

## Measurement of the Optical Properties of Pig Muscle with Sudan Based on the Inverse Adding-Double Method and Integrating Sphere

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In this paper, the adding-double (AD) method and Monte Carlo (MC) method were compared for demonstrating the accuracy of the AD method. The results of the comparison indicated that the error of the AD method relative to the MC method was less than 3%. The running time of the AD method was only several seconds, which was less than the running time of MC method. And the outputs of the Inverse adding-double (IAD) method were consistent with the original data, which were the input of the MC program. After checking the validity of the IAD method, it was used to obtain the optical properties of the pig muscle with different concentrations of Sudan. The results indicated that the absorption and reduced scattering properties were dependent on the wavelength, and at 700 nm there were significant increase in the absorption and reduced scattering values for the samples with Sudan compared to the normal sample.

**Key words:** Near infrared spectroscopy, Integrating sphere, Adding-doubling method, Optical properties, Pig muscle.

Spectroscopy technique is a fast and efficient method for the online analysis. The main features of spectroscopy are as follows: (1) Rapid analysis with high efficiency. (2) By using a suitable container, the samples in different material states (such as liquid, solid, semi-solid, jelly and so on) can be measured directly. (3) Generally the measured samples don't require preprocessing. (4) Noninvasive. That is to say the samples won't be damaged and the original properties can be maintained during the measurement<sup>1-4</sup>.

In recent years, the near-infrared spectroscopy has been widely used in the identification of food, agricultural analysis, disease detection and many other fields<sup>11-14</sup>. The tissues can be characterized by the optical properties, which include the absorption coefficient ( $\mu_a$ ), the

scattering coefficient ( $\mu_s$ ), the phase function ( $P(\theta)$ ), the anisotropy value ( $g$ ) and the reduced scattering coefficient ( $\mu'_s$ ). The optical properties may reveal the morphological and biochemical composition information of the samples and can also be used to identify different kinds of objects. So obtaining the optical properties quickly and accurately is very important for the analysis of the samples<sup>5-7</sup>. The aim of this paper is to obtain the absorption coefficient and the reduced scattering coefficient of the samples by analyzing the near-infrared spectroscopy of the samples.

Different methods have been used to analyze spectroscopy to obtain the optical properties of biological tissues<sup>8</sup>. In this paper IAD method was used to obtain the absorption and reduced scattering properties. The traditional methods such as the diffusion equation, random walk models, Kubelka-Munk, the seven-flux model, and Chan-drasekhar's X and Y functions all need restrictions on one or more of the basic tissue

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properties. However, the optical properties with IAD method were received by repeatedly solving the radiative transport equation until the solution matched the measured transmission and reflection values<sup>8, 9</sup>. So compared with the traditional methods, IAD method is more accuracy and flexibility when modeling turbid samples with mismatched boundary, intermediate albedos, and anisotropic scattering. That's why IAD method was used in this paper to get the optical properties from the samples' near-infrared spectrums.

In this article, the AD method was first compared with the MC method to verify the effectiveness of the AD method. Monte Carlo simulation of photon propagation provides an effective approach toward photon transport in turbid tissues. The method was frequently employed in many studies. From the simulation, specular reflectance, diffuse reflectance, absorbed fraction and transmittance of the turbid medium can be received. In order to ensure the accuracy of the results, about 1000000 photons were used in each simulation. The MC simulation time was about half an hour. The AD method took only a few seconds to obtain the reflectance and the transmittance. After checking the validity of the IAD method, it was used to obtain the optical properties of the pig muscle with different concentrations of Sudan. The results indicated that the absorption and reduced scattering properties were dependent on the wavelength, and at 700 nm there were significant increase in the absorption and reduced scattering values for the samples with Sudan compared to the normal sample. The reflectance and transmittance spectrum of the pig muscle were measured with the integrating sphere system.

## MATERIALS AND METHODS

The experimental system consisted of a tungsten-halogen light source (DH-2000 Ocean Optics), a fiber-optic spectrometer (USB4000 Ocean Optics), an integrating sphere and fiber optic probes. The pork tissues were soaked with different concentrations of Sudan solution. The Monte Carlo simulation program, AD and IAD programs, the Spectra Suite and the MATLAB processing software were used in this paper. The experiment devices were shown in Figure 1.

The pork tissues were cut into slices, and the thickness of the slices was chosen to be 1mm. Then the samples were kept in 5°C for the following experiment. Different concentrations of Sudan solution were prepared by adding different weight of Sudan into 100ml solvent, and the solvent was heated in the boiled water to improve the solubility of Sudan. The weight of Sudan was changed from 0.05g to 1.5g. Each time 0.05g Sudan was added into the solvent to change the concentrations of Sudan.

Monte Carlo simulation and the addingdouble method were compared for demonstrating the accuracy of the AD method. 32 groups of absorption and scattering values were selected based on the values that may be commonly found in tissues<sup>10</sup>. At the wavelength from 650 to 1000 nm, the  $\mu_a$  was varied from 0.053-0.099 mm<sup>-1</sup> and the  $\mu_s$  was varied from 1.04-2.19 mm<sup>-1</sup>. A fixed value of  $g=0.95$  was chosen in all the simulations<sup>11</sup>, which still permitted us to simulate a broad range of by combining different  $\mu_a$  and  $g$ .

In order to compare the two methods, the values of reflection and transmission simulated by the AD<sup>9</sup> method were compared with the results of MC<sup>12</sup> method which were regarded as the true values. The relative error calculations were used to express the deviation of AD from MC. Then the values of reflection and transmission obtained by the MC were input into the IAD program to test the accuracy of the IAD method. The relative errors of the optical properties from IAD relative to the original value were given in this paper.

### Optical properties of pork tissues

The IAD method was used to extract the optical properties of the pig muscle with different concentrations of Sudan. Prior to measurements, pig muscle slices and Sudan solution should be prepared just as described in above. The pig muscle was soaked in Sudan liquor for one hour. In order to keep the samples fresh, the samples were sandwiched between thick glass slides when the measurements were performed. Reflection and transmission measurements were needed to extract the absorption and scattering properties of the pig muscle. The experiment setup for reflection and transmission measurement was shown in Figure 1 and Figure 2 respectively.

The comparison method<sup>13, 14</sup> was used to obtain the reflectance and the transmittance of the

pig muscle. Reflectance spectrum was measured with the integrating sphere just as shown in Figure 1. The total reflectance is defined in terms of the standard reflection and sample reflection measurements:

$$R_{ds} = \frac{I_{rs}}{I_{rr}} R_{dr} \quad \dots (1)$$

Where  $I_{rs}$  and  $I_{rr}$  are the measured intensities of pig muscle sample and the standard with known reflectance  $R_{ds}$  is the reflectance of the sample and  $R_{dr}$  is the known reflectance of the standard. The reflectance of the standard is 0.96. The same method is used in the measurement of transmittance.

$$T_{ds} = \frac{I_{ts}}{I_{tr}} T_{dr} \quad \dots (2)$$

Where  $T_{ts}$  and  $T_{tr}$  are the measured intensities of sample and air respectively.  $T_{dr}$  is the transmittance of air, which is 0.93 according reference<sup>13</sup>.

The IAD method was used to obtain the absorption and scattering values of the pig muscle from the measured reflectance and transmittance of the samples. A data file that contains the properties of the sphere and the reflectance and transmittance of the samples was input into the IAD. The absorption and scattering properties of the samples can be obtained from the produced files of the IAD program<sup>9</sup>.

## RESULTS AND DISCUSSION

Figure 3 and Figure 4 show the comparison of AD method with Monte Carlo method in the wavelength of 650 nm to 1000 nm. The figures indicated that there was excellent

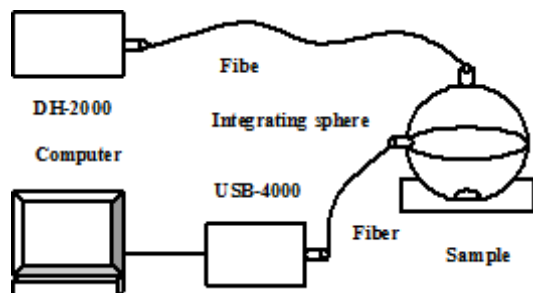


Fig. 1. Experimental setup

agreement between the results of the AD method and the MC method. The relative error was used to express the deviation between the two methods. The relative error was defined as:

$$f_{\text{relative error}} = \left| \frac{\mu_{AD} - \mu_{MC}}{\mu_{MC}} \right| * 100\% \quad \dots (3)$$

The  $\mu_{AD}$  and  $\mu_{MC}$  were the absorption or reduced scattering values obtained by the AD and MC method respectively. The scatter plots of the relative error for the total reflection and total transmission were shown in Figure 5 and Figure 6.

Just as shown in the figures, the relative deviation of the two methods was less than 0.3% for the total reflection and 1.4% for the total transmission. That is to say that the adding-double method has similar accuracy as the Monte Carlo method. Unlike the MC method, the AD method takes only several seconds for a run. In conclusion, it has been proved that the AD method was an effective and real-time method for the simulation of photon transportation.

The Monte Carlo method was commonly used in many studies, so the results of the MC method was considered as the standard of the simulation and the input optical properties (some

**Table 1.** Optical properties of normal pork tissue obtained by IAD method and compared with that of Ref.<sup>16,17</sup>

Location	$\mu_a (mm^{-1})$	$\mu_s' (mm^{-1})$
Pig muscle	$0.59 \pm 0.01$	$24.7 \pm 0.7$
Pig skin epitehe	$1.0 \pm 0.1$	$22.7 \pm 0.8$
Pig skin dermis	$0.89 \pm 0.10$	$21.1 \pm 0.4$
Our study	0.36	26.7

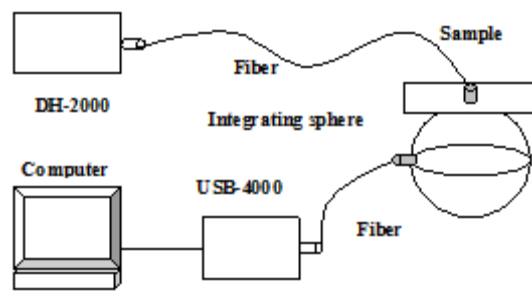
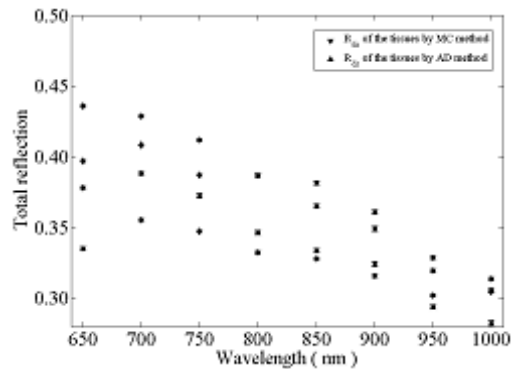


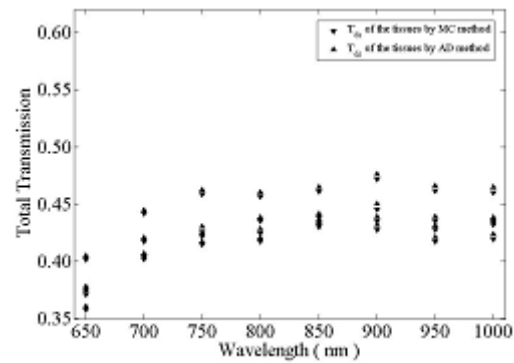
Fig. 2. Experimental setup for transmission measurement

optical properties of tissues in reference<sup>10</sup>) of the MC method was defined as the original values in this article. The IAD method was used to extract the absorption and reduced scattering coefficients from the Monte Carlo simulation. The comparison between the output of IAD and the original values were shown in Figure 7 and Figure 8.

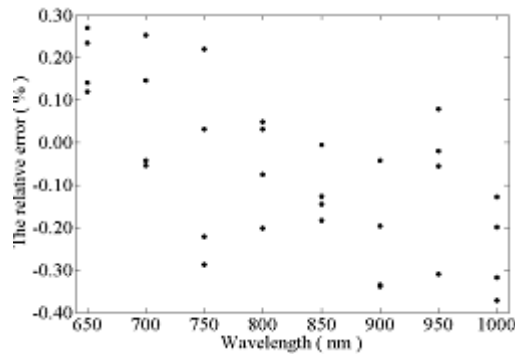
The figures indicate that there is excellent agreement between the results of the IAD program and the original values. To further analyze the accuracy of the IAD method, the relative error was calculated for the absorption and reduced scattering coefficients and shown in Figure 9 and Figure 10 respectively.



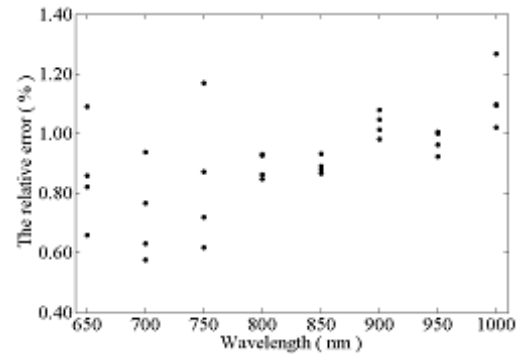
**Fig. 3.** Comparison of the total reflection obtained by the AD and MC method



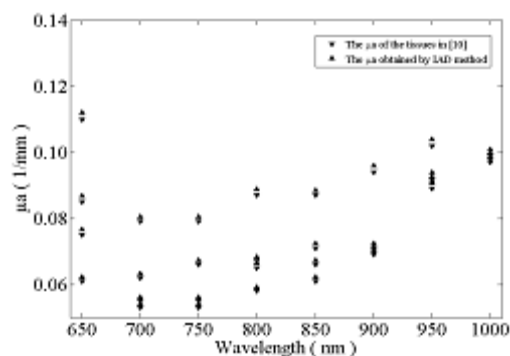
**Fig. 4.** Comparison of the total transmission obtained by the AD and MC method



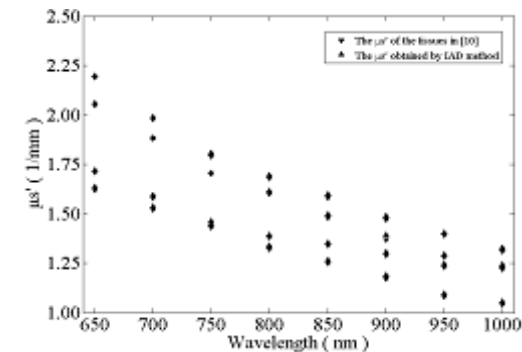
**Fig. 5.** The relative error of the total reflection by AD method



**Fig. 6.** The relative error of the total transmission by AD method



**Fig. 7.** The comparison of the  $\mu_a$  between the values in<sup>10</sup> and the values obtained by the IAD method



**Fig. 8.** The comparison of the  $\mu_{s'}$  between the values in<sup>10</sup> and the values obtained by the IAD method

The relative errors were less than 2.4% and 2.0% for the absorption and reduced scattering coefficients, respectively. The advantage of the IAD method is that it only took several seconds to complete a run. So the IAD method was used to extract the absorption and reduced scattering properties in this paper.

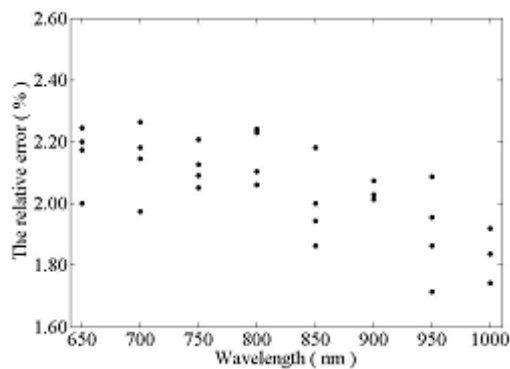


Fig. 9. The relative error of the  $\mu_a$  by the IAD method

In the reflection and transmission measurements of the pig muscle, the tissues were immersed in the liquor of Sudan for one hour. Reflectance and transmittance spectra from each sample were recorded over wavelength range of 350-1050 nm with an integrating sphere. The reflectance and transmittance spectra of the samples with different concentrations of Sudan were shown in Figure 11 and Figure 12.

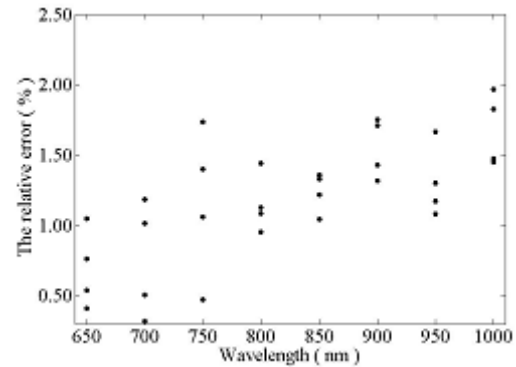


Fig. 10. The relative error of the  $\mu_{s'}$  by the IAD method

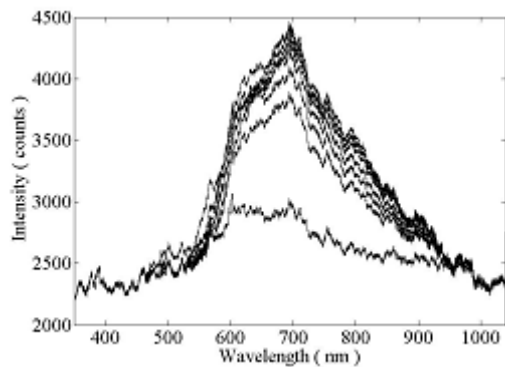


Fig. 11. The reflection of pig muscle samples with different concentrations of Sudan

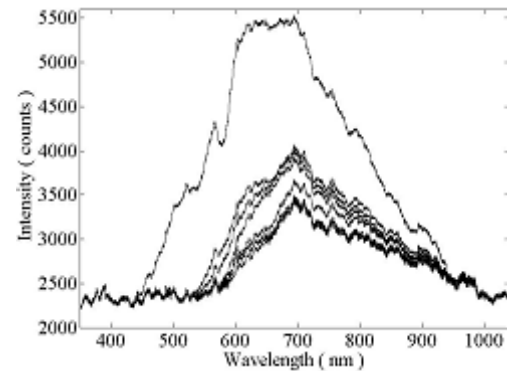


Fig. 12. The transmission of pig muscle samples with different concentrations of Sudan

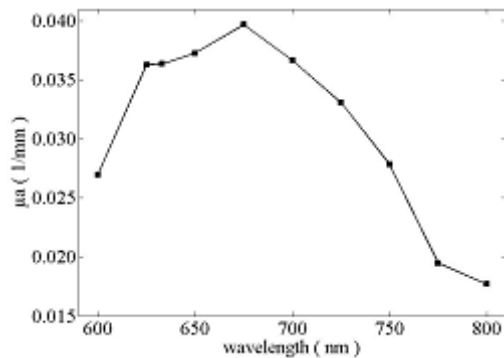


Fig. 13. The  $\mu_a$  of the pork tissue without Sudan versus the wavelength

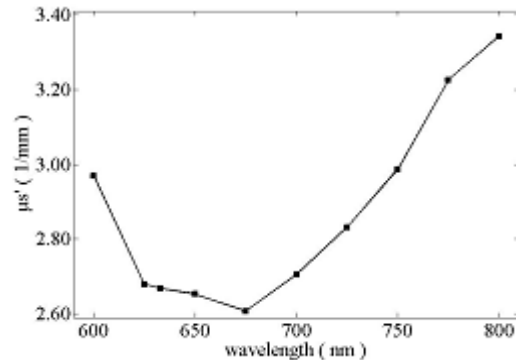
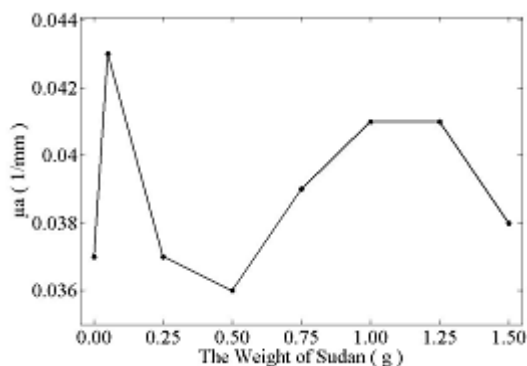


Fig. 14. The  $\mu_{s'}$  of the pork tissue without Sudan versus the wavelength

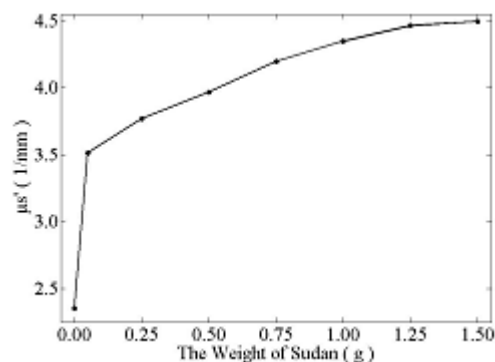


**Fig. 15.** The changing curve of  $\mu_a$  versus the concentration of Sudan

Figure 11 and Figure 12 showed the differences between the spectra of the samples. There was a significant increase in the intensity of the reflectance spectra with increase of Sudan concentration. However, the intensity of the transmittance spectra is decreasing with the increase of Sudan concentration. The differences of the spectra likely reflect the variations of the optical properties. For further study, the IAD method was used to extract the absorption and reduced scattering properties of the samples from the reflectance and transmittance spectra. The total reflection and total transmission of the samples at different wavelength were obtained by the comparison method. The and of the normal pork tissue over the wavelength range of 600-800nm were shown in Figure13 and Figure14.

The Figures indicate that the absorption and reduced scattering properties depended on the wavelength. The increased in the wavelength range of 600-675 nm and decreased in the wavelength range of 675-800 nm. The decreased in the wavelength of 600-675 nm and increased in the wavelength of 675-800 nm. Our results of normal pork tissue at 632.8 nm were compared with the results in reference<sup>16</sup> and <sup>17</sup>. Table 1 showed the detailed data. The lack of agreement between the results was due to the differences of the samples and the experimental conditions.

In order to further analyze the effect of Sudan on the optical properties, the values of  $\mu_a$  and  $\mu_s'$  at 700 nm were obtained over the Sudan weight range of 0.00-1.50 g. And the value of  $\mu_a$  and  $\mu_s'$  versus the concentration of Sudan are shown in Figure 15 and Figure 16.



**Fig. 16.** The changing curve of  $\mu_s'$  versus the concentration of Sudan

In Figure 15 and Figure 16, the abscissa represents the weight of Sudan, which was added into the 100 ml solvent to obtain different concentrations of Sudan solution. The ordinate represents  $\mu_a$  or  $\mu_s'$  of the samples. The results illustrated that there were significant change in the absorption and reduced scattering values for the processed samples compared to the normal sample. The  $\mu_a$  changed between 0.036 mm<sup>-1</sup> and 0.044 mm<sup>-1</sup> with nonlinear relationship, while the  $\mu_s'$  changed between 2.5 mm<sup>-1</sup> and 7.0 mm<sup>-1</sup> with regular pattern. The variation of the optical properties was due to the effect of Sudan. Further study should be aimed at analyzing the quantitative relationship between the optical properties and the weight of Sudan.

## CONCLUSIONS

In this article, the adding-double method and Monte Carlo method were compared for demonstrating the accuracy of the AD method. The results indicated that there was excellent agreement between the results of the AD method and the MC method. The relative deviation of the two methods was less than 0.3% for the total reflection and 1.4% for the total transmission. The IAD method was used in the study to extract the absorption and reduced scattering, and the relative error was less than 2.4% for the absorption and 2.0% for the reduced scattering when compared with the original. The results demonstrated that the IAD method can be used to extract the absorption and reduced scattering properties with high accuracy and efficiency.



In the reflection and transmission measurements of the pig muscle, the results showed that there was a significant increase in the intensity of the reflectance spectra for the processed samples compared to the normal sample. And there was a significant decrease in the intensity of the transmittance spectra for the processed samples compared to the normal sample. The IAD method was used to extract the absorption and reduced scattering properties of the samples from the reflectance and transmittance spectra. The results showed that the absorption and reduced scattering properties were depend on the wavelength, and at 700 nm there were significant change in the absorption and reduced scattering for the processed samples compared to the normal sample. Further study should be made to analyze the quantitative relationship between the optical properties and the weight of Sudan.

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