Detection and characterization of esophagus dysplasia using fluorescence and Raman spectroscopy

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In this paper, we present a three-parametric model using Raman and fluorescence spectroscopy for detection and analysis of esophagus dysphasia and esophagus cancer. The model was set up from more than 1000 samples. And 56 samples were used to test this algorithm of the model prospectively. The serum spectra were excited by laser of the wavelengths 488.0 and 514.5 nm. The apparent differences of auto-fluorescence and Raman spectroscopy were observed for patients compared to the normal: the majority of the fluorescence spectra did not have violent alteration, but three Raman peaks disappeared or were found to be very weak. Moreover, $\Delta\lambda$ value (red shift of fluorescence peak) and α-value (rate of fluorescence intensity) also provide the reference for future research. And I-value (intensity of Raman peak) will decrease with progression of the tumor. The result of spectral analysis is accordance with the clinical diagnosis.

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In recent years, as medimaging and Fourier's integral optical assay technology advanced, spectrum analysis has become a powerful tool to diagnosis patient's status[1]. The laser-induce fluorescence (LIF) and Raman applied to detection of cancerous have made a lot of fruits[2−4].

In this paper, the spectral characteristic of esophagus cancer and normal has been investigated using three parametric modes of LIF and Raman spectroscopy. We attempted to find out some valuable clues by the change of fluorescence shape, position of fluorescence, and position of Raman peak. It may become more difficult to extract valuable information because some chemical components would violently influence the Raman spectra, so the samples were processed beforehand. 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 minutes. Then upper serum was sucked and made into samples. Samples were kept in refrigerator (temperature −4 °C) hermetically for latter investigation but not exceeding one week. While making spectroscopic experiments, specimen about 2 mL was injected into a clean quartz cuvette with a on-off sucker.

Spectra were collected with a double monochromator (it can be precisely controlled by computer) equipped with a photomultiplier tube (PMT). After amplified by a lock-in amplifier, spectral data were input into computer and transacted. The spectral range scanned was from 520 to 640 nm or from 500 to 620 nm at a spectral resolution of 2 cm^{-1}. And the frequency of chopper was 700 Hz. Figure 1 shows the main parts of our instrument: Ar-ion laser (made in 772 Factory in Nanjing), PMT (R928), and lock-in amplifier (SRS-830). The wavelengths of 488.0 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 to 640 nm excited by 514.5 nm; 2) the spectrum from 510 to 530 nm excited by 488.0 nm.

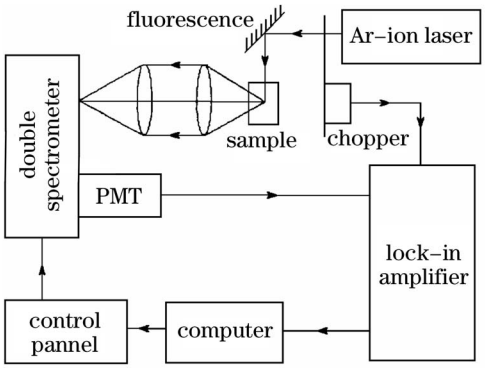

Fig. 1. Experimental instrument.

What we recorded was relative intensity (absolute intensity divided by maximum intensity) 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 recorded the average value. And in the process of original data transaction, the method of least squares was used to smooth spectra. After frequency calibration and spectral correlation in monochromator system detection, we could get a relative intensive-wavelength graph. Among the obtained data, Fig. 2 is a typical normal serum's autofluorescence- Raman spectrum, and Fig. 3 is a typical esophagus cancer serum's autofluorescence-Raman spectrum (before operation). In every figure (a) and (b) are excited by the laser of 514.5-nm wavelength, and (c) are excited by 488.0-nm wavelength.

In Fig. 2, three Raman peaks (mode A, B, and C, mode C is the strongest one) can be observed. With the time of effect, the position of fluorescence peak (excited by 514.5 nm) had a little shift in the direction of long wave. The shape of fluorescence spectrum transformed into ascending curve after excited. In Fig. 3, all the fluorescence spectra are smoother than Fig. 2 and the Raman peaks vanished. The parameter

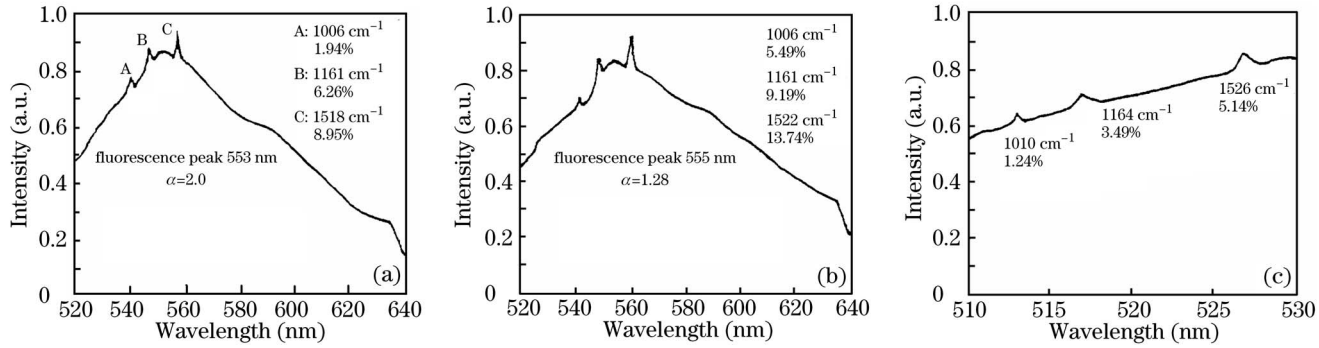

Fig. 2. Typical normal serum auto-fluorescence-resonance spectrum. (a) Excited by 514.5 nm before effect; (b) excited by 514.5 nm after the effect; (c) excited by 488.0 nm after the effect.

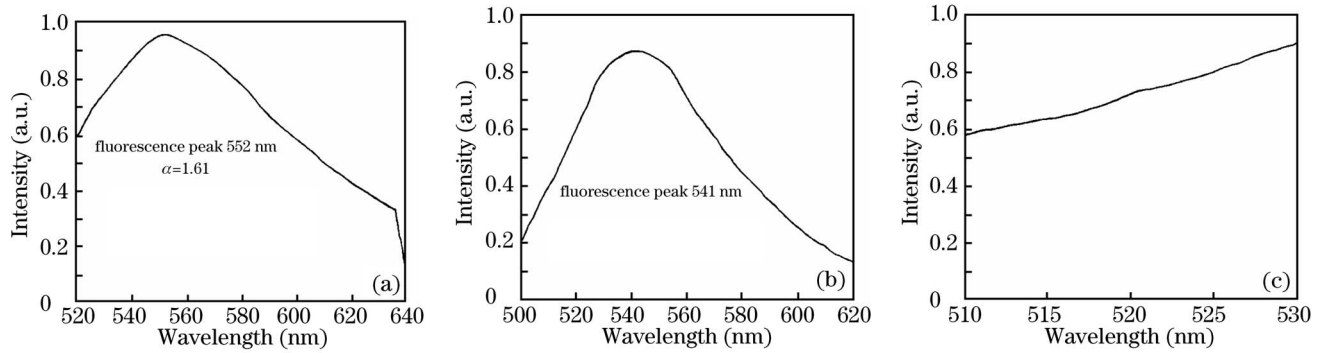

Fig. 3. Typical esophagus cancer serum auto-fluorescence-resonance Raman spectrum. (a) Excited by 514.5 nm before effect; (b) excited by 514.5 nm after the effect; (c) excited by 488.0 nm after the effect.

α ($\alpha = I_{520}/I_{634}$) is the ratio between relative intensity at 520 and 634 nm in spectrum. The parameter β ($\beta = I_{514.5}/I_{488}$) is the ratio between the relative intensities of Raman peak at 1525 cm^{-1} (C peak) using 514.5 and 488.0 nm excitation. Relative intensity of Raman peak is the ratio between Raman intensity and fluorescence intensity at the same wavelength. The parameter $\Delta\lambda$is the red shift for position of fluorescence peak. And for α, it is difficult for us to draw a precise conclusion because it varied irregularly. We consider serum's fluorescence intensity weakening is because serum was affected by photochemical reactions like photolysis under the inducing by laser[5]. In our former research, if $\beta > 1$, $\Delta\lambda \leq 12$ nm, $\alpha > 0.8$ (after excited) we could consider the serum belongs to healthy. From Fig. 3, its $\beta < 1$, $\Delta\lambda > 12$ nm do not agree with the requirement. After excited by laser, the intensity of fluorescence decayed vividly and fluorescence peak had different red shifts opposite to those before excited. Results of some studies indicate that porphyrin concentration occurs in cancerous serum[1,6] fluorescence ranging between 600 and 640 nm (excited by 514.5 nm) comes from resonance π-electron transition of haemoglobin's porphyryl[7,8]. And riboflavin contributes to the fluorescence ranging between 510 and 530 nm[9].

We assumed that three Raman peaks were derived β-carotene[10]. It indicates that the content of β-carotene in normal serum is higher than in cancerous serum. This conclusion agrees well with the result obtained by means of chromatogram[11]. By adopting NARP-HPLC method, the density of beta carotene of the gastroenteric cancer and normal persons in serum is detected. Data showed. that the density of β-carotene in gastroenteric cancer patient's serum (0.3–0.9 μmol/L), the average value is 0.482 μmol/L, was obviously lower than normal one (1.5–3.2 μmol/L). The results indicated that the intensity of Raman peak decreased with aggravation of esophagus cancer. It means that the intensity of Raman peaks 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[12].

Spectral diagnosis for esophagus cancer using β, α, $\Delta\lambda$ three-parametric models, variation of Raman peak in serum is a major factor that we grab the key. Following the aggravation of esophagus cancer, we found that Raman peak would be weaker or disappear. It is the most valuable parameter. Based on the supposition that β is an important role of parameter, α and $\Delta\lambda$ are serve as assistance.

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