Research on the Photography Measurement of the Optical Parameters of the Vegetable Tissues

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Abstract. To measure the optical parameters of biological tissue, the laser imaging system of the diffusing reflection and the transmission measurements based on CCD was designed. The absorption coefficient (μₐ′) and the reduced scattering coefficient (μₛ′) of the biological tissue simulating liquid (Intralipid-20% diluents) were derived from nonlinear regression of the diffusion equation with spatially resolved steady-state diffuse reflectance which was measured by CCD. At the same time, the reduced scattering coefficient (μₛ′) of the biological tissue simulating liquid (Intralipid-20% diluents) was also derived from linear regression of the Beer-Lambert equation with transmission light intensity of the biological tissue simulating liquid (Intralipid-20% diluents) which was measured by CCD for the different titer, which is used as the calibration of the non-invasively method. The optical parameters of the same sample measured by CCD diffusing reflection and transmission measurements were compared. The results of the two methods were approximately consistent. Furthermore, the optical parameters of Eggplant and Murphy were measured by CCD diffusing reflection measurements. So the technique of laser scattering imaging analysis is useful for measuring the optical properties of the vegetable tissues.

1. Introduction
Optical transmission in the biological tissue is one of the research focus on tissue optics [1-3]. By measuring the spatially resolved diffuse light of tissue surface, which is the result of the interaction of the incident light and the effect of absorption and scattering by tissue, we can study the optical properties of the tissue.

The mainly methods for measuring the spatially resolved diffuse reflectance of tissue surface are point by point scanning by optical fiber and CCD video camera measurement. The traditional method of point by point scanning by optical fiber has the following problems: Long measurement time, as a result, optical parameters of biological tissue and the distance between the optical fiber and tissue surface may change in every measurement; the light distribution of the region near source can not be measured; as the integrated optical fibers array will touch the tissue when measuring, the physiological state of the pressurized tissue may change. Measurement of light distribution of tissue diffuse reflectance or transmission with the CCD is new technology appeared in the 90's of the last century [4]. Compared with the traditional method of point by point scanning by optical fiber, the technology of
CCD video camera measurement has greatly improved accuracy and efficiency traditional optical scanning point by point comparison, CCD video camera technology, accuracy and efficiency has been greatly improved and can measure the spatial distribution of light intensity near light source. R. A. Bolt[5] measured the radiance distribution at the surface of the tissue phantoms with CCD and got the Optical Properties with absolute measurement method. Alwin Kienle[6] measured the radiance distribution at the surface of the tissue with the CCD non-contact method and the wavelength of 633nm light.

In this study, the laser imaging system of the diffusing reflection and the transmission measurements based on CCD was designed. The absorption coefficient and the reduced scattering coefficient of the biological tissue simulating liquid (Intralipid-20% diluents) were derived from nonlinear regression of the diffusion equation with spatially resolved steady-state diffuse reflectance which was measured by CCD. At the same time, the reduced scattering coefficient of the biological tissue simulating liquid (Intralipid-20% diluents) was also derived from linear regression of the Beer-Lambert equation with transmission light intensity of the biological tissue simulating liquid (Intralipid-20% diluents) which was measured by CCD for different titer. The absorption coefficient and the reduced scattering coefficient of Murphy and eggplant were measured by the CCD diffusing reflection measurements.

2. Method and Materials

2.1 Measuring $\mu_a$ and $\mu'_s$ by diffuse reflection

![Figure 1](image1.png) **Figure 1** Optical path of experiment setup of CCD diffusing reflection measurement

![Figure 2](image2.png) **Figure 2** Optical path of experiment setup of CCD transmission measurement

The liquid tissue phantom for this experiment is the Intralipid-20% diluents, which is denoted as sample I10. Configuration proportion of the sample I10: 1000ml sample I10 is made by 100ml Intralipid-20% diluted by adding 900ml distilled water. The Experimental device is shown in Fig.1. The laser pass through the attenuator, pinhole ($\phi = 0.1mm$) and the convex lens, at last, it is projected onto the surface of the sample with a smaller incidence angle (5° ~ 10°). The CCD in this measurement is the 150CLM cooled CCD used for digital microscope, pixel resolution of which is 1392×1040, the sensitivity is set to 200, the exposure time is set to 1/10sec. By adjusting the CCD focal length and the amount of light into CCD we can obtain image of diffuse light on the experimental sample surface, the data of which would send to CCD controller for store and simple calculation, then send to computer for further image analysis and processing. Whole experiment is carried out in the darkroom.

Simple-PCI image analysis software developed by Hamamatsu can analyse the CCD image, accurately read the relative intensity value of any point in the image coordinates; it also can directly export relationship between the relative intensity values the image of and spatial pixels to Excel, therefore, in a short period of time we can obtain the analysis data of CCD images.
The method of calibration is that putting a millimeter-scaled ruler at the location at the same focal plane with the experimental samples, and take the photo of the ruler by CCD, then from the scale of the ruler image we can determine the ratio between the length and the number of pixels. Use this constant ratio, we can transform the relationship between relatively light intensity and pixels to the relationship between relative intensity and spatial coordinates.

Light transmission in tissue can be described by the radiative transfer equation\(^7\). As the size tissue sample is much larger than the actual transport distance of photons, the tissue sample can be considered as the semi-infinite medium. When unlimitedly thin collimated beam vertically project to the tissue surface, under the diffusion approximation\(^8\) condition and the extended boundary condition, and define the diffusion coefficient \(D\) as:

\[
D = \frac{1}{3(\mu_a + \mu_s')}
\]  

(1)

The solution of the diffusion approximation is:

\[
R(\rho) = \frac{1}{4\pi} \left[ \frac{z_0}{r_1^2} \exp\left(-\frac{\mu_{eff} r_1}{r_1^2}\right) + \left(\frac{z_0 + 2z_b}{r_2} \exp\left(-\frac{\mu_{eff} r_2}{r_2^2}\right)\right) \right]
\]  

(2)

where: \(z_0=3D\) ; \(\mu_{eff} = (\mu_a/D)^{1/2}\) ; \(r_1^2 = z_o^2 + \rho^2\) ; \(r_2^2 = (z_o + 2z_b)^2 + \rho^2\) ; \(z_b = \frac{1 + R_{eff}}{1 - R_{eff}} \cdot 2D\) ; \(R_{eff}=0.493^{[9]}\), \(\rho\) is the vertical distance at tissue surface from the study point to the incident light beam.

It can be made the non-linear fitting to \(R(\rho)\) that is measured using CCD and equation (2) non-linear fitting by MATLAB program, and the absorption coefficient \(\mu_a\) and the reduced scattering coefficient \(\mu_s'\) of biological tissue can be obtained.

2.2 Measuring scattering coefficient \(\mu_s\) and \(\mu_s'\) by transmission method

The Experimental device is shown in figure.2. It is used to measure the scattering coefficient of liquid tissue phantom I10 and the reduced scattering coefficient, to verify the accuracy of the diffuse reflection method. Laser beam (632.8nm) pass through the attenuator, pinhole \((\phi=0.1mm)\), and the convex lens, then, it is vertically projected in the sample I10 inside the quartz slot. The CCD is in the other side of the slot out of 130cm from the sample to receive the transmitted light intensity. The principle of transmission method which is used to measure the scattering coefficient is based on Beer-Lambert Law:

\[
E_c = E_i \cdot \exp\left[-(\mu_a + \mu_s) \cdot cd\right]
\]  

(3)

in the equation (3), \(E_c\) is the approximately direct transmission light intensity; \(E_i\) is the light intensity which is vertically projected in the sample slot; \(c\) is the concentration of sample 110; \(d\) is the thickness of the sample in the sample slot. Adopt logarithm on both ends of equation (4), then between \(\ln(E_c)\) and \((cd)\) exists a linear relationship, so the negative of its slope is \(\mu_a + \mu_s\). As the intralipid solution is a highly scattering medium with low absorption\(^{[10]}\), \(\mu_s \gg \mu_a\), therefore \(\mu_a + \mu_s \approx \mu_s\). During the experiment, under the fixing condition of the incidence light intensity, the location of sample pool, as well as the CCD focal length and the amount of light which goes through the CCD, then we can obtain the relative transmission light intensity with corresponding solutions of different concentrations of 110, by changing the concentration of sample 110 in sample pool with the distilled water. By making the linear fitting between \(\ln(E_c)\) and \((cd)\) using the MATLAB program
and calculating the negative of its slope, \( \mu_s \). could be acquire. As under the approximate condition of diffuse reflection[9], there exists the relation as follows:

\[
\mu'_s = \mu_s \cdot (1 - g)
\]  

(4)

The anisotropic factor \( g \) of the biological tissue liquid phantom of I10 which used in the experiment is 0.75\(^{[5]}\), put the \( \mu_s \) which obtaind by the transmission method in the equation (4), we could obtain an reduced scattering coefficient (\( \mu'_{s-r} \)).

3. Results and analysis

3.1 Measurement results on samples I10

Diffuse reflection measurement is shown in figure 3. Figure 4 is the nonlinear fitting curve of the measurement data with the equation (2), thus there are:

\( \mu_s = 0.0442 \text{mm}^{-1} \); \( \mu'_s = 2.987 \text{mm}^{-1} \).

![Figure.3 Diffusing reflection distributing of the sample’s surface](image1)

![Figure.4 Nonlinear fitting curve of diffusing reflection measurement](image2)

According to figure 2, we can measure the transmission intensity of sample I10 in different concentration. After the analysis of Simple-PCI software, we can get the relative transmitted light intensity (\( E_c \)) corresponding to sample I10 of different concentration. Taking the linear fitting between \( \ln(E_c) \) and \( (cd) \) by MATLAB software, the slope is -11.353, i.e. the scattering coefficient of sample I10 is \( \mu_s = 11.353 \). Putting \( \mu_s \) into the equation (4), we could obtain the reduced scattering coefficient : \( \mu'_{s-r} = 2.838 \text{mm}^{-1} \).

Comparing the reduced scattering coefficients \( \mu'_s \) and \( \mu'_{s-r} \), which obtained by the methods of diffuse reflection and transmission respectively, the error is: \( \Delta \mu'_s/\mu'_s = 5.25\% \). Taking into account that the different measurement methods and mathematical models used in the experiment may cause some differences to the final outcomes, therefore, the above error is reasonable.

| Sample | Method         | \( \mu'_s/\text{mm}^{-1} \) | \( \mu_s/\text{mm}^{-1} \) | Error               |
|--------|----------------|-----------------------------|-----------------------------|---------------------|
| I10    | this paper     | 2.987                       | 0.0442                      | \( \Delta \mu'_s/\mu'_s : 4.73\% \) |
|        | R. A. Bolt, et al\(^{[4]}\) | 2.852                       | 0.049                       | \( \Delta \mu_s/\mu_s : 9.79\% \) |
Table 1 is the Comparison in this paper’s absorption coefficient $\mu_a$ and reduced scattering coefficient $\mu'_a$ to literature $^4$. From Table 1, It can be seen the optical parameters obtained in this paper is very close to the reported data from RA Bolt, the error of reduced scattering coefficient is 4.73%, the error of absorption coefficient is just about 9.79%, the results which obtained in this paper are allowed within the system error $^{11}$. The reasons for these differences may include the following aspects: the use of Intralipid-20% solution are from different manufacturers, the particle’s size and uniformity will differ in the inside of liquid; CCD’s own dark current as well as the tiny existence of the environment noise source; the error caused by the algorithm of measured data fitting for the optical parameters.

By comparison, we can see that both the devices which designed basing on CCD’s diffuse reflection method to measure the optical properties of biological tissue parameters and the dispose methods all have a high accuracy.

3.2 Measurement results on fresh vegetable tissue
The basis of the above-mentioned study, we also measure optical properties parameters of fresh vegetable tissue such as potatoes, eggplant and so on. The transportation of photon in the vegetable tissues, like other biological tissue, is compliance with the Boltzman transport equation. If the distribution of the light on the surface of the tissue was given, we could use the analytical expression to replay the optical parameters easily, so with the analytical expression of light distribution on the surface and light distribution in the vegetable tissue surface, we could replay the vegetable tissue optical parameters of vegetable tissue. Before the experiment, keep fresh potatoes and eggplants ready, use the surface diffuse reflection optical image to measure them when they are each in full and slice situation, as is shown in Figure 5.

(a) Complete potatoes  (b) Sliced potatoes

(c) Sliced eggplants

Figure 5, The diffuse reflection light distribution on the surface of different vegetable tissue
According to diffuse optical image data, the analysis results are shown in figure 6. Potatoes and eggplants’ fitted optical parameters are in Table 2.

Table 2 the absorption and reduced scattering coefficient of potato and eggplant

| project   | Potato          | Eggplant       |
|-----------|-----------------|----------------|
| complete  | $\mu_a = 0.1011 \, mm^{-1}$ | $\mu_a = 0.0714 \, mm^{-1}$ |
|           | $\mu_s' = 0.9607 \, mm^{-1}$ | $\mu_s' = 0.7809 \, mm^{-1}$ |
| slice     | $\mu_a = 0.0127 \, mm^{-1}$ | $\mu_a = 0.0714 \, mm^{-1}$ |
|           | $\mu_s' = 0.2977 \, mm^{-1}$ | $\mu_s' = 0.7809 \, mm^{-1}$ |

According to the figures 5, there is a big difference in the diffusion images of different types and under different conditions, they are related to the surface texture of vegetable tissue (including: color, moisture, sugar content, smoothness and other conditions). As the transportation of the light in the vegetable tissue decided by the organization optical transmission parameters, therefore, diffuse light images of different types, under different conditions is indicative of different optical parameters.
3. Summary
In short, we have designed that laser image measurement device by CCD to determine the optical parameters of biological tissue. The absorption coefficient ($\mu_a$) and the reduced scattering coefficient ($\mu'_s$) of the biological tissue simulating liquid (Intralipid-20% diluents) were derived from nonlinear regression of the diffusion equation with spatially resolved steady-state diffuse reflectance which was measured by CCD. At the same time, the reduced scattering coefficient ($\mu'_s$) of the biological tissue simulating liquid (Intralipid-20% diluents) was also derived from linear regression of the Beer-Lambert equation with transmission light intensity of the biological tissue simulating liquid (Intralipid-20% diluents) which was measured by CCD for the different titer, which is used as the calibration of the non-invasively method. The optical parameters of the same sample measured by CCD diffusing reflection and transmission measurements were compared. The results of the two methods were approximately consistent. Furthermore, the optical parameters of Eggplant and Murphy were measured by CCD diffusing reflection measurements. So the technique of laser scattering imaging analysis is useful for measuring the optical properties of the vegetable tissues.

Acknowledgements
This work was supported by the Basic Research Operating Expenses of Central University, the Ministry of Education of the People’s Republic of China (No. ZXH2010C003)

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