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Research Article

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Detection and evaluation of esophagus cancer by serum based on LIF-Raman spectroscopy

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ABSTRACT

Laser-induced fluorescence (LIF) and Raman spectroscopy were used to measure serum of esophagus cancer patients and healthy people. Eighteen esophagus cancer patients and 21 healthy volunteers participated in this research. Raman peak intensities between different groups were detected and compared. Three parameters were selected as the standard for comparison. This preliminary results show that Raman spectroscopy has the potential to target esophagus cancer.

Keywords: Raman spectroscopy, fluorescence, esophagus cancer

INTRODUCTION

The incidence of esophagus cancer is estimated 18 thousand in USA. Five-year survival rate for esophagus cancer is less than 20%[1]. Radical treatment for early esophagus cancer is associated with long term survival. Conventional detection method such as endoscopy is time consuming and has a low detection rate because of inflammation and high inter-observer variability[2]. New approaches are in urgent need.

Fluorescence spectroscopy has been used to distinguish cancerous tissues from normal in many organs such as lung, breast, and cervical[3–5]. Raman spectroscopy is a vibrational spectroscopic technique which has received considerable attention regarding applications in cancer screening. Raman spectroscopy is non-invasive, rapid and convenient, and is ideal for population screening[6]. A number of studies have been conducted on the potential of Raman spectroscopy for cancer diagnosis[7–9].

In this paper, LIF and Raman spectroscopy were used in tandem on the analysis of serum from esophagus cancer patients before and after operation and healthy people. Spectral results show that Raman spectroscopy of esophagus cancer can be differentiated from those of esophagus cancer after operation and healthy controls. Three parameters of LIF-Raman spectroscopy were selected among groups to see the changes with the development of esophagus cancer.

EXPERIMENTAL SECTION

It may become more difficult to extract valuable information because some chemical components would violently influent the Raman spectra, so we must process the samples beforehand. Twenty-one healthy controls, 18 esophagus cancer and 18 esophagus cancer after operation participated in this experiment. To minimize the interference, all the samples were extracted before breakfast. 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. While making spectroscopic experiments, specimen about 2ml was injected into a clean quartz cuvette with a one-off sucker.

Spectra were collected with a double spectrometer (it can be precisely controlled by computer) equipped with a PMT. After amplified by a lock-in amplifier, spectral data were input into computer and transacted. The spectral range scanned was from 520 nm to 640 nm or 500 nm to 620 nm at a spectral resolution of $2cm^{-1}$. And the frequency of chopper was 700Hz. Fig 1 shows the main parts of our instrument: an Ar-ion laser (made in 772 factory in Nanjing), a PMT (R456 model), a lock-in amplifier (391A model), and a double spectrometer (HRD-1 model). The wavelengths of 488.0 nm and 514.5 nm were chosen for excitation.

We collected the samples once a week, and divided them into normal, cancer and the ones after operation. All the samples were scanned, so two 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. What we recorded was relative intensity of Raman peaks in order to reduce such interference as the undulation of laser power. And for the purpose of lessening influence of other harmful factors, we sampled several data at each wavelength. Then average values were recorded. And in the process of original data transaction, method of least squares was used to smooth spectra.

Described spectrometer collected the needed spectra and transformed them, so we could observe a spectral band from the fluorescence spectrum in the PMT system. In the band, region of fluorescence spectrum was wider than Raman spectrum. The noise mainly composed by system shot noise. After frequency calibration and spectral correlation in spectrometer system detection, we could get a relative intensive-wavelength graph.

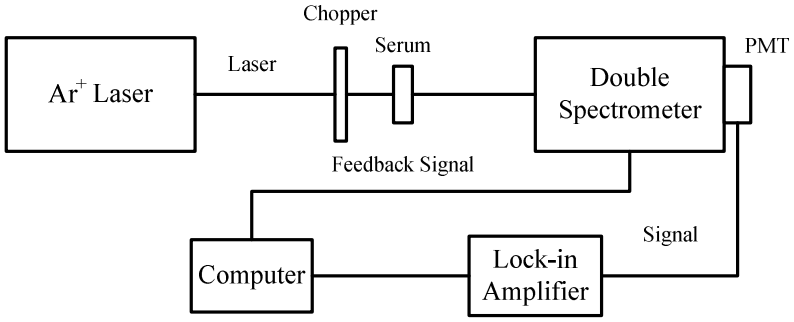

Figure 1. Experimental setup of spectroscopy system

RESULTS AND DISCUSSION

Among the got data, Figure 2 is a typical normal body's serum's autofluorescence-resonance Raman spectrum. Figure 3 is a typical esophagus cancer serum's autofluorescence-resonance Raman spectrum (before operation). **Error! Reference source not found.** is got from the same patients' spectrum after the operation (among all patients, he recovered the best). In each Figure, (a) is excited by the laser of 514.5 nm wavelength, and (b) are excited by 488.0 nm wavelength.

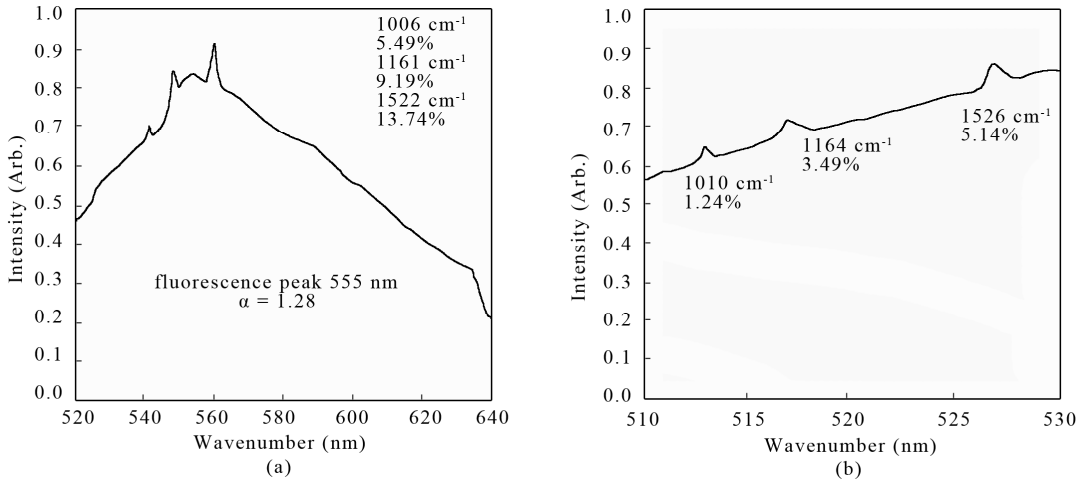

Figure 2. Typical LIF-Raman spectra of normal people: (a), excited by 514.5 nm; (b), excited by 488.0 nm

In Figure 2, three Raman peaks (mode A, B, and C, mode C is the strongest one) can be observed. With the effect of time, the position of fluorescence peak (excited by 5l4.5 nm) had a little shift in the direction of long wave. In the same time, the intensity of fluorescence peak weakened. To the end, mode C shifted to the left of fluorescence peak. No fluorescence peak appeared in the spectrum of esophagus cancer excited by 488.0 nm (Figure 3). The shape of fluorescence spectrum transformed into ascending line after affected. For spectra of esophagus cancer, all the fluorescence spectra are smoother than normal and the Raman peaks vanished. α-value decreases about 0.4 (α is the ratio of scanning start value and inflection point at about 634 nm of fluorescence spectra). At the same time, fluorescence spectrum's starting point fell down and became lower than end point (excited by 514.5 nm).

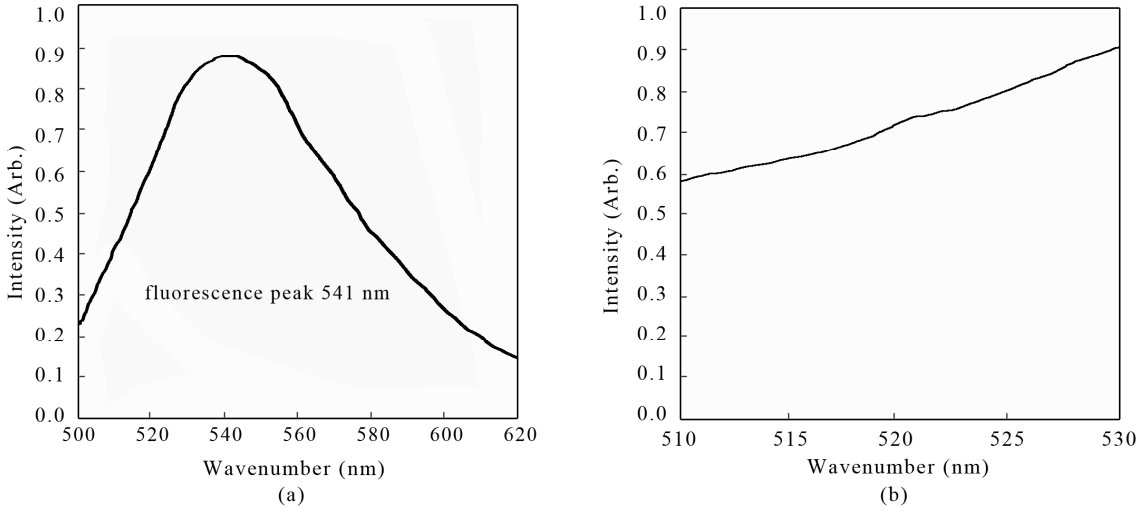

Figure 3. Typical LIF-Raman spectra of esophagus cancer: (a), excited by 514.5 nm; (b), excited by 488.0 nm

After operation, three Raman peaks recovered in different degrees. Fluorescence spectrum keeps the same trend and we know that the two end points return to the state that the front is high, the behind is low. And for α, it is impossible for us to draw a certain conclusion because it varied irregularly. But we could point out that it increased. After excited by laser, the intensity of fluorescence decayed vividly and the fluorescence peak had different red shifts compared to those before excited. We consider serum's fluorescence intensity weakening is because serum was affected by photochemical reactions like photolysis under the inducing by laser. In our former research, if $\Delta\lambda \leq 12$nm ($\Delta\lambda$ is the red shift absolute value of fluorescence peak), we could consider the serum belongs to the healthy. α-value is also an important parameter. All the normal serum $\alpha > 0.8$ (after excited). From spectra of esophagus cancer, its $\Delta\lambda$ and α do not agree with the value of normal people. From table 1, we could know the patient is still ill although his serum α-value has already been partly similar to the normal.

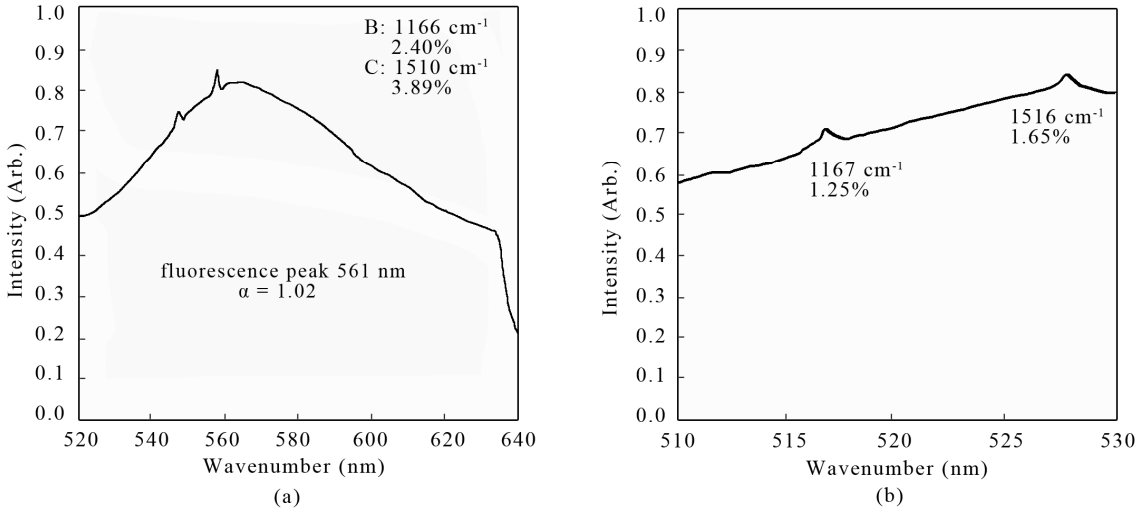

Figure 4 Typical LIF-Raman spectra of esophagus cancer after operation: (a), excited by 514.5 nm; (b), excited by 488.0 nm

From the β-value (β equals to Raman peak absolute intensity/fluorescence peak absolute intensity of the same position), we also could get abundant information. Comparing the graphs in the same figure, β (514.5 nm) is greater than β (488.0 nm) in serum Raman-fluorescence spectrum (C peak after excited) if a person is healthy. However the patient's β state is on the contrary.

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). **Error! Reference source not found.** 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	Esophagus cancer	Esophagus cancer after operation	
Δλ	9.857(1.245)	13.056(1.649)	10.222(0.533)	<0.001
α	0.962(0.084)	0.711(0.065)	0.722(0.053)	<0.001
β	1.052(0.073)	0.867(0.082)	1.067(0.058)	<0.001

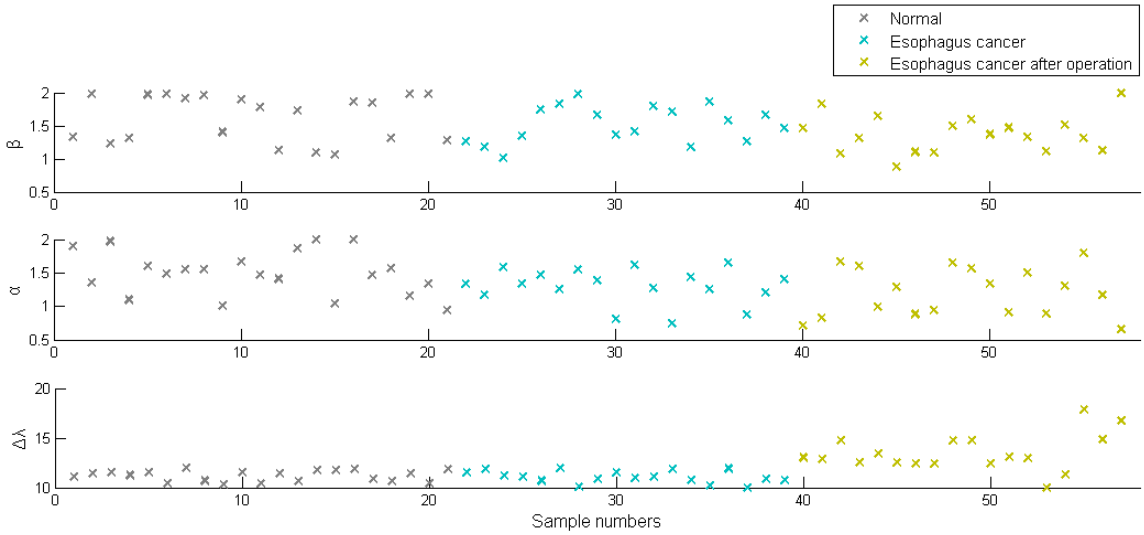

Figure 5. Values of the three parameters of the three groups

Integrating the research result of biological tissue's autofluoresence, we regards the fluorescence partly comes from the serum's lactochrome (Vitamin B_2) at wavenumber of 510-530 nm[10]. And the fluorescence in 600-640 nm (excited by 514.5 nm) comes from resonance π-electron transition of haemoglobin's porphyryl[11]. From the variation of Raman peak like intensity and position, we assumed that they were derived from the progression of esophagus cancers.

In epidemiology, studies showed that the incidence of cancer is closely relevant to the Raman peak intensity. The stronger, more distinct the Raman peak, the less incidence of cancer. By comparing the chemical component predicted by the spectra, we found the Raman peak is very similar to β-carotene's[12]. Norman E. Marcon found that as normal tissue becomes dysplastic, its fluorescent signal decreases. Changes are wavelength dependent, and those differences in these spectral wavelengths correlate with changes in histology. Research found that low level of β-carotene caused by low intake of vitamin C in serum contribute the induction of gastric cancer[13]. Our result indicated that the height of Raman peak decreased with the aggravation of esophagus cancer. It means that the intensity of Raman spectra has close relation with cancer. The reason perhaps is the component of β-carotene. Such conclusion agrees well with former studies in chromatogram and epidemiology.

CONCLUSION

The variation of Raman peak in serum is a major factor for the detection of esophagus cancer. Following the aggravation of esophagus cancer, we found that Raman peak would be weaker or even disappear. We have demonstrated parameters β together with α and Δλ be used for diagnosing esophagus cancer. Due to the endogenous

fluorescence background presented in blood plasma, which is of the order of a million times more intense, the relatively weak Raman signals are difficult to extract. Therefore, future technique will resolve it.

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