelatin powder was totally dissolved. The gelatin solution was left at room temperature for one to two hours to cool, then it turned into a firm piece of gel. After measuring the total weight again, water content remaining in the gel was calculated. The gel was clear, translucent, and slightly yellowish in color. It was taken out from beaker, weighed, and left in freezer for two days to dehydrate. Since the weight of dehydrated gelatin was the same as the original weight of gelatin powder, there was hardly any water left in the dehydrated gelatin sample. The dehydrated gelatin was still yellowish and translucent but darker in color. Thickness of gelatin sample was measured with a micrometer. The sample was directly clamped on a film sample holder for measurement with air as reference. Its absorption spectrum was compared with that of elastin.

Results

The light absorption property of water has been intensively studied by many researchers.[4-5] The magnitude of the absorption spectra shown in Figure 2 are close to those found in literature. The absorption spectra of saline under different temperature are shown in Figure 3. The absorption property at 970 nm and 1160 nm increased with temperature. This temperature dependency also matches the measurement by Buijs et al.[10] The absorption property for wavelength longer than 1300 nm increases greatly. At 1390 nm, the absorption property would reach $10 \, \text{cm}^{-1}$. The transmission intensity of 1390 nm is less than 0.005% after traveling through 1 cm thick of water.

The absorption spectra of oleic acid, fatty acid mixture, and pork lard are found to be quite similar in this spectral region, see Figure 4. There are two relatively weak absorption peaks at 930 nm and 1040 nm. The absorption peak at around 1200 nm shows evidence of double peaks at 1170 nm, and 1210 nm. Aside from the absorption peaks, these absorption spectra were very flat comparing with that of water. Since the fatty acid mixture only became melt and translucent at temperature above 40 °C, measurement was taken at 40°C, 43°C, and 46°C. In Figure 5, no obvious change in the absorption property of
Near-infrared Absorption of Tissue

Fig. 5: Absorption spectra of melted fatty acid mixture at 40°C, 43°C, and 46°C. Wavelengths of absorption peaks are 930 nm, 1040 nm, 1165 nm, and 1210 nm.

Fig. 6: Spectra of $\ln[I_{\text{water}}(\lambda)/I_{\text{lens}}(\lambda)]$ (solid line), $a_{\text{water}}(\lambda)xd$ (dash line), and $a_{\text{elastin}}(\lambda)xd$ (bold line). The absorption peak of water at 970 nm (slash area) was used to estimate the water content needed for compensation. Absorbance spectrum of elastin is revealed after the contribution of water was compensated.

The absorption spectrum of eye lens was measured by using the same thickness of saline as the reference. The $\ln[I_{\text{water}}(\lambda)/I_{\text{lens}}(\lambda)]$ spectrum in Figure 6 was the average of five eye lens sample. Besides the baseline offset, the spectrum of each lens was the same in spectral profile. Because of different water content in two light paths, it caused an interference in the $\ln[I_{\text{water}}(\lambda)/I_{\text{lens}}(\lambda)]$ spectrum. This interference could be canceled out if the volume fraction of water in the eye lens were known. Since it is difficult to accurately measure the water volume fraction by dehydrating the eye lens, an alternative method was used. As shown in Figure 6, the dent at 970 nm in the measured $\ln[I_{\text{water}}(\lambda)/I_{\text{lens}}(\lambda)]$ spectrum was caused by extra water absorption term, $a_{\text{water}}(\lambda)xd$, in equation (4). By assuming that collagen has a relatively flat absorption spectrum in the region between 900 nm and 1070 nm, the volume fraction of collagen was estimated based on the absorption peak of water at 970 nm. The volume fraction ($x$) of elastin in the eye lens samples was calculated to be 39.6%. With this volume fraction, the absorption coefficient of elastin, $a_{\text{elastin}}(\lambda)$, was calculated using equation (4).

The absorption coefficient spectra of saline, mixed fatty
acid, dehydrated gelatin, and elastin are shown together in Figure 7 for comparison. Both gelatin and elastin had a small absorption peak at about 1190 nm.

**Discussion**

In the NIR region with wavelength longer than 1300 nm, absorption properties of tissue constituents are about one to two orders larger than those shown in this study. The strong absorption of NIR by water generally limits the penetration depth of light in tissue. It would require either a very high intensity light source or a very sensitive detecting circuit for probing the inside of tissue. However, high energy of light might cause thermal damage. Therefore, only the light with wavelength shorter than 1300 nm is suitable for sending light deep into biological tissue. As mentioned before, the light absorption still can not be ignored, especially when path length of light increases. Accurate absorption property is of great importance in choosing the proper wavelength and power as well as quantifying light distribution and path length in tissue. If absorption measurement were done on a bulky intact tissue, light scattering would make the accuracy of measurement very poor. Even though a thin layer of intact tissue can be sliced down for measurement to reduce scattering effect, the absorption peaks at below 1300 nm will be too low to be seen. Light path length has to be long enough to reveal the absorption property of the short wavelength NIR. This is why the purified major soft tissue constituents were used in this study to measure the elements of absorption property of tissue.

The near infrared spectrum of biological materials basically results from the overtones and combinations of O-H, C-H, and N-H groups’ stretching vibrations.[11] Since water and fatty acids are the major tissue components in tissue and they consist similar O-H and C-H intramolecular hydrogen-bonds, water and fatty acids become the major NIR absorbing materials in soft tissues. The absorption peaks in water transmission spectra are related to the O-H bond in water molecules. Two major absorption peaks of water shown in Figure 3 are the overtones at 960 nm and the combination band at 1190 nm.[4,11] In Figure 4, the two small absorption peaks at 930 nm and 1040 nm in the transmission spectra of pure pork fat are the combinations of stretching and bending of the methyl and methylene groups in fatty acids.[12] The large absorption peak near 1200 nm is the second overtone of the C-H stretching vibration also in fatty acids. For unsaturated fatty acids, the 1200 nm absorption peak is accompanied by another peak at 1180 nm and appears as double peaks.[13,14]

Elastin and gelatin are found to have a higher baseline of absorption properties than those of water and fatty acid. Because gelatin has a very complicated chemical structure which does not have a specifically abundant type of intramolecular O-H or C-H bonding, the C-H related absorption peak at around 1190 nm shown in Figure 5 is relatively small. In addition, the volume fraction of proteins in soft tissue is relatively small, about 20% to 30% in skin and probably less than 10% in fat tissue. The contribution by this absorption peak in intact tissues is very small and buried in the strong absorption peak of water and fatty acids at the same wavelength region.

Although temperature, ion content, ion concentration, and many other factors might affect the intrinsic absorption spectra of tissue components,[10,15] most of these factors should insignificant in *in vivo* measurements. In a living body with homeostatic conditions, these factors are either stable or variable only within a small range. The absorption properties of hemoglobin and its derivatives are lower than that of water and fat in the wavelength region beyond 1000 nm. Volume fraction of hemoglobin is also relatively small in tissue. Therefore, these
pigments and their oxygen saturation should be very small in long wavelength region.

Conclusion

Absorption spectra of standardized samples of biological soft tissue constituents including water, fat, and protein were measured in this study. The absorption peaks of these spectra in the NIR range from 900 nm to 1300 nm are identified. These absorption spectra can be used as references for estimating the volume fractions of different tissue constituents in an unknown tissue sample. For applications in biomedical optics, absorption property of a biological soft tissue also can be estimated based on its constituents and their volume fractions. With better determined absorption property, scattering property of biological tissue also can be more accurately determined from mathematical models involving both absorption and scattering factors.

Acknowledgments

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References