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Research Article

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Characteristics of LIF and Raman spectroscopy of serum for the detection of colon cancer

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ABSTRACT

Raman spectroscopy is a promising optical technique for blood diagnosis. This study assessed the diagnostic potential of Raman spectroscopy of serum by evaluating its ability to distinguish colon cancer patients and normal people. Raman spectroscopy of serum taken from normal people, colon cancer patients, and colon cancer patients after operation was collected by using an Ar-ion Raman spectroscopy system. Three parameters - Δλ, α, β - were used for the comparison among different groups. Thirty-five colon cancer patients were tested before and after operation, 22 normal people were measured as controls. The results show that Raman spectroscopy differentiated colon cancer from colon cancer after operation, and the controls.

Keywords: fluorescence spectroscopy, Raman spectroscopy, serum, colon cancer, diagnosis

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INTRODUCTION

Colon cancer ranks as the third most common cancer worldwide, predicted in 2013 to account for 100 thousand for newly diagnosed cancers[1]. Currently, colonoscopy is the most commonly used technique for early detection, but this method cannot detect under-surface pre-cancerous changes[2]. Endoscopic ultrasound and optical coherence tomography (OTC) are two other usual methods for colon cancer detection. However, ultrasound has too low resolution and OTC is complex to operate[3,4].

Raman spectroscopy can provide information about the biochemical composition of the biological sample. It is possible for Raman spectroscopy to become a rapid, non-invasive and convenient method to detect molecular changes at a precarcinogenesis stage[5]. Raman spectroscopy can effectively provide chemical variation information about the structure and the composition of biological materials at molecular level[6]. Many researches have been made in the detection of colon cancer using Raman spectroscopy[7–9].

Our study applied LIF-Raman spectroscopy on serum of colon cancer before and after operation and the control group. Spectral differences and parameter changes were recorded and compared among groups. The results show that LIF-Raman spectroscopy can be used as a non-invasive, rapid and convenient analysis for colon cancer diagnosis.

EXPERIMENTAL SECTION

In this paper 22 normal human, 35 colon cancer human, 35 and cancer human after being operated were researched. All sample were obtained from the Tumor Hospital of Liaoning Province, subjects were phlebotomized before breakfast in the morning. The vein blood obtained was separated in segregator at a speed of 3000 rot/min for 10 min. Then upper serum was sucked and made into samples. Samples were kept in refrigerator (temperature -4^{o}C) hermetically for latter investigation but not exceeding three weeks.

The spectral range scanned was from 520 nm to 640 nm and from 500 nm to 620 nm, or from 510 nm to 530 nm and from 540 nm to 560 nm at the resolution of 2 cm. The frequency of chopper was 700 Hz. The laser source for this set-up was an Ar-ion laser operating at 488 nm or 514.5 nm.

Figure 1 shows the main parts of our instrument: an Ar-ion laser (made in 722 factory in Nanjing), a PMT(R928 model), a lock-in amplifier (SRS-830 model), and a double spectrometer (HRD-1 model).

Spectra were collected with a double spectrometer equipped with a PMT. The sample chip was exited directly by the laser beam and the Raman radiation from sample was collected and put into the spectrometer. Then the signal was amplified by a lock-in amplifier. All spectra data were input into computer and saved. A personal computer controls the entire system, saves and output the Raman spectrograph.

For each sample, four spectra were measured (1) The spectrum from 520 nm to 640 nm excited by 514.5 nm, (2) The spectrum from 500 nm to 620 nm excited by 488.0 nm, (3) The spectrum from 510 nm to 530 nm excited by 488.0 nm after being radiated by laser (488.0 nm spectrum), (4) The spectrum from 540 nm to 560 nm excited by 514.5 nm (514.5 nm spectrum). At the same time we measure a colon cancer human's serum, excited by 514.5 nm and 488.0 nm before operation and after operation.

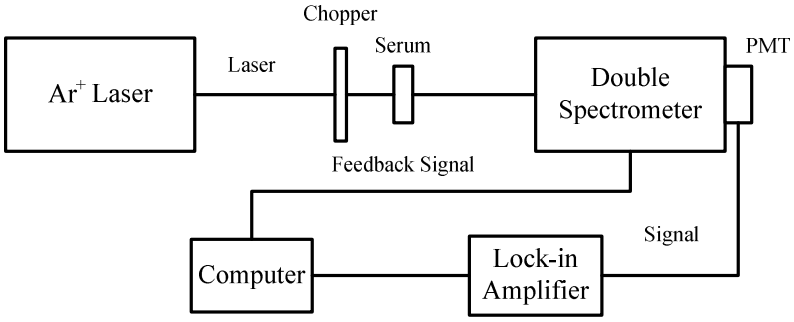

Figure 1. Experimental setup of spectroscopy system

RESULTS AND DISCUSSION

Figure 2(a) shows the Raman spectrum of normal man's serum excited by 514.5 nm. In the spectral range of 520-640 nm, the three Raman peaks present in this region are well distinguished, there is slight peaks A, but peak B and C is clear. The relative intensity of Raman peak C (I_{r5145}) is 10.04%. The fluorescence peak is 547 nm.

Figure **1**(b) shows the Raman spectrum of colon cancer's serum excited by 514.5 nm. Though the spectrum ranges 520-640 nm, there is no clear Raman peaks present observed in this region. The fluorescence peak is 550 nm.

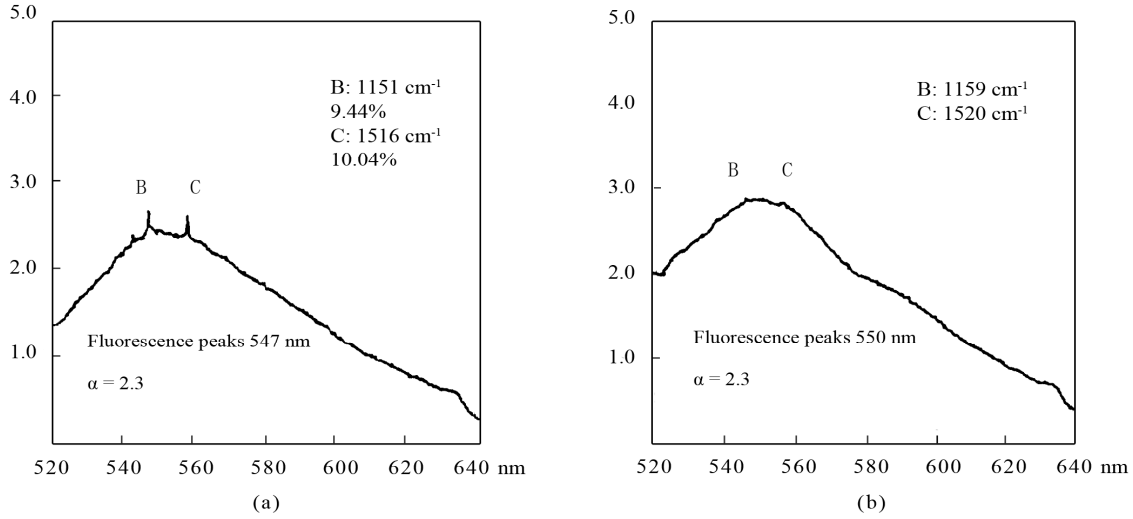

Figure 2. Raman spectrum of serum excited by 514.5 nm

Figure 3(a) shows the Raman spectrum of normal man's serum excited by 488.0 nm. The spectral range is 500-620 nm, and there are Raman peaks present in this region. The parameter I_{r4880} (relative intensity of peak C) is 8.24%. Figure 3(b) shows the Raman spectrum of colon cancer's serum excited by 488.0 nm. The spectra ranges 500-620 nm, there are two slight Raman peaks present in this region. There is no peak A present. The parameter I_{r4880} is 2.45%.

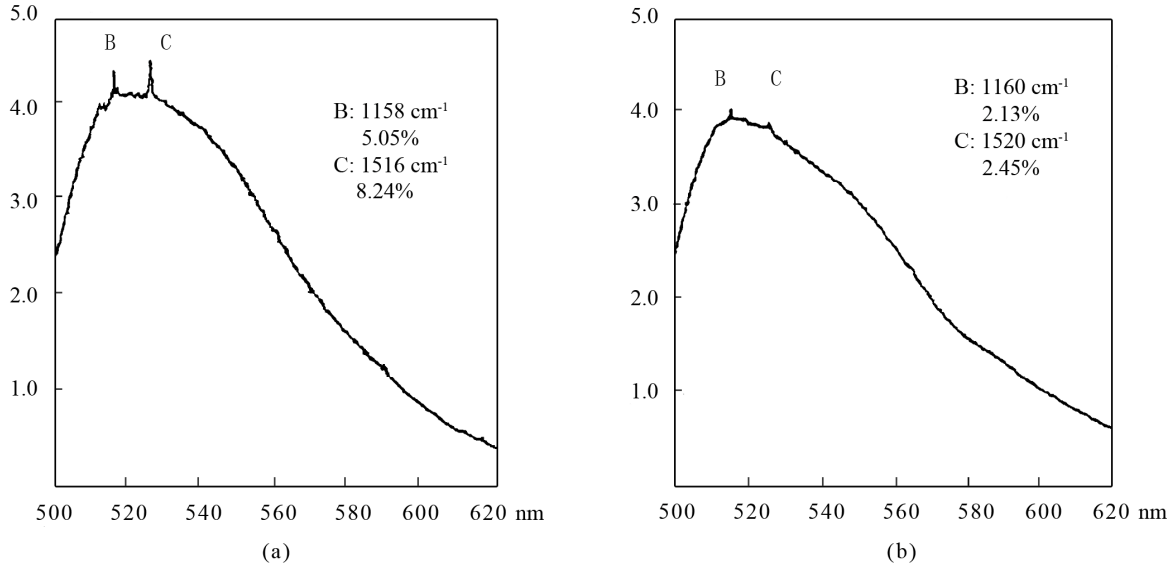

Figure 3. Raman spectrum of serum excited by 488.0 nm

Figure 4(a) shows the Raman spectrum of normal man's serum excited by 488.0 nm after sample being radiated by laser (488.0 nm). The spectra ranges 510-530 nm, there are two clear Raman peaks, Raman peak B at 1144 cm^{-1} and Raman peak C at 1518 cm^{-1}. Figure 4(b) shows the Raman spectrum of colon cancer's serum excited by 488.0 nm after sample being radiated by laser (488.0 nm) too. The spectral range is 510-530 nm. There are two slight Raman peaks: Raman peak B at 1157 cm^{-1}, Raman peak C at 1518 cm^{-1}.

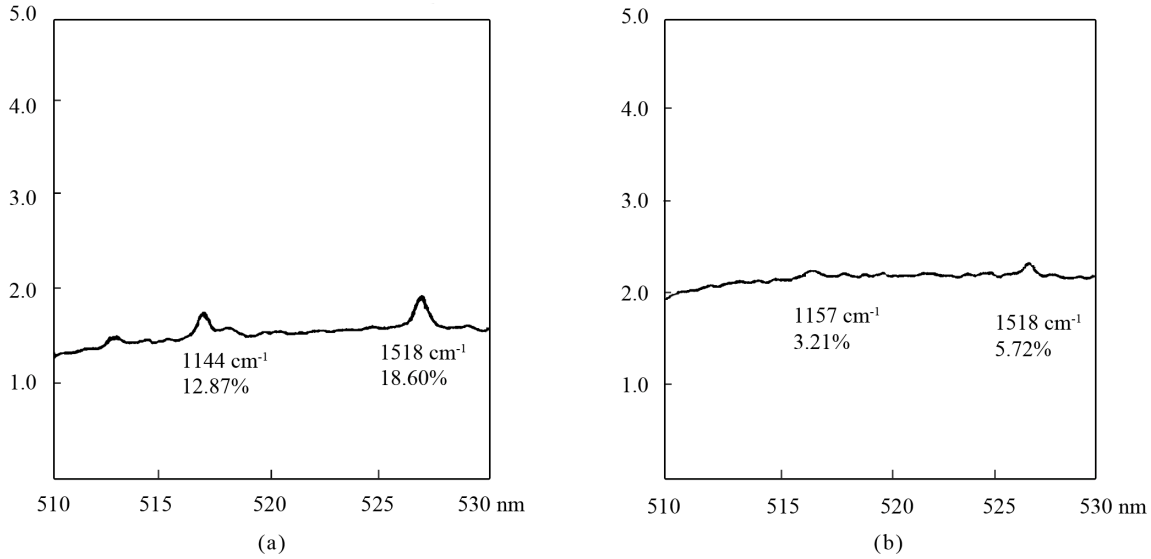

Figure 4. Raman spectrum of serum excited by 488.0 nm after sample being radiated by laser (488.0 nm)

Figure 5(a) shows the Raman spectrum of normal man's serum excited by 514.5 nm after sample being radiated by laser (488.0 nm). The spectral range is 540-560 nm. There are three huge clear Raman peaks: Raman peak A at 1000 cm^{-1}, Raman peak B at 1144 cm^{-1}, and Raman peak C at 1518 cm^{-1}. Figure 5(b) shows the Raman spectrum of colon cancer's serum excited by 514.5 nm after sample being radiated by laser (488.0 nm). The spectral range is 540-560 nm too, there are two slight Raman peaks - Raman peak B at 1144 cm^{-1}, Raman peak C at 1518 cm^{-1}, and no Raman peak A. There are big differences from Figure 5(a) and Figure 5(b).

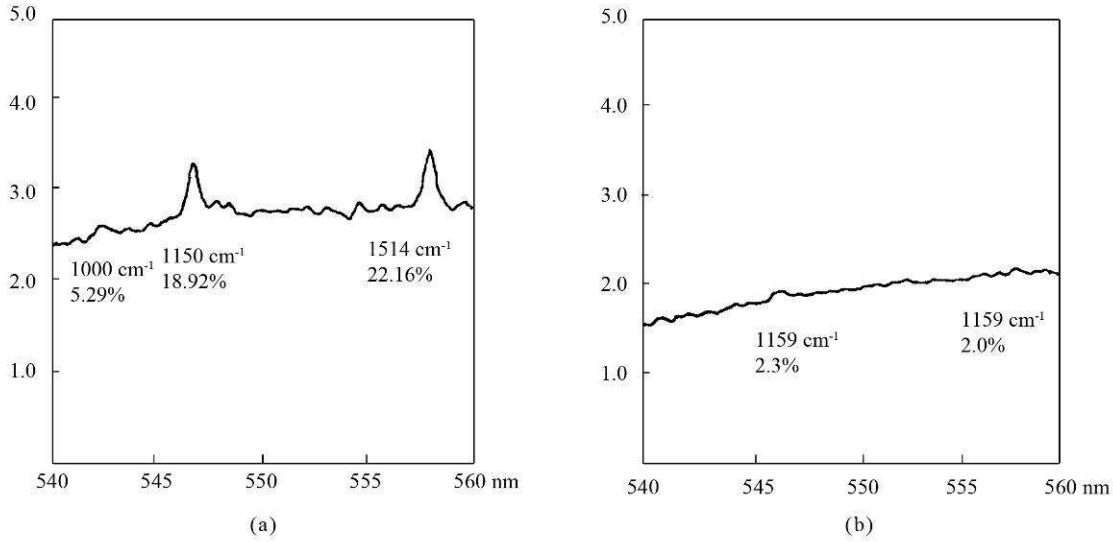

Figure 5. Raman spectrum of serum excited by 514.5 nm after sample being radiated by laser (488.0 nm)

For quantitative evaluation of the spectra, three parameters were designed. The three parameters are Δλ, α and β. Among them, Δλ is the red shift of fluorescence peak. α is the ratio between the fluorescence peak intensity at 520nm and the intensity at 634 nm – I_{520}/I_{634}. β is the radio between the relative intensity of Raman peak C excited by 514.5nm and the one excited by 488.0 nm – $I_{514.5}/I_{488.0}$. Three parameters of the three groups (normal, colon cancer and colon cancer after operation) were calculated and the results were shown in Table 1. From the table we can see that, all the three parameters have statistically significant difference between groups (*p* value<0.001). Figure 6 is the three parameters of all the samples from three groups. From the figure we can see that, parameters belonging to different groups have apparent different values. The results show that the three parameters are useful in the prediction of groups.

Table 1. Three parameters and the SD value of three groups

Parameters	Value (mean ± SD)			*p* value
	Normal	Colon cancer	Colon cancer after operation	
Δλ	10.136(1.358)	13.029(2.443)	10.143(1.149)	<0.001
α	0.923(0.085)	0.731(0.095)	0.717(0.038)	<0.001
β	1.032(0.076)	0.837(0.083)	1.060(0.068)	<0.001

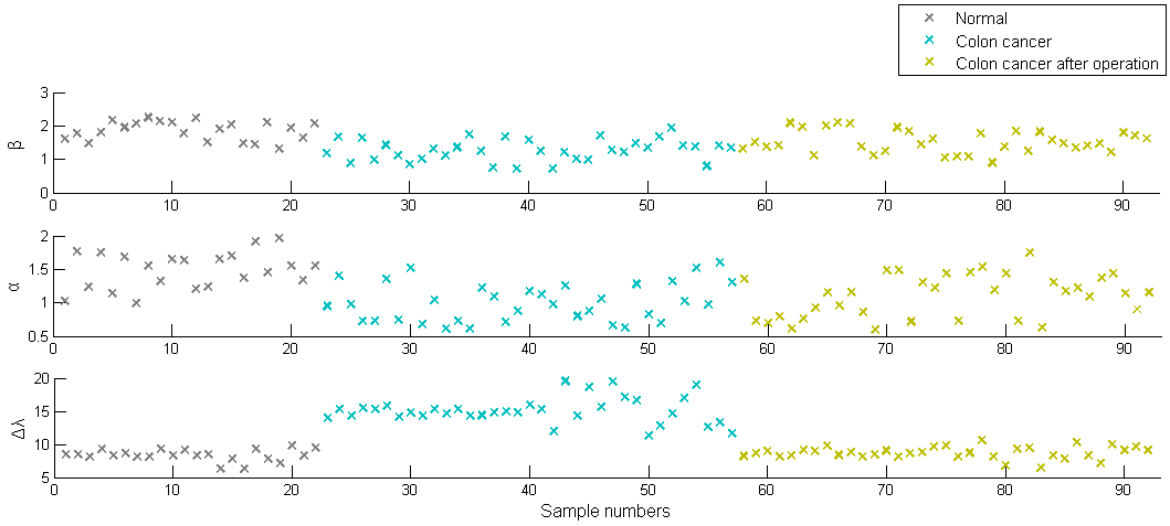

Figure 6 Distribution of the three parameters of all the samples

LIF is a valid way to detect the normal tissue cell and extraordinary chemical structure of the physical character of tissue cell. At the same time, using the effect of laser on the molecule can help us obtain the structure and energy

change dynamics of molecule. The spectral theory of laser is an important way and technique to the analysis of the content and structure of bio material. The Raman spectroscopy is a valid tool to the detection of the changes of biology tissue too. Furthermore, pathology theory and molecule biology theory research indicated that there are much difference between the normal tissue cell and cancerous cell of its chemical structure and physical character. The cancerous cell states of metabolism and chemical structure led to the change of cell environment. Furthermore, all these occurred before the cell were changed. It's the theory base of cancer diagnosis by LIF. In the process of cancer, products of metabolism and gene of cancerous cells went into blood. Then they led to the change of fluorescence material and ingredient in serum. Therefore normal and cancer's LIF and Raman spectra must be different. The former part what we state is the basis of that LIF can diagnosis cancer, so the photophysical of native fluorescence of tissue cell and their structure can be considered as a useful parameter. So in colon cancer and other cancers some chemical compositions in serum may change dramatically as disease progress and provide important clues for the diagnosis. Therefore it is essential to find such changes and determine the connection between them and cancer evolution for diagnosis of cancer using LIF.

Results of some studies indicate that porphyrin concentration occurs in cancerous serum. Fluorescence ranging between 600 nm and 640 nm may be derived from the transition of electron in porphyrin in heme protein[10]. And riboflavin contributes to the fluorescence ranging between 510 nm and 530 nm. Raman peak B was assigned to amino acids tryptophan and phenylalanine, and peak C was assigned to beta carotenen[11]. The decrease of these Raman peaks was caused by the decrease of the corresponding biomolecules with the deterioration of colon cancer.

CONCLUSION

The content change of chemical components in serum is a major factor in serum spectral changes. Through all we state, we can make a conclusion that the content of riboflavin, porphyrin and other fluorescence materials in serum is different between colon cancer and normal, which induced Raman spectrum intensity and fluorescence is different obviously. Through three parameters $\Delta\lambda$, α, and β, we can diagnosis the colon cancer accurately by Raman spectrum and LIF. It's useful in diagnosis of cancer.

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