Monte Carlo Simulation of Colon Tissue Autofluorescence Spectrum

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Abstract

We have developed a Monte Carlo simulation method for estimating the autofluorescence spectra of colon tissues with different mucosa and submucosa layer thickness. The simulated excitation light wavelength is at 330 nm wavelength and the fluorescence emission spectrum is from 370 nm to 550 nm. Through the simulation process, we have obtained better understanding of the optical properties of the colon tissues, as well as the relationship between the fluorescence spectrum and the development of adenoma in different stages.

***Keywords*:** Monte Carlo simulation, Laser-induced autofluorescence (LIAF), Fluorophores.

Introduction

Background

Cancers have always been the leading cause of death in recent years. The cure rate of cancer is higher if it can be diagnosed earlier. In addition, early cancer diagnosis plays an important role in cancer therapy. Autofluorescence technique detects the emission of the natural fluorophores of tissues under the excitation of ultraviolet light .The distribution and concentration of the natural fluorophores changes along with pathological developments. Therefore, through the measurement of the autofluorescence spectrum, we may recognize the status of tissue. In addition, the advantage of the minimum invasion diagnostic procedure has made the autofluorescence technique become an applicable and promising early cancer diagnostic technique.

Colon early cancer diagnosis

Colon tissues consists of two layers, mucosa and submucosa layer. The average mucosa layer thickness is about 500 μm [1]. Its thickness increases with the development of dysphasia and pathologic tissue change. Adenoma is the most common pathologic change of colon tissues [4]. It consists of two categories, polypoid adenoma and non-polypoid adenoma. Both types result from the abnormal hyperplasia within the mucosa layer. Polypoid adenoma can be easily diagnosed because of its convex type appearance. However, non-polypoid adenoma could not be easily diagnosed because of its flat manifestation. In addition, the close relationship between adenoma and adenocarcinoma has been reported. Therefore, an early diagnosis of adenoma and adenocarcinoma is very important in the treatment of early colon cancers.

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Autofluorescence of biologic tissues

Collagen, NADH, and FAD are major natural fluorophores in colon tissue. By using the ultraviolet light excitation, autofluorescence emission can be generated. It is observed that the fluorescent peaks for these fluorophores are at 380 nm, 440 nm, and 530 nm at 330 nm excitation [9]. There are three factors affecting the autofluorescence spectrum in biologic tissues: (1) the distribution of the excitation light in the tissue, (2) the distribution and emission intensity of fluorophores of the tissue, and (3) the absorption and scattering properties of the tissue will affect the degree of the fluorescence escape function of the tissue.

Monte Carlo Simulation principle

In biological tissues, the main factors that affect the fluorescence spectrum are the concentration and distribution the fluorophores and the absorption and scattering properties of the tissue [2]. These factors need to be considered in the light induced autofluorescence (LIAF) model. In addition, the anisotropic forward scattering characteristics of biological tissue also affects the forward scattering. Therefore, the anisotropic coefficient (g) also needs to be considered in the model [3].

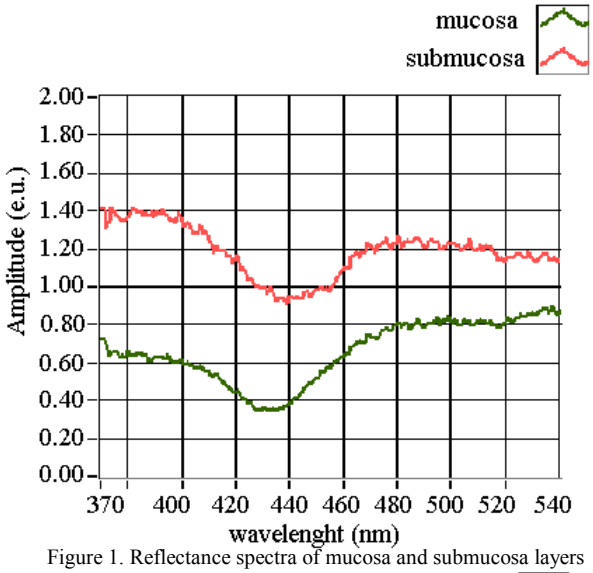
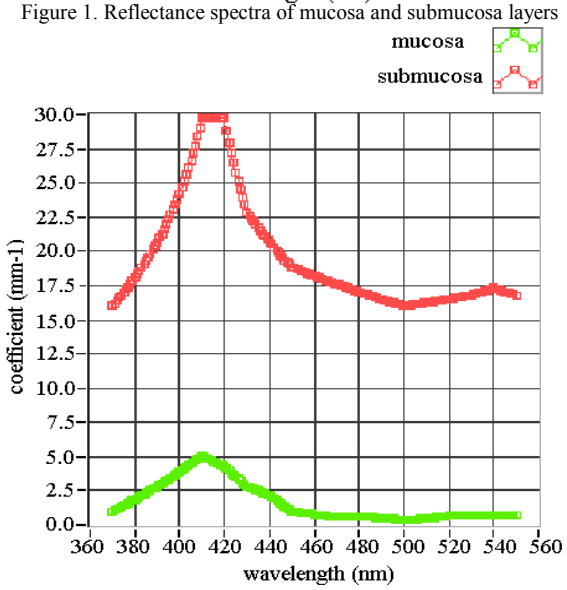
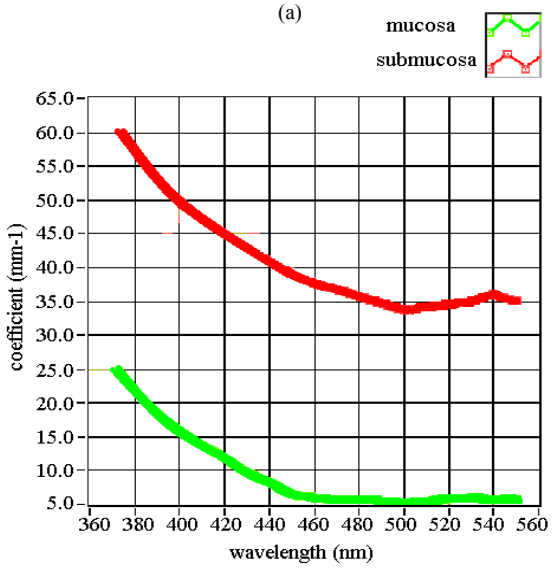


Figure 1. Reflectance spectra of mucosa and submucosa layers



(a)



(b)

Figure 2. (a) Absorption coefficient of the colon tissue; (b) Scattering coefficient of the colon tissue

Monte Carlo simulation (MCS) [2,5,7] could describe photon distributions in a turbid medium. Its major concept involves the consideration of absorption, scattering, and anisotropic scattering properties of the tissue and the calculation of the resulted photon distribution. Advantages of the Monte Carol method are its simple in use and the flexibility in applications. Nevertheless, the calculation efficiency of this method is a great concern. For letter understanding of the progressive process of pathological developments, we could constitute a mathematic tissue model for simulating the fluorescence spectrum of tissue of different stages. By changing the thickness of mucosa and submucosa layer of colon tissue, we could have estimate autofluorescence spectrum of colon tissues of different compositions and could obtain a better understanding of these spectra.

Monte Carlo simulation (MCS) is a numeric analysis method that is often used in estimating the behavior of photons in the tissue in a macroscopic view. The simplification and basic hypothesis of the MCS model is: (1) biologic tissues are arranged in axial symmetry, (2) the tissue optical property is homogeneous in the same layer of the tissue, (3) the angle of the excitation photon is parallel to the normal vector of the tissue.

There are three major parameters used in this simulation, absorption coefficient (μ_a), scattering coefficient (μ_s), and anisotropic coefficient (g). These parameters are wavelength dependent. μ_a represents the degree of the absorption for a group of photons for being absorbed after a propagation of a unit length in the tissue. μ_s represents the degree of scattering effect for a group of photons after a unit length propagation. Anisotropic coefficient, g, represents the cosine value of the averaged scattering angle. The isotropic scattering coefficient μ_s' is defined as $\mu_s' = \mu_s(1\text{-}g)$ [10,11], where g is around 0.7 to 0.99 in biological tissue. The scattering angle is between $\pm 8^\circ$ to $\pm 45^\circ$.

The reflectance coefficient, Rd(λ), and the transmittance coefficient, Tt(λ), can be obtained from the transmission and reflection spectroscopic measurements from mucosa and submucosa layers. Fig. 1 shows the reflectance spectra. In this paper, since it is very difficult to remove the submucosa layer for transmittance measurement, so Tt(λ) is estimated obtained from published data [6]. After Rd (λ) and Tt (λ) are determined: the μ_a (λ) and μ_s (λ) coefficients, which can be obtained through the theory of Kubelka-Munk Two Flux [8,12,13], and are shown in Fig. 2, The Kubelka-Munk Two Flux theory is shown below:

$$S = \frac{1}{bt}\ln\left[\frac{1 - Rd(a-b)}{Tt}\right] \tag{1}$$

$$a = \frac{1 - Tt^2 + Rd^2}{2Rd} \tag{2}$$

$$b = \sqrt{a^2 - 1} \tag{3}$$

$$K = S(a-1) \tag{4}$$

$$\mu_a = K/2 \tag{5}$$

$$\mu_s = \frac{4}{3}(S + \frac{1}{4}\mu_a)/(1-g) \tag{6}$$

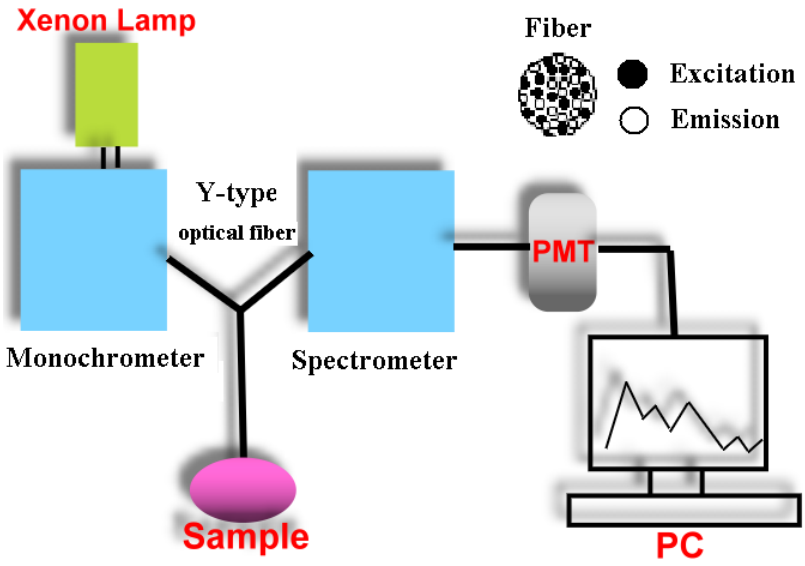


Figure 3. Experiment setup

Method

Simulation of autofluorescence spectrum

We have grouped the colon tissue into two different layers according to their optical structure: mucosa and submucosa layers. We have applied Monte Carlo simulation method for calculating the distribution of a group of photons after penetrating the tissue, as well as the escape of the fluorescence photons after the excitation on the fluorophores [6,7]. The autofluorescence distribution can be described as:

$$\Psi_F(\lambda,r) = \sum_{i=0}[\Phi_{exci}(r',Z_i)\times\rho(Z_i)]\otimes\Gamma_{escape}(\lambda,r',Z_i) \times\beta_{normal}(\lambda,Z_i)\times\Delta Z \quad (7)$$

where $\phi_{exci}(r', Z_i)$ represents the distribution of the excitation photons in the tissue, ρ(Zi) represents the total concentration of fluorophores distribution in the tissue. The distribution of the fluorophores is assumed to be homogeneous in each of the mucosa and submucosa layer. However, the concentration of the fluorescent substance is measured to be much stronger in the submucosa layer than in the mucosa layer. $\Gamma_{esc}(\lambda, r', z)$ represents the escape function of the fluorescence photons after their outgrowth from biological tissue through the excitation [1]. $\beta_{normal}(\lambda, z)$ represents the normalized autofluorescence spectrum, from 370 nm to 550 nm, in each of the mucosa and submucosa layers. The autofluorescence spectrum in each layer at different depth can be measured individually by the frozen slicing approach. The relative strength of the total contribution from each fluorophores at different depth can be shown by the normalized parameters, $\beta_{normal}(\lambda, z)$.

Colon specimen autofluorescence measurements

Experimental procedure

In this paper, pork colon specimen was used in the experiment [6]. The frozen preserved pork colon tissue was removed from the −70℃ refrigerator and immersed in a buffer solution with the concentration of 0.1M. The total autofluorescence signal of the bulk colon tissue was measured first; then the autofluorescence signal of each of the mucosa and submucosa layers was measured separately. We have used the technique of frozen slicing to slice a 1 mm thick colon tissue into 100 μm thick tissue specimen.

Fig. 3 represented the experimental structure of spectrometers, a light source, and a Y-type optical fiber. Through the Y-type optical fiber, the tissue signal was excited by the excitation beam conducted through a fiber waveguide onto the layered specimen, and was sent back through the other fiber to the spectroscope. The excitation light source is a Xenon Lamp (CERMAX, ILC Technology, Sunnyvale California USA). The light source passes through a spectrometer system (D10, Jobin Yvon, Arpajon_France) and generates a narrowband band light at 330 ± 0 nm for to excitation. Because the intensity of the excitation light source is much stronger than the emission signals and the bandwidth of excitation beam overlaps with the emission spectrum, therefore, we insert a long-pass filter, 365 nm, before the entrance of spectroscope to reduce the overlapped interference from the excitation beam. We collect the emission light through long-pass filter and apply photo-multiplier tube (R928, Hamamastu, Japan) to detect the intensities at different wavelengths. The Y-shape optic fiber was constituted by eighteen excitation fibers and nineteen receiving fibers, which are arranged randomly. The diameter of the quartz fiber is 200 μm, N.A.=0.22.

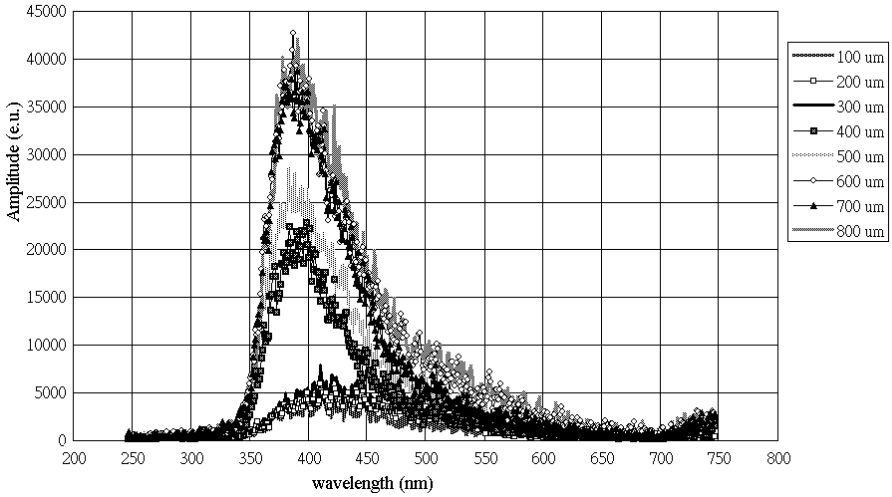


Figure 4. Measures autofluorescent spectra of sliced colon tissue at different depth, from 100 mm to 800 mm .

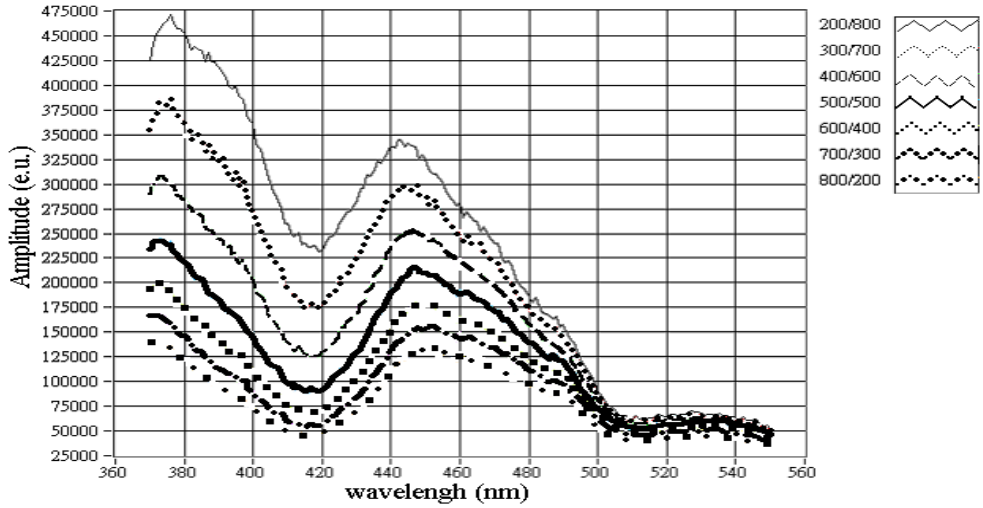


Figure 5. Simulated spectra of autofluorescence with different mucosa/submucosa thickness composition.

Results and discussion

In this paper, we have used MCS modeling to simulate the photon interactions in colon tissue and acquired the simulated autofluorescence spectra of different colon tissues by applying the mimic distribution of photon energy by the MCS modeling. The controlled factor was the thickness of mucosa and submucosa layers, μ_a , μ_s , and g, tissue optical parameters. We have assumed that the total thickness of the mucosa and submucosa layers was 1mm, and the thickness of mucosa layer was increased from 200 μm to 800 μm by adding 100 μm in each step. The thickness of submucosa layer was varied from 800 μm to 200 μm respectively.

ρ(Zi) measurements

We have sliced colon tissue into slices of 100μm thick specimen and measured their autofluorescence spectra. Fig. 4 shows the autofluorescence spectra of each of the 100 μm thick colon tissue at different depth. We can find that there are 3 groups of these spectra, (1) the mucosa group, at 100~300 μm depth, with the weakest fluorescence intensity, (2) the intermediate group, at 400~500 μm depth, represents the middle region between mucosa/submucosa layer with moderate intensity, and (3) the submucosa group at 600~800 μm depth, with the strongest intensity. The measured autofluorescence spectra of the mucosa layer and the mucosa layer groups are couple of time different in their fluorescent intensity, but the variation in the intensity in each of the group is quite minimum. In addition, fluorescent intensities of the submucosa group is 8~9 times stronger than that of the mucosa group. Therefore, the concentration of the fluorephores in the submucosa layer, at 600 ~800 μm depth are much stronger than the mucosa layer at 100 μm~300 μm depth.

Simulated results

Fig. 5 shows the autofluorescence spectra of different colon tissues with different mucosa/submucosa thickness. We have found that the intensity of the fluorescent spectrum

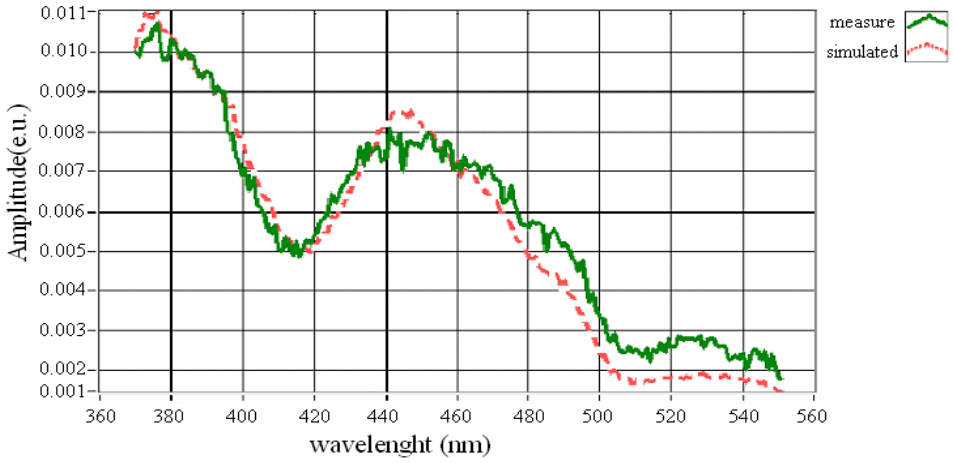


Figure 6. The autofluorescence spectra of a measured colon tissue and a simulated colon tissue with 300 mm / 700 mm mucosa and submucosa layers.

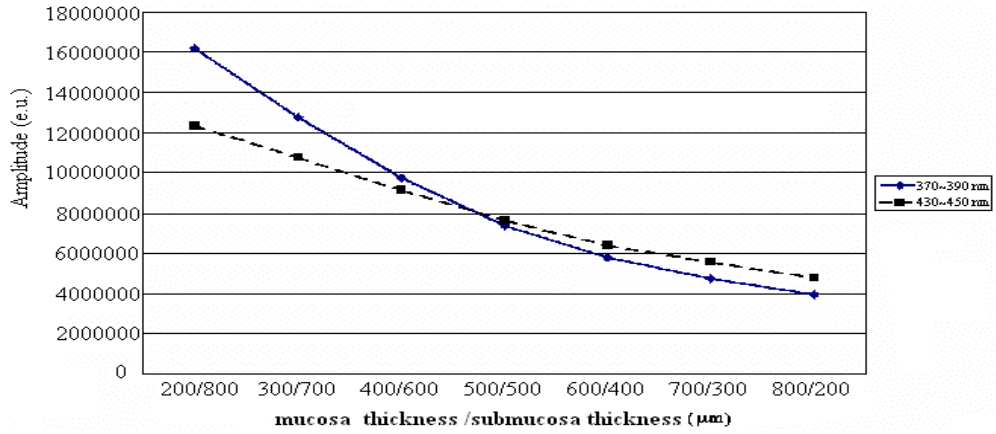


Figure 7. Integrated autofluorescence spectra of collagen and NADH emission changes with different mucosa and submucosa layer thickness.

decreases as the thickness of the mucosa layer increases. This finding may result from (1) the increased mucosa layer reduced the probability for the excited photons reaching the submucosa layer, (2) the escape of the fluorescence in the submucosa layer reaching the tissue surface was also reduced by the increased absorption and scattering effects from the increased thickness of the mucosa layer, and (3) the contribution of the autofluorescence spectrum from the submucosa layer is much stronger than the mucosa layer, and the increasing in the thickness of the mucosa layer will reduce the exposure of the submucosa layer to the excitation photons. The major fluorescence emissions at 330 nm excitation in the mucosa layer are from collagen and NADH, and the fluorescence emission in the submucosa layer is from collagen that is much strong or the submucosa layer than in the mucosa layer. When the thickness of the mucosa layer increases, the reduction of the fluorescence emission of collagen at 380 nm is more affected than the NADH's emission at 460 nm. We have compared the autofluorescence spectrum of the normal colon tissue between the measured and the MCS simulated spectrum of the composition of the mucosa layer, 300 μm, and the submucosa layer, 700 μm, as shown in Fig. 6. These two spectra appear to be very similar.

Ratio of autofluorescence intensity of Collagen/NADH

Fig. 7 shows the integrated autofluorescence intensity of collagen and NADH with different composition of the mucosa and submucosa layer thickness. The integrated intensity of the collagen covers 380 ± 10 nm, and the NADH covers 440 ± 10 nm. We observed that the integrated autofluorescence intensity of both collagen and NADH intensity reduces as the thickness of the mucosa layer increases. In addition, the autofluorescence integrated intensity of collagen is weaker than that of NADH when the thickness of the mucosa layer is thicker than 500 μm. For comparing the influence of the mucosa/submucosa thickness to the autofluorescence intensity of collagen and NADH, we compared the ratio of their intensities, collagen / NADH, as shown in Fig. 8. It displays that the thicker mucosa layer is, the lower collagen/NADH ratio is. When the thickness of the mucosa layer is thicker than 500 μm, the value of ratio becomes less than 1 that represents the fluorescent intensity of the collagen become weaker than that of the NADH.

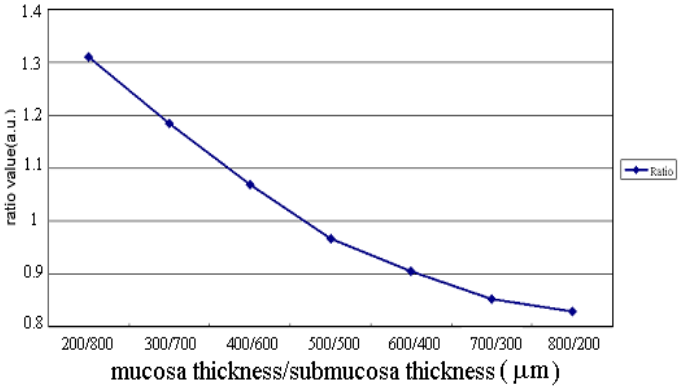


Figure 8. The ratio of the integrated autofluorescence intensity of collagen/ NADH with different thickness of the mucosa and submucosa layer thickness.

Conclusion

In this paper, we have applied the MCS to simulate the interactions between photons and colon tissues. We could simulate the complicated physical phenomena by using the MCS mathematic method. The MCS approach has been successfully used for estimating the energy distribution of excitation photons in colon tissues, the distribution of fluorescence emission from the excited fluorephores, and the effects of the absorption and scattering of the colon tissues. The study of MCS could help the understanding of the photons and tissue interactions under various conditions, and the mechanism and characteristics of tissue fluorescence. Through this autofluorescent MCS modeling, we could simulate the process of the pathological change of tissue in different developing stages. This study could provide valuable information in the future research and development of noninvasive biophotonic diagnostic techniques.

Acknowledgements

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References

[1] Renato Marchesini, Emanuele Pignoli, Stefano Tomatis, Sabrina Fumagalli, Adele E. Sichirollo, Silvana Di Palma, Marko Dal Fante, Pasquale Spinelli, Anna C. Croce, and Giovanni Borttiroli, “Ex vivo Optical Properties of Human Colon Tissue “ , Lasers in Surgery and Medicine , 15: 351-357, 1994.

[2] Lihong Wang , Steven L. Jacques , Liqiong Zheng , “MCML－Monte Carlo modeling of light transport in multi-layered tissues”, Computer Methods and Programs in Biomedicine, 47: 131-146, 1995.

[3] Wai-Fung Cheong , Scott A. Prahl , Ashley J. Welch , “A review of the Optical Properties of Biological Tissue”, IEEE Journal of Quantum Electronics , 26: 2166-2185, 1990.

[4] Thomas D. Wang , Jacques Van Dam , James M. Crawford , Elizabeth A. Prieisinger , Yang Wang , Michael S. Feld , “Fluorescence Endoscopic Imaging of Human Colonic Adenomas” , Gastroenterology , 111: 1182-1191, 1996.

[5] M.L. de Jode , “Monte Carlo simulations of Light Distributions in an Embedded Tumor Model : Studies of Selectivity in Photodynamic Therapy” , Lasers Med. Sci., 15: 49-56, 2000.

[6] George I. Zonios , Robert M. Cothren , Joseph T. Arendt , Jun Wu , Jacques Van Dam , James M. Crawford , Ramasamy Manoharan , Michael S. Feld , “Morphological Model of Human Colon Tissue Fluorescence” , IEEE Transactions on Biomedical engineering , 43: 113-122, 1996.

[7] Jianan Qu , Calum MacAulay , Stephen Lam , Branko Palcic , “Laser-induced fluorescence spectroscopy at endoscopy : tissue optics , Monte Carlo modeling , and in vivo measurements” , Optical Engineering , 34: 3334-3343, 1995.

[8] Valery Tuchin , “Tissue Optics – Light Scattering Methods and Instruments for Medical Diagnosis” , SPIE Press , Bellingham , Washington , 2000.

[9] A. Pradhan , P. Pal , G. Durocher , L. Villeneuve , A. Balassy , F. Babai , L. Gaboury , L. Blanchard , “Steady state and Tissue resolved Fluorescence Properties of Metastatic and Non-metastatic Malignant Cells from Different Species” , J. Photochem. Photobiol. B: Biol., 3: 101-112, 1998.

[10] S. Chandrasekhar, “Radiative Transfer”, Oxford University Press, Oxford, 1950.

[11] A. Ishimaru, “Wave Propagation and Scattering in Random Media”, Academic Press, New York, 1987.

[12] P. Kubelka, “New Contributions to the Optics of Intensely Light Scattering Materials. Part II: Nonhomogeneous Layers”, J. Opt. Soc. Am., 344: 330-335, 1954.

[13] C.M. Gardner, S.L. Jacques, A.J. Welch, “Fluorescence Spectroscopy of Tissue: Recovery of Intrinsic Fluorescence from Measured Fluorescence”, Appl. Opt., 35: 1780-1792, 1996.

[14] Irene Georgakoudi, Brian C. Jacobson, Jacques Van Dam, ”Fluorescence, Reflectance, and Light-Scattering Spectroscopy for Evaluating Dysplasia in Patients with Barrett’s Esophagus” , Gastroenterology , 120: 1620-1629, 2001