

Differences in optical properties between healthy and pathological human colon tissues using a Ti:sapphire laser: an *in vitro* study using the Monte Carlo inversion technique

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Abstract. The purpose of the study is to analyze and compare differences in the optical properties between normal and adenomatous human colon tissues *in vitro* at 630-, 680-, 720-, 780-, 850-, and 890-nm wavelengths using a Ti:sapphire laser. The optical parameters of tissue samples are determined using a double integrating sphere setup at seven different laser wavelengths. The inverse Monte Carlo simulation is used to determine the optical properties from the measurements. The results of measurement show that the optical properties and their differences vary with a change of laser wavelength for normal and adenomatous colon mucosa/submucosa and normal and adenomatous colon muscle layer/chorion. The maximum absorption coefficients for normal and adenomatous human colon mucosa/submucosa are 680 nm, and the minimum absorption coefficients for both are 890 nm. The maximum difference of the absorption coefficients between both is 56.8% at 780 nm. The maximum scattering coefficients for normal and adenomatous colon mucosa/submucosa are 890 nm, and the minimum scattering coefficients for both are 780 nm. The maximum difference of the scattering coefficients between both is 10.6% at 780 nm. The maximum absorption coefficients for normal and adenomatous colon muscle layer/chorion are 680 nm, and the minimum absorption coefficients for both are 890 nm. The maximum difference of the absorption coefficients between both is 47.9% at 780 nm. The maximum scattering coefficients for normal and adenomatous colon muscle layer/chorion are 890 nm, and the minimum scattering coefficients for both are 680 nm. The maximum difference of the scattering coefficients between both is 9.61% at 850 nm. The differences in absorption coefficients between normal and adenomatous tissues are more significant than those in scattering coefficients. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1990125]

Keywords: laser; adenomatous human colon tissue; optical properties; integrating sphere.

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1 Introduction

More than 85% of all cancers originate in the epithelia lining the internal surfaces of the human body. The majority of such lesions are readily treatable if diagnosed at an early state.¹ Apart from conventional methods of cancer diagnosis,²⁻⁴ there is a need to develop new approaches that are simple, objective, and noninvasive. The use of optical techniques for diagnostic purposes relies on the capability to measure the optical properties of healthy and pathological tissues and in appreciating their relative differences. In fact, a degree of con-

trast must exist between absorption and scattering coefficients for detection of tissue alteration using optical detecting and optical imaging.⁵ In recent years, an increasing group of researchers has been interested in nonionizing, near-infrared (NIR) approaches for detecting and imaging diseased tissues. The proposed techniques range from continuous wave^{6,7} to frequency-domain^{8,9} or time-dependent measurements of scattered light.^{10,11} These techniques are based on the determination of optical properties of scattering media. The optical properties are represented by the absorption coefficient μ_a , the scattering coefficient μ_s , and the anisotropy factor g . Since optical detecting and optical imaging are based on se-

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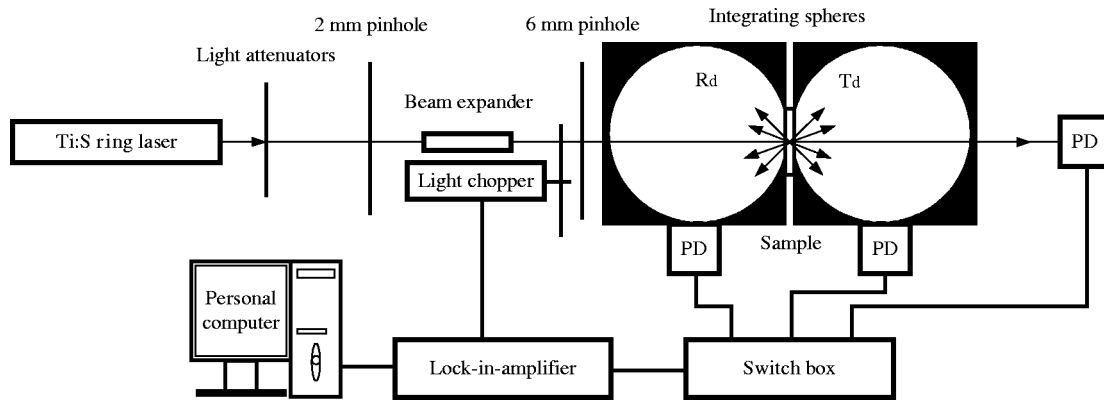


Fig. 1 Experimental setup consisting of double-integrating-sphere with an intervening sample.

lective differences existing in optical properties of healthy and pathological tissues, it is particularly important for diagnostic purposes. For example, laser-induced autofluorescence (LIAF) spectroscopy has been found to be a promising tool for early cancer diagnosis in colons and other organs.^{12,13} Consequently, tissue optical properties of healthy and pathological human colon tissues are important for medical applications in diagnosis and therapy.¹⁴ The purpose of the present study is to compare differences in the absorption and scattering properties between normal and adenomatous human colon mucosa/submucosa, and between normal and adenomatous muscle layer/chorion *in vitro* at 630, 680, 720, 780, 810, 850, and 890 nm. The results were analyzed and compared from the experimental data we obtained.

2 Materials and Methods

2.1 Sample Preparation

In vitro optical properties were investigated for four kinds of tissues: normal and adenomatous human colon mucosa/submucosa, and normal and adenomatous human colon muscle layer/chorion, of which there are two tissue types (namely, human colon mucosa/submucosa and muscle layer/chorion) for four kinds of tissues. Tissue samples were taken from six human colons (six adenomatous, six normal), immediately after excision of the tissues. Each removed colon sample was immediately rinsed briefly in saline to remove surface excess blood and peeled off surface fats, placed in a bottle with saline as soon as possible, stored in a refrigerator at -70°C , and then sectioned by microtome before measurement. All tissue samples were respectively clamped between two glass slides 0.16 and 0.65 mm thick, and then were placed between the two integrating spheres before tissue samples were measured. From the normal human colons, a total of 12 tissue samples, with a mean thickness of 0.40 mm, were used (six from mucosa/submucosa and six from muscle layer/chorion). From the adenomatous human colons, a total of 12 tissue samples, with a mean thickness of 0.40 mm, were used (six from mucosa/submucosa and six from muscle layer/chorion). Tissue samples were prepared and measurements were taken within 16 h after removal.

2.2 Methods

2.2.1 Measurement of tissue optical properties

An established method for measuring the optical parameters of turbid materials is the integrating-sphere technique. This technique is widely used for the determination of the optical properties of biological tissues *in vitro*.^{15,16} The experimental setup is schematically shown in Fig. 1. The colon samples were placed between two identical integrating spheres (Anhui Institute of Optics and Fine Mechanics, Academia Sinica, China, model F4) 50 mm in diameter with a circular sample port 12 mm in diameter, and illuminated with collimated light from a 2.5-mm beam diameter, 630-, 680-, 720-, 780-, 810-, 850-, 890-nm Ti:S ring laser (Coherent, USA, model 899-05) operating at 0 to 0.7 W. The outputs of all the lasers were collimated and attenuated (to a power at most 10 mW) by a 2-mm pinhole and light attenuators, respectively. The light fluences within each sphere (the diffuse reflectance in the first sphere and the diffuse transmission in the second sphere) and the collimated transmission (at a distance of 70 cm beyond the second sphere) were measured using standard light measuring techniques.¹⁷ The input light beams divergence was approximately 1.5 deg, and the light beams were chopped mechanically at 500 Hz using a light chopper (SRS, USA, model SR540). The collimated transmittance T_c , the diffuse reflectance R_d , and the diffuse transmittance T_d were measured by using photodiode detectors (APP, Hamamatsu, Japan, model C5460) at 630, 680, 720, 780, 810, 850, and 890 nm. The signals were amplified using a lock-in amplifier (SRS, USA, model SR830) and processed by a personal computer. All measurements within the spheres were made relative to the signal when a 99% reflecting plate (Anhui Institute of Optics and Fine Mechanics, Academia Sinica, China, model F4) was placed at the sample aperture, and the collimated transmission measurement was made relative to a measurement with no sample. For a more detailed description of the measurement method of a double integrating-sphere setup, see Pickering et al.,¹⁸⁻²⁰ and van Hillegerberg et al.²¹ The experiments involved a tissue sample held between two glass slides and submerged in saline. Therefore, the specular reflectance and refraction at the tissue/glass/saline boundary must

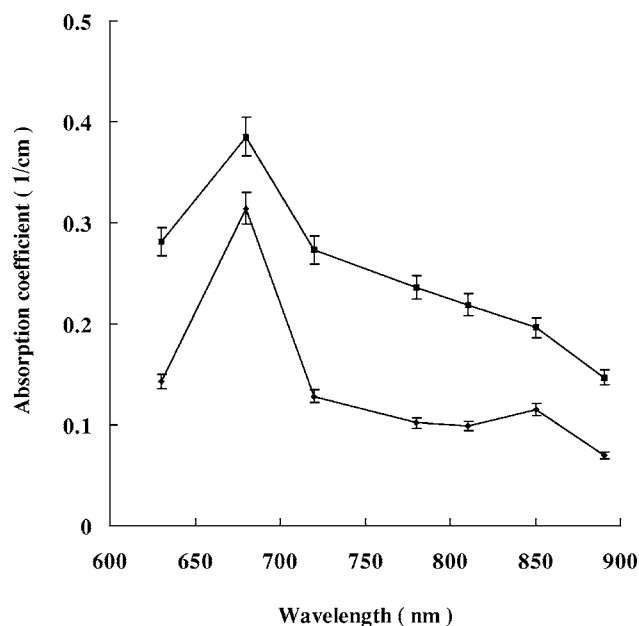


Fig. 2 The absorption coefficients of normal (◆-◆) and adenomatous (■-■) human colon mucosa/submucosa, calculated with inverse Monte Carlo simulation.

be corrected. A complete description has been given by Jacques, Alter, and Prahl.²²

2.2.2 Calculation of tissue optical parameters

Inverse Monte Carlo simulation²³⁻²⁵ was applied to calculate the optical parameters of colon tissue samples from the measured data. The basic concept and implementation of the Monte Carlo technique have been described earlier.²⁶⁻²⁸ The Monte Carlo technique allows us to take into account a real-

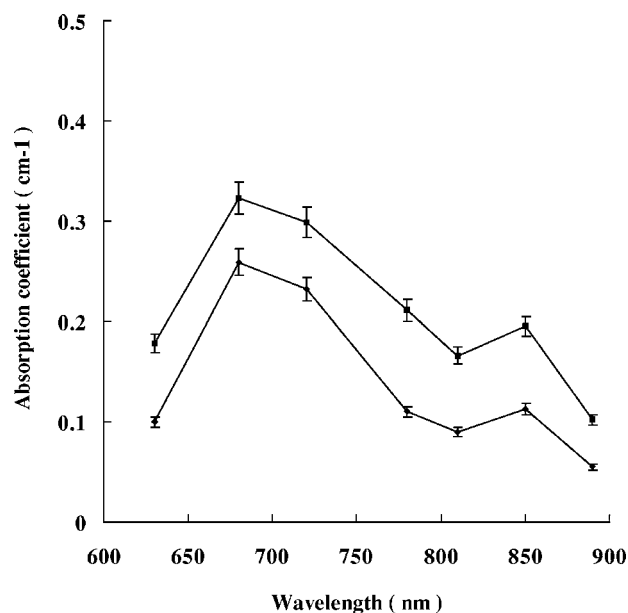


Fig. 3 The absorption coefficients of normal (◆-◆) and adenomatous (■-■) human colon muscle layer/chorion, calculated with inverse Monte Carlo simulation.

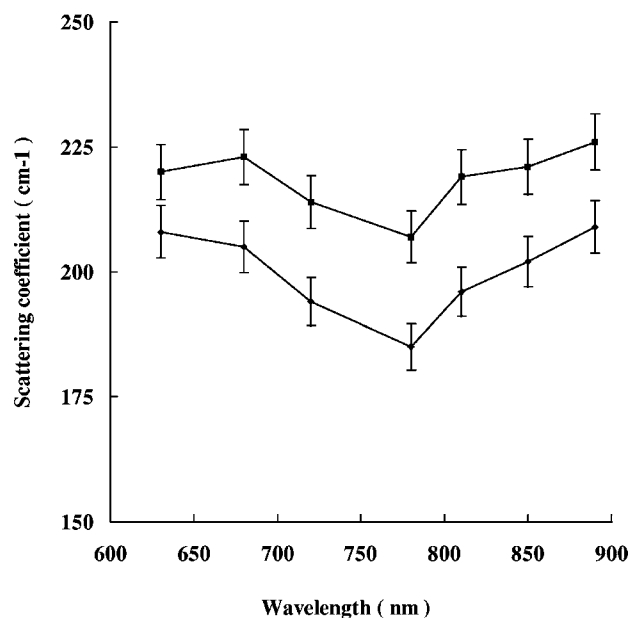


Fig. 4 The scattering coefficients of normal (◆-◆) and adenomatous (■-■) human colon mucosa/submucosa, calculated with inverse Monte Carlo simulation.

istic 3-D geometry of the experimental configuration, including the side losses of light at the edges of the sample. For the present study, the Monte Carlo method has been modified according to the particular geometry of the experimental setup. To calculate the optical parameters with Monte Carlo simulations, an initial set of optical properties has been estimated²⁹ on the basis of the measurements obtained by an analytical model (Kubelka-Munk theory).³⁰⁻³² These values were used to calculate the transmission and reflection properties of the samples, assuming the Henyey-Greenstein phase function.³³ If the differences between calculated and measured data were within 2%, the estimated optical parameters were regarded to represent the real optical properties of the tissue sample. If the expected data did not match the experimental results, the optical parameters were corrected iteratively until the true optical parameters were found.³⁴ The algorithm accounts for the refractive index of the glass slides ($n \approx 1.55$) and of tissue ($n = 1.4$).³⁵ A student's t test was used to compare the data obtained from the normal and adenomatous human colon tissue samples.

3 Results

In this investigation, seven different wavelengths of laser were used for radiating the two incision areas of tissue slices. Each measured value at each wavelength in the same condition of experimentation was the mean value gained by 24 repeated measurements for each kind of tissue sample (a total of six tissue samples at the same wavelength), and each tissue sample was measured twice for each incision area of the tissue slice, respectively. The position of the luminous spot of incident light radiation on the sample was changed after each measured datum was obtained. The two incision areas of samples per tissue type were radiated at each wavelength of laser, respectively, and the measured results were reproducible

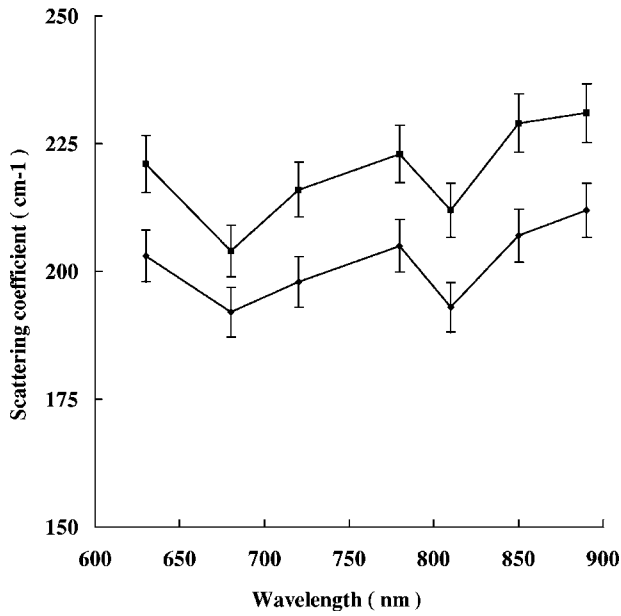


Fig. 5 The scattering coefficients of normal (◆-◆) and adenomatous (■-■) human colon muscle layer/chorion, calculated with inverse Monte Carlo simulation.

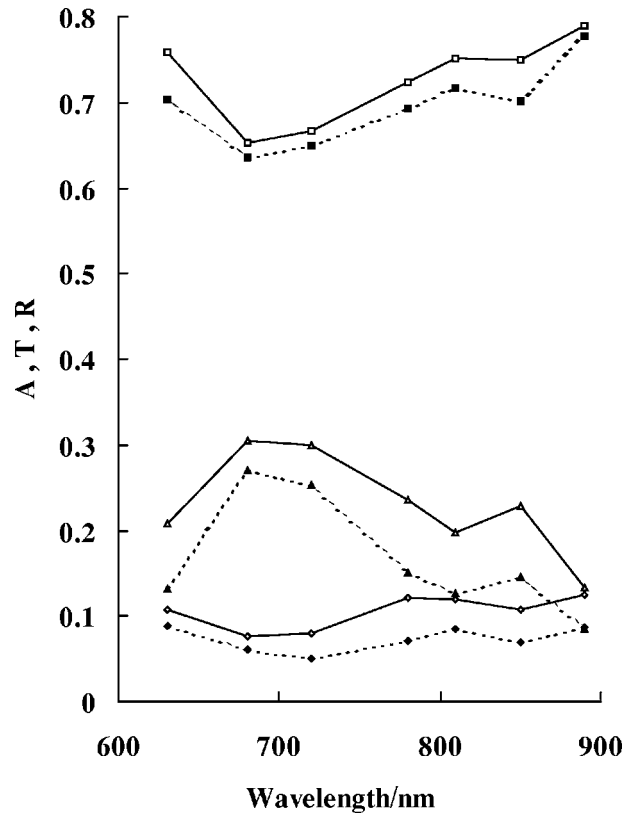


Fig. 7 The results of a Monte Carlo simulation for normal and adenomatous human colon muscle layer/chorion with the same thickness of 0.4 mm: ▲-▲, absorption for normal human colon muscle layer/chorion; △-△, absorption for adenomatous human colon muscle layer/chorion; ◇-◇, transmission for normal human colon muscle layer/chorion; ◆-◆, transmission for adenomatous human colon muscle layer/chorion; □-□, reflection for normal human colon muscle layer/chorion; and ■-■, reflection for adenomatous human colon muscle layer/chorion.

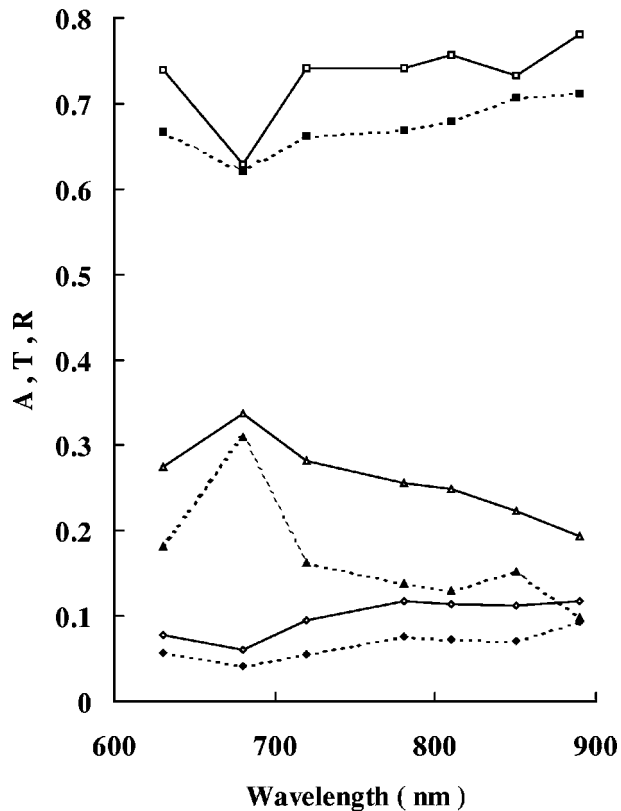


Fig. 6 The results of a Monte Carlo simulation for normal and adenomatous human colon mucosa/submucosa with the same thickness of 0.4 mm: ▲-▲, absorption for normal human colon mucosa/submucosa; △-△, absorption for adenomatous human colon mucosa/submucosa; ◇-◇, transmission for normal human colon mucosa/submucosa; ◆-◆, transmission for adenomatous human colon mucosa/submucosa; □-□, reflection for normal human colon mucosa/submucosa; and ■-■, reflection for adenomatous human colon mucosa/submucosa.

for a specific sample at a specific wavelength. There were no significant differences in the measured values R_d or T_d and/or T_c of the two incision areas of all samples per tissue type at the same wavelength. Consequently, the mean values of T_c , R_d , and T_d of the two incision areas of all samples per tissue type at the same wavelength were obtained from these measurement data. By the inverse Monte Carlo simulation, we gained the wavelength dependence of the absorption coefficients and the scattering coefficients of the transport theory for these tissues from these measurements. The optical properties are expressed as the mean±standard deviation for all measurements within one group of samples (e.g., normal colon mucosa/submucosa at 780 nm). Figures 2 and 3 show differences in the absorption coefficients between both normal and adenomatous human colon mucosa/submucosa and both normal and adenomatous human colon muscle layer/chorion at seven different wavelengths. Figures 4 and 5 show differences in the scattering coefficients between two kinds of colon mucosa/submucosa and two kinds of colon muscle layer/chorion at seven different wavelengths. Figures 6 and 7 show differences in the absorption A, transmission T, and reflection R between both normal and adenomatous human colon mucosa/submucosa and both normal and adenomatous human

Table 1 The μ_a and μ_s were determined for normal and adenomatous human colon mucosa/submucosa by the inverse Monte Carlo simulation from these measurements, and A, T, and R were determined by Monte Carlo simulation.

λ (nm)	Normal colon mucosa/submucosa					Adenomatous colon mucosa/submucosa									
	$\mu_a(\text{cm}^{-1})$	$\mu_s(\text{cm}^{-1})$	A	T	R	$\mu_a(\text{cm}^{-1})$	$\mu_s(\text{cm}^{-1})$	A	T	R	$D_1\%$	$D_2\%$	$D_3\%$	$D_4\%$	$D_5\%$
630	0.143±0.004	208±5.27	0.181	0.0779	0.739	0.281±0.007	220±5.52	0.275	0.0577	0.667	49.1	5.46	34.2	25.9	9.74
680	0.315±0.008	205±5.13	0.310	0.0608	0.629	0.384±0.011	223±5.58	0.337	0.0420	0.621	18	8.07	8.01	30.9	1.27
720	0.128±0.003	194±4.85	0.162	0.0944	0.742	0.273±0.007	214±5.35	0.282	0.0559	0.662	53.1	8.49	42.6	40.8	10.8
780	0.102±0.003	185±4.63	0.138	0.118	0.741	0.236±0.006	207±5.19	0.255	0.0757	0.669	56.8	10.6	45.2	35.9	9.72
810	0.0988±0.0025	196±4.91	0.129	0.114	0.756	0.219±0.006	219±5.48	0.248	0.0729	0.679	54.9	10.5	50	36.1	10.2
850	0.115±0.003	202±5.05	0.152	0.112	0.732	0.196±0.005	221±5.53	0.223	0.0706	0.706	41.3	8.6	31.8	37	3.55
890	0.0694±0.0017	209±5.23	0.0982	0.118	0.781	0.147±0.004	226±5.65	0.194	0.0929	0.712	52.8	7.52	49.4	21.3	8.84

colon muscle layer/chorion at seven different wavelengths.

Tables 1 and 2 summarize the results of our measurements for four kinds of tissues at the seven different wavelengths, where D_1 , D_2 , D_3 , D_4 , and D_5 represent the differences of the absorption coefficients, scattering coefficients, absorption, transmission, and reflection, respectively.

4 Discussion and Conclusion

In vitro optical properties of normal and adenomatous human colon mucosa/submucosa, and normal and adenomatous human colon muscle layer/chorion, were determined at 630, 680, 720, 780, 810, 850, and 890 nm. The measurements were performed using a double-integrating-sphere setup, and the optical properties were assessed from these measurements using inverse Monte Carlo simulation. In our study, it is interesting to note the differences in absorption and scattering properties measured between normal and adenomatous human colon tissues at seven different laser wavelengths. We believe these differences in absorption and scattering properties

should help to differentiate diagnosis for human colon tissues by using optical methods.

For normal and adenomatous colon mucosa/submucosa, the results of this study show that the optical properties and their differences vary with a change of laser wavelength, as shown in Table 1. The maximum absorption coefficient for normal colon mucosa/submucosa is 0.315 cm^{-1} at 680 nm, and for adenomatous colon mucosa/submucosa is 0.384 cm^{-1} at 680 nm. The difference of the absorption coefficients between both colon mucosa/submucosa is only 18%, and the difference is also the minimum difference. The minimum absorption coefficient for normal colon mucosa/submucosa is 0.0694 cm^{-1} at 890 nm, and for adenomatous colon mucosa/submucosa is 0.147 cm^{-1} at 890 nm. The difference of the absorption coefficients between both is 52.8%, whereas the maximum difference of the absorption coefficients between both is 56.8% at 780 nm. Figure 2 shows the laser wavelength dependence of the absorption coefficients of normal and adenomatous colon mucosa/submucosa. The maximum

Table 2 The μ_a and μ_s were determined for normal and adenomatous human colon muscle layer/chorion by the inverse Monte Carlo simulation from these measurements, and A, T, and R were determined by Monte Carlo simulation.

λ (nm)	Normal colon muscle layer/chorion					Adenomatous colon muscle layer/chorion									
	$\mu_a(\text{cm}^{-1})$	$\mu_s(\text{cm}^{-1})$	A	T	R	$\mu_a(\text{cm}^{-1})$	$\mu_s(\text{cm}^{-1})$	A	T	R	$D_1\%$	$D_2\%$	$D_3\%$	$D_4\%$	$D_5\%$
630	0.0997±0.0025	203±5.08	0.131	0.108	0.759	0.178±0.005	221±5.53	0.208	0.0878	0.703	44	8.15	37	18.7	7.38
680	0.259±0.007	192±4.82	0.270	0.0769	0.653	0.323±0.008	204±5.12	0.305	0.0605	0.635	19.8	5.88	11.5	47.4	2.76
720	0.232±0.006	198±4.95	0.253	0.0797	0.667	0.299±0.008	216±5.48	0.299	0.0502	0.650	22.4	8.33	15.4	37	2.55
780	0.110±0.003	205±5.14	0.151	0.122	0.724	0.211±0.005	223±5.59	0.236	0.0706	0.693	47.9	8.07	36	42.1	4.28
810	0.0894±0.0023	193±4.83	0.126	0.120	0.752	0.166±0.004	212±5.35	0.198	0.0843	0.717	46.2	8.96	36.4	29.8	4.65
850	0.113±0.003	207±5.18	0.146	0.108	0.750	0.195±0.005	229±5.74	0.229	0.0694	0.702	42.1	9.61	36.3	35.7	6.4
890	0.0547±0.0014	212±5.36	0.0843	0.125	0.789	0.102±0.003	231±5.79	0.133	0.0867	0.777	46.4	8.23	36.6	30.6	1.52

scattering coefficient for normal colon mucosa/submucosa is 209 cm^{-1} at 890 nm, and for adenomatous colon mucosa/submucosa is 226 cm^{-1} at 890 nm. The difference of the scattering coefficients between both is only 7.52%. The minimum scattering coefficient for normal colon mucosa/submucosa is 185 cm^{-1} at 780 nm, and for adenomatous colon mucosa/submucosa is 207 cm^{-1} at 780 nm. The difference of the scattering coefficients between both is 10.6%. It is obvious that the difference at 780 nm is also the maximum difference, and the minimum difference of the scattering coefficients between both is 5.46% at 630 nm. Figure 4 shows the laser wavelength dependence of the scattering coefficients of normal and adenomatous colon mucosa/submucosa. The maximum absorption for normal colon mucosa/submucosa is 0.310 at 680 nm, and for adenomatous colon mucosa/submucosa is 0.337 at 680 nm. The difference of the absorption between both is only 8.01%, whereas the difference at 680 nm is also the minimum difference. The minimum absorption for normal colon mucosa/submucosa is 0.0982 at 890 nm, and for adenomatous colon mucosa/submucosa is 0.194 at 890 nm. The difference of the absorption between both is 49.4%, whereas the maximum difference of the absorption between both is 50% at 810 nm. Figure 6 shows the laser wavelength dependence of the absorption of normal and adenomatous colon mucosa/submucosa. The maximum transmission for normal colon mucosa/submucosa is 0.118 at 780 and 890 nm, respectively, and for adenomatous colon mucosa/submucosa is 0.0929 at 890 nm. The difference of the transmission between both at 890 nm is 21.8%, whereas the difference at 890 nm is also the minimum difference. The minimum transmission for normal colon mucosa/submucosa is 0.0608 at 680 nm, and for adenomatous colon mucosa/submucosa is 0.0420 at 680 nm. The difference of the transmission between both is 30.9%, and the maximum difference of the transmission between both is 40.8% at 720 nm. Figure 6 also shows the laser wavelength dependence of the transmission of normal and adenomatous colon mucosa/submucosa. The maximum reflection for normal colon mucosa/submucosa is 0.781 at 890 nm, and for adenomatous colon mucosa/submucosa is 0.712 at 890 nm. The differences of the reflection between both is 8.84%. The minimum reflection for normal colon mucosa/submucosa is 0.629 at 680 nm, and for adenomatous colon mucosa/submucosa is 0.621 at 680 nm. The differences of the reflection between both is 1.27%, and the difference at 680 nm is also the minimum difference, whereas the maximum difference of the reflection between both is 10.8% at 720 nm. Figure 6 also shows the laser wavelength dependence of the reflection of normal and adenomatous colon mucosa/submucosa.

For normal and adenomatous colon muscle layer/chorion, the results of this study show that the optical properties and their differences also vary with a change of laser wavelength, as shown in Table 2. The maximum absorption coefficient for normal colon muscle layer/chorion is 0.259 cm^{-1} at 680 nm, and for adenomatous colon muscle layer/chorion is 0.323 cm^{-1} at 680 nm. The difference of the absorption coefficients between both is 19.8%, whereas the difference at 680 nm is the minimum difference. The minimum absorption coefficient for normal colon muscle layer/chorion is 0.0547 cm^{-1} at 890 nm, and for adenomatous colon muscle

layer/chorion is 0.102 cm^{-1} at 890 nm. The difference of the absorption coefficients between both is 46.4%. The maximum difference of the absorption coefficients between both is 47.9% at 780 nm. Figure 3 shows the laser wavelength dependence of the absorption coefficients of normal and adenomatous colon muscle layer/chorion. The maximum of the scattering coefficient for normal colon muscle layer/chorion is 212 cm^{-1} at 890 nm, and for adenomatous colon muscle layer/chorion is 231 cm^{-1} at 890 nm. The difference of the scattering coefficients between both is 8.23%. The minimum scattering coefficient for normal colon muscle layer/chorion is 192 cm^{-1} at 680 nm, and for adenomatous colon muscle layer/chorion is 204 cm^{-1} at 680 nm. The difference of the scattering coefficients between both is 5.88%, whereas the difference at 680 nm is also the minimum difference, and the maximum difference of the scattering coefficients between both is 9.61% at 850 nm. Figure 5 shows the laser wavelength dependence of the scattering coefficients of normal and adenomatous colon muscle layer/chorion. The maximum absorption for normal colon muscle layer/chorion is 0.270 at 680 nm, and for adenomatous colon muscle layer/chorion is 0.305 at 680 nm. The difference of the absorption between both is 11.5%, whereas the difference at 680 nm is also the minimum difference. The minimum absorption for normal colon muscle layer/chorion is 0.0843 at 890 nm, and for adenomatous colon muscle layer/chorion is 0.133 at 890 nm. The difference of the absorption between both is 36.6%. The maximum difference of the absorption between both is 37% at 630 nm. Figure 7 shows the laser wavelength dependence of the absorption of normal and adenomatous colon muscle layer/chorion. The maximum transmission for normal colon muscle layer/chorion is 0.125 at 890 nm, and for adenomatous colon muscle layer/chorion is 0.0878 at 630 nm. The minimum transmission for normal colon muscle layer/chorion is 0.0769 at 680 nm, and for adenomatous colon muscle layer/chorion is 0.0502 at 720 nm. The maximum difference of the transmission between both is 47.4% at 680 nm, and the minimum difference is 18.7% at 630 nm. Figure 7 also shows the laser wavelength dependence of the transmission of normal and adenomatous colon muscle layer/chorion. The maximum reflection for normal colon muscle layer/chorion is 0.789 at 890 nm, and for adenomatous colon muscle layer/chorion is 0.777 at 890 nm. The difference of the reflection between both is 1.52%, whereas the difference at 890 nm is also the minimum difference. The minimum reflection for normal colon muscle layer/chorion is 0.653 at 680 nm, and for adenomatous colon muscle layer/chorion is 0.635 at 680 nm. The difference of the reflection between both is 2.76%. The maximum difference of the reflection between both is 7.38% at 630 nm. Figure 7 also shows the laser wavelength dependence of the reflection of normal and adenomatous colon muscle layer/chorion.

It is obvious that there were large differences in the absorption coefficients between normal and adenomatous colon mucosa/submucosa, and between normal and adenomatous colon muscle layer/chorion at the same laser wavelength. There were also obvious differences in the scattering coefficients between normal and adenomatous colon mucosa/submucosa, and between normal and adenomatous colon muscle layer/chorion at the same laser wavelength. There

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