Spectral analysis of abnormal liver tissue using fluorescence and Raman spectroscopy

Xiaozhou Li (李晓舟)[1,2], Junxiu Lin (林均岫)[1], Jianhua Ding (丁建华)[1], Deli Wang (王德力)[2], Yue Wang (王 玥)[2], and Xinyu Li (李新雨)[2]

[1] *Institute of applied Physics, Dalian University of Technology, Dalian 116024*

[2] *Physics Department, Shenyang Ligong University, Shenyang 110168*

In this paper, laser induced human serum Raman spectra of liver cancer are measured. The differences in serum spectra between normal people and liver cancer patients are analyzed. For the typical spectrum of normal serum, there are three sharp Raman peaks and relative intensity of Raman peaks excited by 514.5 nm is higher than that excited by 488.0 nm. However, for the Raman spectrum of liver cancer serum there are no peaks or very weak Raman peaks at the same positions. To liver cirrhosis, the shape of Raman peak is similar to normal and fluorescence spectrum is similar to that of liver cancer from statistic data. Moreover, the liver fibrosis and liver cirrhosis were studied using the technology of laser-induced fluorescence (LIF). The experiment indicates that the blue shift of fluorescence peak differences between the normal, liver fibrosis and liver cirrhosis were observed. These results have important reference values to explore the method of laser spectrum diagnosis.

OCIS codes: 170.0170, 300.0300, 000.1430.

The photophysical properties of native fluorophores of cells, tissues, and their structures have been considered as a useful parameter to study alterations in the functional, morphological, and microenviromental changes[1−3]. And the auto-fluorescence and Raman spectrum has been researched for the purpose of differentiating cancer from normal cases[4−6].

The method of laser-induced fluorescence (LIF) spectroscopy has been investigated for cancer diagnosis. Serum diagnosis is a method to detect liver fibrosis which is applied in wide studying. In chemical compositions in serum may change dramatically as disease progresses. And biochemical changes associating with disease provide important clues for diagnosis. Therefore it is essential to find such changes and the connection between them with the cancer evolution using LIF.

All samples were obtained from Tumor Hospital of Liaoning Province. Subjects were phlebotomized before breakfast in morning. The vein blood obtained was separated in into segregator at a speed of 3000 rot/min for 10 minutes. Then upper serum was sucked and made samples. Samples were kept in refrigerator (temperature 4 °C) hermetically for latter investigation but not exceeding three weeks.

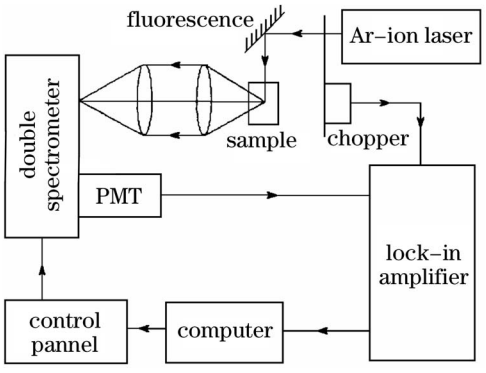

Fig. 1. Instrument.

The spectral were collected with a double monochromator equipped with a photomultiplier tube (PMT). After amplified by a lock-in amplifier, spectral data was input into computer and transacted. The scanned spectral range 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. Figuer 1 shows the main parts of our instrument: Ar-ion laser, lock-in amplifier, PMT, and double spectrometer. The wavelengths of 488.0 and 514.5 nm were chosen for excitation.

The typical auto-fluorescence and Raman spectrum obtained from the normal serum was displayed in Fig. 2.

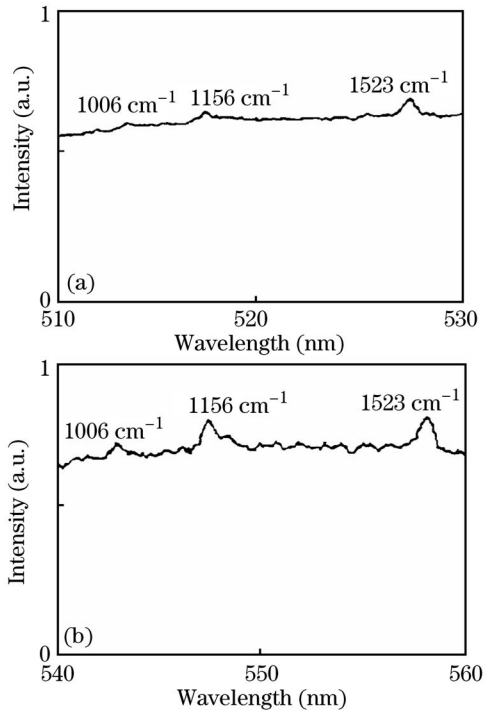

Fig. 2. Typical spectrum of serum for normal case.

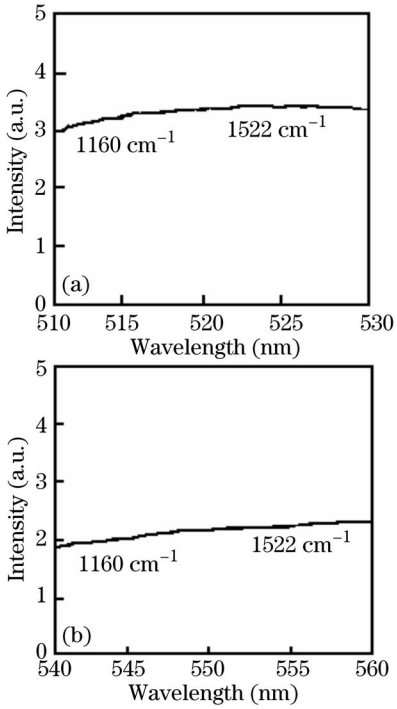

Fig. 3. Typical serum spectrum of liver cancer.

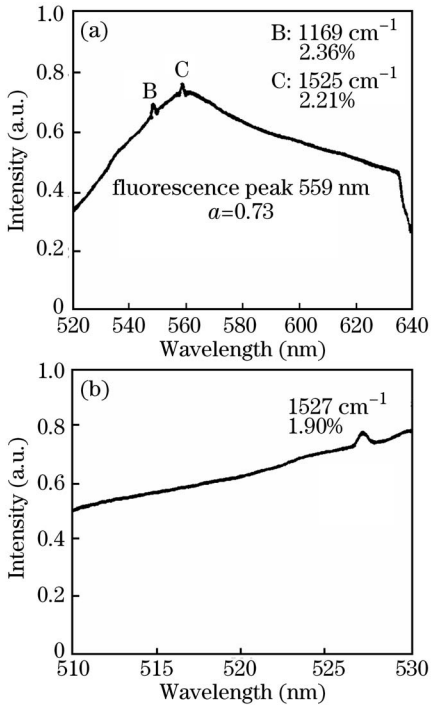

Fig. 4. Fluorescence spectrum (liver cirrhosis). (a) Excited by 514.5 nm; (b) excited by 488.0 nm.

The fluorescence peak is at about 553 nm. There are three resonance Raman peaks in the fluorescence spectral band (the peak by 1523 cm^{-1} is the strongest, and the one by 1006 cm^{-1} is the weakest). β-value (the relative intensity, equaling to the ratio of Raman peak intensity and fluorescence intensity in the same position) of the three Raman peaks are respectively 1.94%, 8.28% and 8.96%.

Figuer 3 shows the spectrum from liver cancer serum. 8 of 9 serum specimens' fluorescence peaks are very plain so we could not discover it. And the most spectroscopes from liver cancer serums have no Raman shift and very weak resonance Raman signal. The main fluorescence peak excited by 514.5 nm is from 549 to 555 nm. The normal serum spectra have three strong resonance Raman peaks, which are averagely shifted by 1010 , 1160, and 1523 cm^{-1} respectively, while the fluorescence spectra from cancer serum contour no Raman peak or weaker than the spectra from normal serum.

From Fig. 4, differences appear immediately. The trend of fluorescence is similar to liver cancer, for example, the shape of fluorescence spectroscopy described by α-value (the ratio of scanning start point and end point). But its β-value is similar to the normal.

In addition, we compare liver fibrosis and liver cirrhosis at the same time (Fig. 5). To liver cirrhosis, its blue shift is more violent, average bigger than 7.5 nm compared with liver fibrosis based on normal position. Under the same radiation case, the trend between them is similar, such as fluorescence spectrum shape, tubercle in 630–640 nm, the pathological tissues are sure to exist native connection.

The liver fibrosis is studied by applying the technology of LIF. The fluorescence spectrum of liver tissue is shown in Fig. 5. The average fluorescence peak wavelength, for normal liver tissue is 571.1 nm in the range of 570.0–573.0 nm, for liver fibrosis is 564.0 nm in the range of 558.7–570.8 nm, and for liver sclerosis tissue is 556.1 nm in the range of 550.3–559.0 nm. From the cancer research, the most cancer serum's fluorescence intensity will undulate violently. Liver pathological tissues are like this. So considering liver fibrosis and liver cirrhosis are the considerable omens of liver cancer. But the liver pathological tissues' fluorescence peak blue shift is very rare, and liver cirrhosis exceeds normal liver very much (average = 15 nm), so blue shift perhaps is the key to get more development.

The same serum sample was excited by different wavelength, as shown in Fig. 6. It was studied that the fluorescence spectra are partly from fiavin, cytochrome C, and bilirubin, which is the metabolite of porphyrin ring in proteins. For β carotene's fluorescence

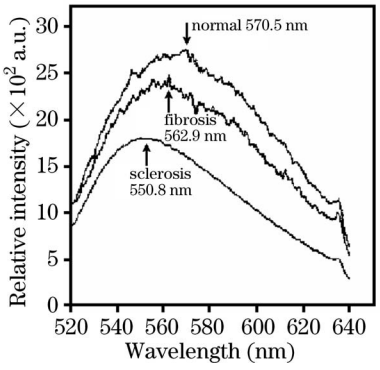

Fig. 5. Fluorescence spectra of liver tissue.

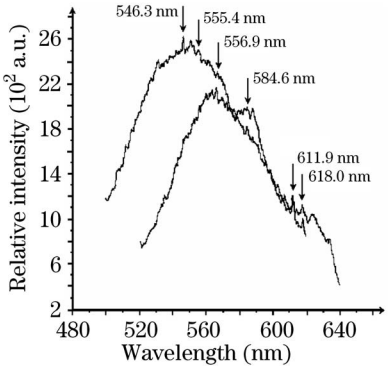

Fig. 6. Fluorescence radiated by different wavelengths.

like the spectrum we got and with the progression of disease, its density fell, we assume β carotene have connection with disease. The higher density is healthful. In human blood, there are a great variety of fluorescence materials. And only a small part of them have been made certain of fluorescence. Serum spectrum is the combination of these fluorescence materials' radiation.

The experiment results showed that for the typical spectrum of normal serum, there are three sharp Raman peaks and, that relative intensity of Raman peak C excited by 514.5 nm is higher than that by 488.0 nm. To the liver cancerous serum, the state is on the opposition or has no Raman peaks or very weak Raman peaks. The results may be useful in cancer diagnosis.

The experiment indicates that there is notable fluorescence difference between the abnormal and normal liver tissue, there is blue shift in abnormal tissue compared with normal liver tissue. These results have important reference value to explore the method of laser spectrum diagnosis[7−9].

X. Li's e-mail address is xzlee@tom.com.

References

1. Q. Luo, H. Gong, X. Liu, and Z. Li, Spectroscopy and Spectra Analysis **17,** (3) (1997).
2. K. Kneipp, A. S. Haka, and M. S. Feld, Appl. Spectrosc. **56,** 150 (2002).
3. E. B. Hanlon, R. Manoharan, T.-W. Koo, K. E. Shafer, J. T. Motz, M. Fitzmaurice, J. R. Kramer, I. Itzkan, R. R. Dasari, and M. S. Feld, Physics in Medicine and Biology **45,** R1 (2000).
4. W. Bai, Journal of World Chinese (8) (2000).
5. V. Backman, M. B. Wallace, L. T. Perelman, J. T. Arendt, R. Gurjar, M. G. Müller, Q. Zhang, G. Zonios, E. Kline, T. McGillican, S. Shapshay, T. Valdez, K. Badizadegan, J. M. Crawford, M. Fitzmaurice, S. Kabani, H. S. Levin, M. Seiler, R. R. Dasari, I. Itzkan, J. van Dam, and M. S. Feld, Nature **406,** 35 (2000).
6. J. K. Dhingra, D. F. Perrault, Jr., K. McMillan, E. E. Rebeiz, S. Kabani, R. Manoharan, I. Itzkan, M. S. Feld, and S. M. Shapshay, Arch. Otolaryngol. Head Neck Surg. **122,** 1181 (1996).
7. H. Yamazaki, S. Kaminaka, E. Kohda, M. Mukai, and H. Hamaguchi, Radiation Medicine **21,** 1 (2003).
8. M.-J. Anita and R. R.-K. Rebecca, J. Biomedical Optics **1,** 31 (1996).
9. R. Manoharan, K. Shafer, L. Perelman, J. Wu, K. Chen, G. Deinum, M. Fitzmaurice, J. Myles, J. Crowe, R. R., Dasari, and M. S. Feld, Photochem. Photobiol. **67,** 15 (1998).