

# Spectral Imaging System for Diffusive Reflectance

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## Abstract

The light distribution and light path in a biological tissue is determined by its optical properties. In most applications of biomedical optics, the intensity of light source has to be well controlled to achieve the best effect and to prevent possible harm. The key factors for making decision are the optical properties. The purpose of this study is to build a spectral image measurement system to capture the diffusive reflectance image when monochromatic light is projected on the surface of a scattering media. A Monte Carlo model is also used to simulate the image of diffusive reflectance. The effect of absorption and scattering coefficients on the diffusive reflectance is studied by modifying their values in model. The reflectance intensity at the center position and the slope of radial intensity profile are features that can be used to evaluate the optical coefficients.

**Keywords:** Diffusive reflection, Tissue optics, Optical coefficients, Monte Carlo simulation

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## Introduction

Optical properties of biological tissue are crucial factors for applications in biomedical optics. These optical parameters determine the light energy distribution and light path in tissue. In most applications, the power of light source has to be strong enough to deliver certain amount of energy to a specific depth in target tissue. Although a very strong light source can be used to send as much energy as wanted, it also will cause undesired photochemical or photothermal reaction. Such as in photodynamic therapy application, the illuminated region has to be properly controlled to achieve the best therapeutic effect without causing possible harm on the surrounding tissue. In order to regulate the power of light source, the intensity of light distribution in tissue should be accurately predicted.

To predict the light distribution in tissue, a mathematical model has to be used to simulate the propagation of light in a scattering media. Optical parameters, such as absorption, scattering, anisotropic coefficients, and refraction index are generally used in a model to describe the optical characteristic of biological tissue [1-4]. These representative parameters control the propagation direction of light and its energy dissipation in a scattering media.

The distribution of light in tissue is a group behavior of numerous of photons. When light enters a turbid media like biological tissue, photons will encounter a series of scattering process that change their direction of propagation. The frequency of scattering is quantified by a scattering coefficient. The anisotropic scattering coefficient indicates the tendency of

forward or backward scattering. Light energy is also gradually absorbed while traveling in the media. The fraction of light absorbed within a unit of length is defined as the absorption coefficient. In a thick tissue, all the injected photons are either absorbed inside the tissue or backscattered and finally escape from the surface. The reflected photons form a light distribution of diffusive reflectance on the surface. By simulating the mechanism of random scattering of photons, a mathematical model can produce the diffusive distribution of light in tissue. Thus, the light distribution is simply determined only by a few parameters. Therefore, the accuracy of simulation of light distribution depends on how accurate these parameters are evaluated.

There are several methods have been reported for measuring the optical parameters of a biological tissue [2]. An integrating sphere is a standardized instrument for measuring diffusive reflectance. However, only one reading can be obtained from the measurement. Since there are at least an absorption coefficient and a scattering coefficient needed for describing a diffusive light propagation, it is an underdetermined problem to evaluate more than one optical parameter from a single measurement. The size of window of integrating sphere also has to be much larger than the light penetration depth in order to minimize the effect of light escape from outside of the window. The penetration depth of visible and near infrared light is about several hundreds microns to a few mini-meters in biological tissue. This requires either very large integrating spheres or a very thin tissue sample for the measurement. Therefore, two integrating spheres measurement of the diffusive transmission and reflectance from a thin layer of tissue sample is also developed to determine the absorption and scattering coefficients [5]. But,

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it is an *ex vivo* measurement that only can be applied to an excised tissue sample.

Although some typical optical parameters acquired from biological tissue can be found in literature, these optical coefficients have a great variation depending on the measurement methods [2]. Unfortunately, it is difficult to choose from these typical optical coefficients for optimal light dose control. To solve this problem, the optical coefficients of the target tissue should be determined from an *in vivo* measurement. Such a measurement should be minimal invasive and can be easily performed. One way of doing this is to probe the tissue through a standardized process with a low intensity of light. The optical coefficients are then evaluated from the diffusive reflectance image on tissue surface.

The radial profile of diffusive reflectance is mostly determined by the light absorption and scattering properties [7-12]. The effect of anisotropic scattering is not a significant factor at the far field where more than about 25 times of scattering has encountered. The light intensity distribution image is a two dimension measurement that offers much more information than a single reading of measurement. Therefore, the absorption and scattering coefficients can be more accurately determined from such a measurement. The purpose of this research is to use tissue phantom and mathematical model to study the relationship between optical parameters and distribution of diffusive reflectance.

## Materials and Methods

### Diffusive reflectance imaging system

To study the optical characteristics of turbid media, light is focused on the surface of sample as a small light spot. The multiple scattering of photons results in a diffusive reflectance that appears as a larger light spot. The intensity profile of this diffusive reflectance can be analyzed by taking the image of the spot. Since the optical coefficients of biological tissue vary with wavelength, a spectral imaging system was established for capturing the reflectance image. The block diagram shown in Figure 1 is the system setup for measuring the spectral light intensity distribution of diffusive reflectance. A 250W halogen-tungsten lamp (TS-428, Acton Research) works as a strong and broadband light source that covers the visible and near-infrared spectral region (450 nm to 800 nm). A monochromator (SpectraPro-150, Acton Research) divides the white light into narrow band source with different wavelengths. A personal computer controls a grating in the monochromator to scan through the interested spectral range. The exiting slit of monochromator is replaced by a circular hole with 0.5 mm of diameter. A convex lens focuses the monochromatic light on the surface of tissue to form a 0.5 mm diameter of incident light spot. A 50:50 beam splitter placed between the lens and tissue sample reflects half of the incident light to a powermeter (NOVA, Ophir) for monitoring the intensity of light. The rest of light projects on tissue sample and is back-scattered to produce a diffusive reflectance. The backscattering light from tissue surface is also half reflected by the beam splitter before captured by the CCD camera. The beam splitter makes it

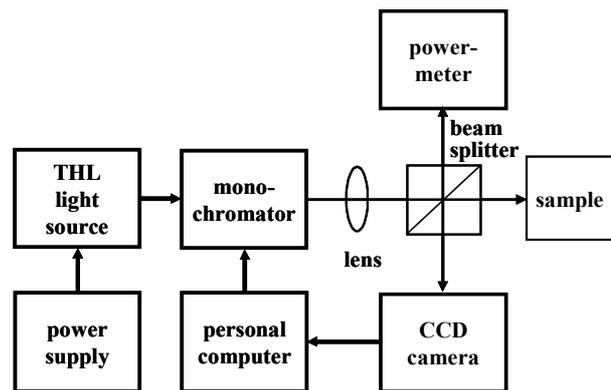


Figure 1. System setup for measuring the light intensity distribution of diffusive reflectance on tissue surface.

possible to capture the circular reflectance image from being blocked by light guiding devices. Since the intensity of diffusive reflectance decreases exponentially away from the incident center, the imaging system should have a large dynamic range. A 16 bits CCD camera (KX1E, Apogee Instruments) captures the diffusive reflectance image and transfers it to a computer for carrying out analysis. Theoretically, the tissue sample has to be a semi-infinite space to prevent any influence by the reflection from the sample-holder boundary. By controlling the incident power at several  $\mu\text{W}$ , most power of the reflectance is limited within the range of about  $1 \text{ cm}^3$ . The volume of tissue phantom,  $2.5\text{cm} \times 5\text{cm} \times 5\text{cm}$ , is large enough for ignoring the influence of reflection from the boundaries.

### Tissue Phantom

Lipovenös-10% (Fresenius Pharma, Austria) is used as a tissue phantom with controlled optical properties. It has the same content as Intralipid-10%, and the optical properties of Intralipid-10% have been well studied and reported in literature. Other advantages of using Intralipid-10% as a tissue phantom are its similar content with tissue and it is a very homogeneous scattering media. It contains mostly fat, protein, and water, and the concentration of these contents are relatively constant. The scattering property can be controlled by diluting with distilled water.

### Mathematical simulation

The random multiple scattering model of photon in a turbid media was established to simulate the diffusive reflectance of light on tissue surface. It records the loss of light energy through the multiple scattering process and plots the distribution of absorbed light energy for evaluating the static distribution of light intensity in tissue. The simulation is controlled by four major optical properties: refraction index, absorption, scattering, and anisotropic coefficients. Each scattering event is simulated as a random process determined by several random variables [13]. The probability density functions of these random variables are functions of the optical properties. The Monte Carlo model was coded and executed using MATLAB<sup>®</sup> (Mathworks). To achieve accurate

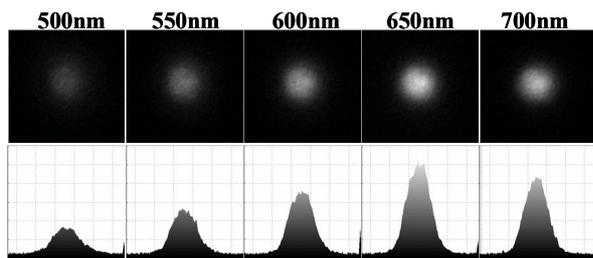


Figure 2. Diffusive reflectance images of different wavelengths of light on the surface of tissue phantom (Lipovenös-10%). The intensity is normalized to the total incident power of light monitored by the power meter.

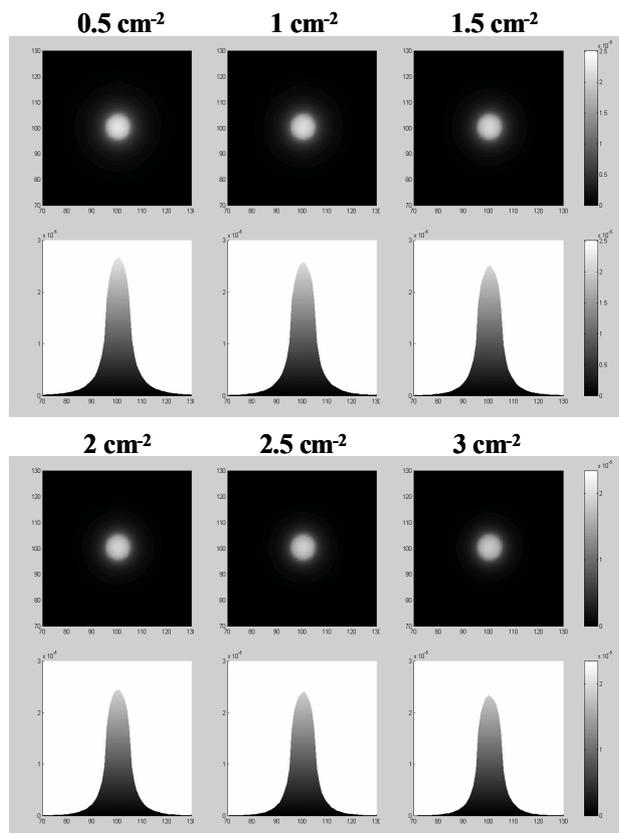


Figure 3. Simulation of diffusive reflectance image with absorption coefficient ranges from  $0.5 \text{ cm}^{-1}$  to  $3.0 \text{ cm}^{-1}$ . The scattering and anisotropic coefficients are  $300 \text{ cm}^{-1}$  and  $0.8$  respectively. Shown in the figures are reflectance images and the radial distribution of the reflectance intensity.

simulation results, four hundred thousands of photons were used in each simulation. The values of optical parameters used in simulation are referred to literatures.[1-5]

### Results

The diffusive reflectance images of light with different wavelengths on tissue phantom (Lipovenös-10%) are shown in the upper column of Figure 2. The lower column of Figure 2 shows the respective radial profile of diffusive reflectance.

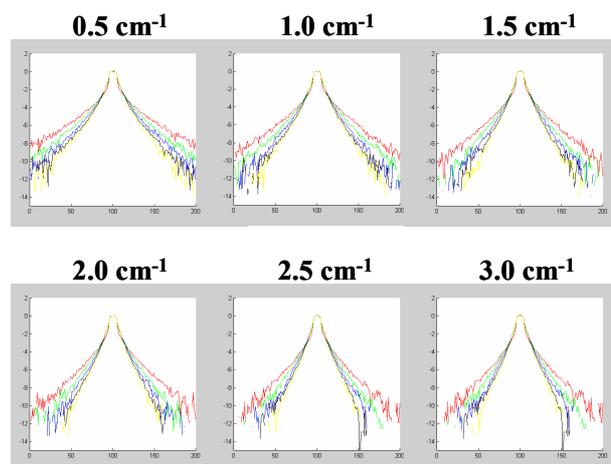


Figure 4. Simulation of diffusive reflection with different absorption coefficients (from  $0.5 \text{ cm}^{-1}$  to  $3.0 \text{ cm}^{-1}$ ) and scattering coefficients (from  $100 \text{ cm}^{-1}$  to  $500 \text{ cm}^{-1}$ ). In all figures, the slope of the radial distribution at far field is the lowest for  $100 \text{ cm}^{-1}$ . The slope increases as the scattering coefficient increases.

Since the intensity of reflectance varies with the intensity of light source, the reflectance image is normalized to the total incident power of source monitored by the powermeter. The intensity of reflectance increases as the light source scans toward longer wavelength.

Figure 3 shows the simulation results of diffusive reflectance images and radial intensity profiles by the Monte Carlo model. The absorption coefficient ranges from  $0.5 \text{ cm}^{-1}$  to  $3.0 \text{ cm}^{-1}$ . In order to see the effect of absorption coefficient alone on diffusive reflectance, the scattering coefficient is all set to be  $300 \text{ cm}^{-1}$  and the anisotropic coefficient is  $0.8$ . The reflectance intensity at the center position decreases as the absorption coefficient increases.

The effect of scattering coefficient on the reflectance profile is shown in Figure 4. The absorption coefficient is kept the same in the simulation of each graph, and the scattering coefficient is varied from  $100 \text{ cm}^{-1}$  to  $500 \text{ cm}^{-1}$  with an interval of  $100 \text{ cm}^{-1}$ . The intensity profiles are divided by the intensity of the center position for normalization. The difference in decreasing rate at far field is more clearly shown by taking the logarithm of intensity. The anisotropic coefficient is all set to be  $0.8$ .

The reflectance intensity at the center position is shown as a function of the absorption and scattering coefficient, see Figure 5. The simulated reflectance intensity decreases as the absorption coefficient increases, whereas it decreases as the scattering coefficient increases.

### Discussion

The path of light in a scattering media is mainly determined by the scattering properties. The scattering coefficient and anisotropic coefficient control the scattering direction of a traveling photon. The scattering coefficient relates to the mean distance between two continuous scattering

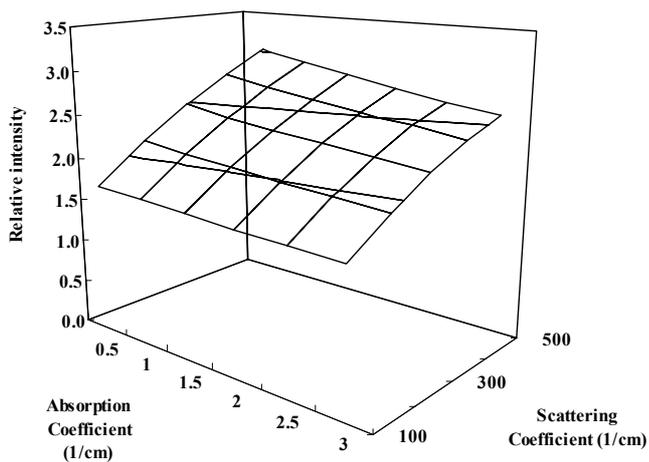


Figure 5. The intensity of diffusive reflection at the incident center as a function of absorption and scattering coefficients.

events. A larger scattering coefficient stands for a higher frequency of scattering events. Since light has a strong forward scattering tendency in most biological tissues, an anisotropic coefficient is used to quantify this scattering characteristic. The value of +1, 0, and -1 represent totally forward, isotropic, and totally backward scattering, respectively. The typical value of anisotropic coefficient ranges from about 0.7 to 0.95 for biological tissue. However, even for a highly forward scattering media, its anisotropic scattering character will gradually lose with the multiple scattering process. The criterion is about 20 to 25 times of scattering for biological tissue. Beyond this criterion, the scattering of light can be treated as a diffusive scattering that has no preference to propagate in any specific direction. The diffusive scattering is equivalent to an isotropic scattering. Therefore, the anisotropic coefficient becomes an insignificant factor in determining the reflectance image at the surrounding area about 1 mm away from the center.

The absorption coefficient determines how fast the light energy is absorbed while traveling in a light absorbing media. The light energy dissipation follows the Beer's law, therefore, the intensity of light decreases exponentially. When the path length is comparatively smaller than the value of absorption coefficient, the decrease of intensity with distance is relatively linear. Since most of the photons that are reflected back to the surface inside the incident area do not penetrate deep into the scattering sample, the reflectance intensity at the center position changes slightly linear with the absorption coefficient. This tendency is better seen with the intensity profile shown in linear scale. The simulation results of diffusive reflectance in Figure 3 show the intensity at center position decreases with the increase of absorption coefficient. Whereas, the intensity decreases exponentially when light propagates away from the center position. In order to see the difference in decreasing rate for different scattering coefficient, the intensity profile should be shown in a logarithm scale as in Figure 4. In each figure, all the simulated radial intensity profiles are with the same

absorption coefficient. The results of five different values of scattering coefficient, from  $100 \text{ cm}^{-1}$  to  $500 \text{ cm}^{-1}$ , are overlapped together. The results in all six graphs are quite similar. The slope of radial intensity profile is lower for smaller scattering coefficient and higher for larger scattering coefficient.

To estimate the optical properties of biological tissue, a great number of model simulations have to be carried out to obtain the relationship between optical properties and the intensity distribution images. The reflectance intensity at the center of image is shown as a function of the absorption and scattering coefficients, see Figure 5. The result shows that a strong reflectance is with either a strong scattering property or a low absorption property, or both. Material with a large scattering coefficient strongly reflects light within a small depth without much energy loss, and the reflectance intensity is very high. For material with a small scattering coefficient and a very small absorption coefficient, it allows light to penetrate deep into the scattering media. Eventually, it still reflects most of light back to surface and without a great loss of energy. In this case, the reflectance is also strong and a large diffusive reflectance spot is form.

## Conclusion

An optical system for measuring the diffusive reflectance image on a turbid media is established in this study. The image of diffusive reflectance on sample surface is a two-dimension measurement. It provides more information for determining the optical properties than a single value of reflectance measured by an integrating sphere. The measurement of diffusive reflectance image is also non-invasive. Based on the results of simulation, the reflectance intensity at the center position and the slope of radial intensity profile at the far field are two major features for determining the absorption and scattering properties of a turbid media. The scattering and absorption coefficient both have a great contribution in determining the reflectance intensity at the center of image. When the reflectance intensity at the center position is normalized, the slope of radial intensity profile at the far field can be used to determine the scattering coefficient. Since a low power of light is used to evaluate the optical properties, its application should be limited in the area of linear optics that optical properties do not change with the intensity of light source.

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