PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(3):327-330
World Journal of Gastroenterology ISSN 1007-9327

In vivo and *in situ* detection of colorectal cancer using Fourier transform infrared spectroscopy

Qing-Bo Li, Zhi Xu, Neng-Wei Zhang, Li Zhang, Fan Wang, Li-Min Yang, Jian-Sheng Wang, Su Zhou, Yuan-Fu Zhang, Xiao-Si Zhou, Jing-Sen Shi, Jin-Guang Wu

Qing-Bo Li, Li Zhang, Fan Wang, Li-Min Yang, Yuan-Fu Zhang, Jin-Guang Wu, The State Key Laboratory of Rare Earth Materials Chemistry and Applications, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
Zhi Xu, Neng-Wei Zhang, Su Zhou, Xiao-Si Zhou, Department of General Surgery, Third Hospital of Peking University, Beijing 100083, China
Jian-Sheng Wang, Jing-Sen Shi, Department of Oncological Surgery, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Supported by the National Natural Science Foundation of China, No. 30371604 and State Key Project of China, No. 2002CCA01900
Correspondence to: Jin-Guang Wu, The State Key Laboratory of Rare Earth Materials Chemistry and Applications, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. wjg@chem.pku.edu.cn
Telephone: +86-10-62757951 **Fax:** +86-10-62751708
Received: 2004-04-09 **Accepted:** 2004-05-31

Abstract

AIM: Real-time and rapid identification of the malignant tissue can be performed during or before surgical operation. Here we aimed to detect *in vivo* and *in situ* colorectal cancer by using Fourier transform infrared (FTIR) spectroscopy and fiber-optic technology.

METHODS: A total of five patients with large intestine cancer were detected *in vivo* and *in situ*. Of them, three cases of colon cancer and one case of cecum cancer were detected intraoperatively and *in vivo* by using a FTIR spectrometer during surgical operation, and one case of rectum cancer was explored non-invasively and *in vivo* before the surgical operation. Normal and malignant colorectal tissues were detected *in vivo* and *in situ* using FTIR spectroscopy on the basis of fundamental studies.

RESULTS: There were significant differences between FTIR spectra of normal and malignant colorectal tissues detected *in vivo* and *in situ*. Experimental results revealed that the spectral characteristics of normal and malignant tissues found *in vivo* and *in situ* were similar to those obtained from *in vitro* measurement in our previous fundamental research.

CONCLUSION: FTIR fiber-optic attenuated total reflectance (ATR) spectroscopy can identify *in situ* and *in vivo* colorectal cancer. FTIR spectroscopic method with fiber optics is a non-invasive, rapid, accurate and *in vivo* cancer detection technique in clinical diagnosis.

Key words: Colorectal cancer; Fourier transform infrared spectroscopy

Li QB, Xu Z, Zhang NW, Zhang L, Wang F, Yang LM, Wang JS, Zhou S, Zhang YF, Zhou XS, Shi JS, Wu JG. *In vivo* and *in situ* detection of colorectal cancer using Fourier transform infrared spectroscopy. *World J Gastroenterol* 2005; 11(3): 327-330
http://www.wjgnet.com/1007-9327/11/327.asp

INTRODUCTION

Cancer is one of the leading causes of death in the world. The mortality rate from malignant neoplasms has increased markedly in the last four decades. The important goal in cancer research is to develop an accurate, quick, convenient, and inexpensive cancer detection method to increase the survival probability.

Fourier transform infrared spectroscopy, as an effective tool for investigating chemical changes at molecular level, has been utilized to detect carcinoma[1-7]. This method has many advantages, for example, it possesses a promising perspective in detecting early cancer, which is very important for the survival of cancer patients. We have investigated the detection of malignant tissues, such as stomach, esophagus, gallbladder, colon, lung, liver, parotid gland carcinomas, *etc*. with FTIR spectroscopy since 1995[8-17]. Our research work consisted of three steps. The first step was to study the differences between malignant and normal tissues, which were stored in liquid nitrogen. The specimens were thawed at room temperature and measured *in vitro* using the FTIR method. In the second step of study, fresh samples obtained during surgical operation were measured by the FTIR method *in vitro* immediately. In these two steps of fundamental research, we demonstrated that the malignant tissues could be distinguished from the normal tissues measured *in vitro* using the FTIR method. The third step was the aim of this study, which was to measure the tumor *in vivo* and *in situ* using fiber optics with the FTIR method. FTIR fiber-optic technique can exhibit perspectives in cancer diagnosis *in vivo* and *in situ* because of its advantages[18].

Colorectal cancer is one of the most frequent cancers in Western countries[19]. It is the fourth leading cause of cancer deaths and tends to increase in China, especially in big cities like Shanghai. Reducing the mortality poses a big challenge for clinicians and researchers. It is of great importance to diagnose colorectal malignant tumors *in vivo* and *in situ* using the FTIR method and fiber optics with an ATR probe. Real-time and rapid identification of the malignant tissues would be performed during surgical operation. It is helpful for the surgeons to reduce the waiting time for the pathological results. Furthermore, it allows accurately and rapidly to determine the proper operative treatment, for example, the rapid determination of cut edges of surgically resected specimens, which is also a goal of surgery in the removal of neoplasms. The technique of FTIR with fiber optics has a promising perspective as a new non-invasive and early detection method of colorectal cancer.

MATERIALS AND METHODS

Patients and materials

Three colon cancer patients and one cecum cancer patient were measured *in vivo* using a FTIR spectrometer during surgical operation, and a rectum cancer patient was measured non-invasively *in vivo* and *in situ* before surgical operation in the Department of General Surgery, Third Hospital of Peking University, China. These five cases, including 4 males and 1 female, aged from 52 to 77 years (mean, 64.2 years), were analyzed in the present study. The consents of the patients were obtained before the experiments.

Spectral measurements

The spectra were measured in the Department of General Surgery, Third Hospital of Peking University by using a mobile WQF-500 FTIR spectrometer made in Beijing Second Optical Instrument Factory with a mid-IR fiber optics and ZnSe ATR probe (Spectra-Tech Corporation). A mercury cadmium telluride (MCT) detector was used and cooled by liquid nitrogen. Scans were performed with 4/cm resolution and 32 scans were co-added to increase the signal-to-noise ratio. Sterilization was strictly made at first.

The spectra of four colon and cecum cancer patients were measured *in vivo* and *in situ* during surgical operation. After strict sterilization, the ATR probe was put on the surface of the detected tissues by the surgeons and one spectrum was recorded for about one minute using a FTIR spectrometer. The spectra of the samples were collected from paired carcinomas and adjacent normal tissues in colorectal cancer patients. The FTIR measurement was non-invasive and harmless to the patients.

One rectum cancer case was measured non-invasively and *in vivo* before surgical operation. After strict sterilization, the fiber optics with ATR probe was inserted into the rectum through anus and put on the cancerous tissue 3 cm away from the anus. It took about one minute to measure the spectrum non-invasively. For the comparative analysis, the spectrum of the fresh tissue sample from the same site obtained during surgery was measured *in vitro* immediately.

Each FTIR analysis result was compared with the corresponding histological result, that is to say, the double blind method was used for FTIR and biopsy measurements.

RESULTS

For the systematic report of these colorectal cancer detection research projects, the detection of malignant colorectal tumor samples *in vitro* was introduced first. In our previous fundamental study[20-24], a large amount of frozen samples stored in liquid nitrogen and fresh tissues during surgical operation immediately were measured *in vitro* by using a FTIR spectrometer. The research results showed that there were obvious differences between the FTIR spectra of malignant and normal tissues measured *in vitro*. Taking the colon tissue specimens measured *in vitro* as examples, the spectra are shown in Figure 1. Through the spectral analysis, the spectral characteristics of malignancy were as follows. The bands in the C-H stretching vibration in the region 2 800-3 000/cm and C = O band near 1 700-1 750/cm became weak and even disappeared. The peak of amide I band shifted to a lower wave number. The intensity of the amides II bands became weak, and the intensity of bands near 1 400/cm was stronger than that of the bands near 1 460/cm. The variations of the FTIR spectra between the normal and malignant colon tissues provided a basis and an opportunity for clinical application.

On the basis of the fundamental research, the FTIR spectra of malignant colon and cecum tissues measured *in vivo* and *in situ* during surgical operation were investigated. All the spectra of the measured colon and cecum cancerous tissues are shown in Figure 2. We could see that the relative intensity of $I_{1\,456}/I_{1\,413}$ became smaller in the spectra of the malignant colon and cecum tissues. In addition, there was a weak amide II band near 1 550/cm, a shift of the amide I band to a lower wavenumber near 1 640/cm in the spectra of the cancerous colon and cecum tissues. The peaks of 2 924/cm, 2 989/cm, assigned to CH_2 and CH_3 vibration bands, and the band near 1 740/cm related to C = O vibrations, became weak and even disappeared in the spectra of the malignant colon and cecum tissues. These spectral features indicated the malignancy of the detected tissues. The spectral characteristics and FTIR analysis results of all these cases are listed in Table 1. After the FTIR measurement, the detected colorectal tissues were resected and histologically verified as colorectal carcinomas. The FTIR analysis results were in agreement with the pathological results. Experimental results revealed that the spectral characteristics of normal and malignant colorectal tissues measured *in vivo* and *in situ* were similar to those obtained from *in vitro* measurement.

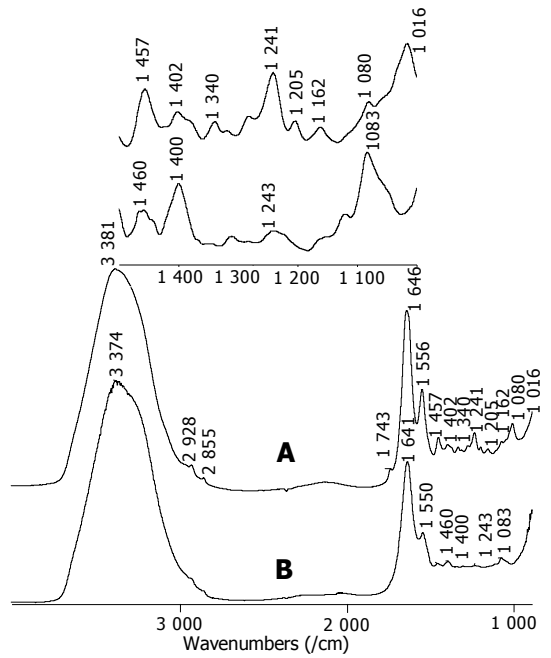

Figure 1 Typical FTIR spectra of colon tissues measured *in vitro*. A: spectrum of normal colon tissue sample; B: spectrum of malignant colon tissue sample.

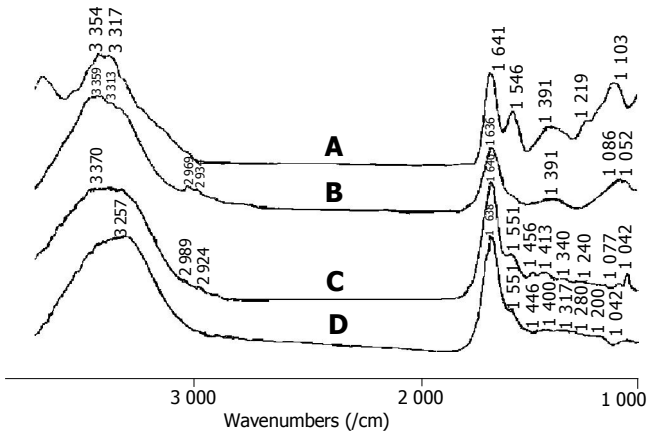

Figure 2 FTIR spectra of malignant cecum and colon tissues measured *in vivo* and *in situ* during surgical operation. A: spectrum of malignant cecum tissue sample; B, C and D: spectra of malignant colon tissue samples.

Table 1 Results of FTIR spectral analysis and corresponding pathological detection of measured colorectal tissues

Case number	Tissue	Sex	Age (yr)	Measurement date	FTIR measurement mode	Characteristics of FTIR spectra	FTIR analysis results	Pathological results
1	Cecum	F	69	2003-07-21	*in vivo* during surgical operation	The bands of C-H stretching vibration and C = O vibration disappeared; peak position of amide I = 1 641/cm; $I_{amide\ II}$ (middle weak); $I_{1\ 393}$/cm>$I_{1\ 460}$/cm	Cecum cancer	Adenocarcinoma of cecum
2	Sigmoid colon	M	63	2003-07-22	*in vivo* during surgical operation	$I_{2\ 969}$/cm (weak) , $I_{2\ 934}$/cm (weak); peak position of amide I = 1 636/cm; $I_{amide\ II}$ (very weak); $I_{1\ 391}$ /cm>$I_{1\ 460}$/cm	Colon cancer	Moderately differentiated adenocarcinoma of sigmoid colon
3	Sigmoid colon	M	52	2003-11-03	*in vivo* during surgical operation	$I_{2\ 989}$/cm (weak) , $I_{2\ 924}$/cm (weak); peak position of amide I = 1 640/cm; $I_{amide\ II}$ (very weak); $I_{1\ 413}$/cm>≈$I_{1\ 456}$/cm	Colon cancer	Carcinoma of sigmoid
4	Transverse colon	M	77	2003-12-05	*in vivo* during surgical operation	The bands of C-H stretching vibration and C = O vibration disappear; peak position of amide I = 1 638/cm; $I_{amide\ II}$ (very weak); $I_{1\ 400}$/cm>$I_{1\ 446}$/cm	Colon cancer	Adenocarcinoma of transverse colon
5	Rectum	M	60	2003-07-22	*in vivo* before surgical operation	$I_{2\ 924}$/cm (weak), $I_{2\ 850}$/cm (weak), $I_{1\ 731\ cm\text{-}1}$ (weak); peak position of amideI = 1 641/cm; $I_{amide\ II}$ (weak); $I_{1\ 402}$ /cm>$I_{1\ 469}$/cm	Rectum cancer	Moderately differentiated adenocarcinoma of rectum

In addition, one rectum cancer patient was measured non-invasively and *in vivo* before surgical operation. The spectrum before surgical operation was similar to that of the fresh tissue from the same site measured *in vitro* immediately (Figure 3). The C = O band near 1 740/cm, nearly disappeared in the spectrum as shown in Figure 3A. The band of amide I of protein was located at a lower wavenumber near 1 640/cm. The intensity of amide II bands located at -1 556/cm in the malignant tissue samples was less intense. The intensity of 1 469/cm band decreased. These spectral characteristics of the FTIR spectrum of malignant rectum tissue measured *in vivo* before surgical operation were consistent with those measured *in vitro*. The result of the FTIR detection for the rectum tumor was consistent with the biopsy test.

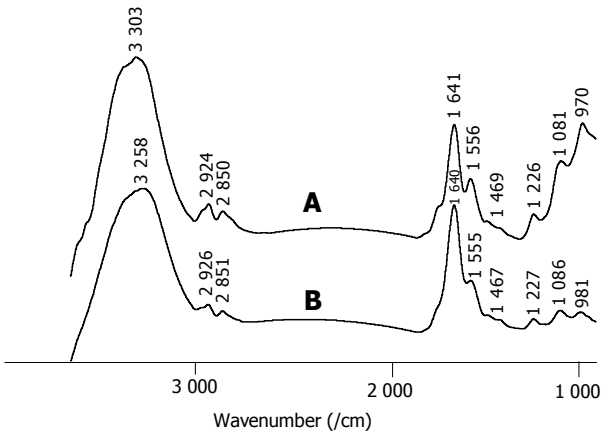

Figure 3 FTIR spectra of malignant rectum tissues. A: spectrum measured non-invasively, *in vivo* before surgical operation; B: spectrum of fresh resected rectum tissue from the same site measured *in vitro*.

DISCUSSION

FTIR spectroscopy can provide information of molecular structure and composition. The development of cancer is always along the following sequences. Gene mutation is the first event, the second step is the alternation of biomolecules in both composition and molecular structure aspects. After that, the variation on cells and morphology of biological tissues will take place and can be detected by iconographic and pathological techniques. Vibrational spectroscopic methods, which are sensitive to the chemical changes at molecular level, may be developed as a powerful method to detect cancer at the second step of its development process, prior to most cancer diagnostic methods available today[25].

FTIR spectra are sensitive to the changes of biomolecules so as to diagnose the cancerous tissues[26]. The spectra of normal tissues often have a stretching vibration of carbonyl located near 1 745/cm, and the symmetry and asymmetry stretching vibrations of methylene located around 2 852/cm and 2 930/cm, as well as methyl at 2 873/cm and 2 958/cm. However, these peaks mentioned above often decrease, even disappear in the spectra of malignant tissues, because triglyceride contains a large proportion of methyl, methylene and carbonyl, and the fat in the region of the malignant tissue is consumed because of the increased nutritional and energy requirement in the development of carcinoma. The bands near 1 645/cm assigned to amide I of protein and deformation vibration of water molecule are located at a higher wavenumber in the normal colorectal tissue than those in the malignant tissue. The peak of amide II bands located at 1 545/cm in the malignant colorectal tissue is less intense and much broader than that in the normal tissue.

In conclusion, FTIR fiber-optic ATR spectroscopy can identify colorectal cancers *in situ* and *in vivo*. It provides real-time results for operating surgeons. FTIR spectroscopy can be applied to *in vivo* and *in situ* detection of not only colorectal cancer but also other malignant tissues of the digestive system. In addition, with further research, the technique of FTIR with fiber optics may exhibit its potential for non-invasive, *in situ* and *in vivo* detection of cancerous tissues before surgical operation. FTIR spectroscopic method with fiber optics

possesses a promising perspective to be a non-invasive, rapid, accurate and *in vivo* cancer detection technique.

REFERENCES

1 **Rigas B**, Morgello S, Goldman IS, Wong PT. Human colorectal cancers display abnormal Fourier-transform infrared spectra. *Proc Natl Acad Sci USA* 1990; **87**: 8140-8144

2 **Koenig F**, Larne R, Enquist H, McGovern FJ, Schomacker KT, Kollias N, Deutsch TF. Spectroscopic measurement of diffuse reflectance for enhanced detection of bladder carcinoma. *Urology* 1998; **51**: 342-345

3 **Rahman J**, Hewlett A, Taylor DR, Xiao SY, Wu JG, Soloway RD. Reproducible, rapid quantitation of hepatic fibrosis in human liver biopsies using Fourier transform infrared (FTIR) spectroscopy. *Gastroenterology* 2002; **122**: A303

4 **Wang HP**, Wang HC, Huang YJ. Microscopic FTIR studies of lung cancer cells in pleural fluid. *Sci Total Environ* 1997; **204**: 283-287

5 **Huleihel M**, Salman A, Erukhimovitch V, Ramesh J, Hammody Z, Mordechai S. Novel spectral method for the study of viral carcinogenesis *in vitro*. *J Biochem Biophys Methods* 2002; **50**: 111-121

6 **Fung Kee Fung M**, Senterman M, Eid P, Faught W, Mikhael NZ, Wong PT. Comparison of Fourier-transform infrared spectroscopic screening of exfoliated cervical cells with standard Papanicolaou screening. *Gynecol Oncol* 1997; **66**: 10-15

7 **Argov S**, Ramesh J, Salman A, Sinelnikov I, Goldstein J, Guterman H, Mordechai S. Diagnostic potential of Fourier-transform infrared microspectroscopy and advanced computational methods in colon cancer patients. *J Biomed Opt* 2002; **7**: 248-254

8 **Wang JS**, Shi JS, Xu YZ, Duan XY, Zhang L, Wang J, Yang LM, Weng SF, Wu JG. FT-IR spectroscopic analysis of normal and cancerous tissues of esophagus. *World J Gastroenterol* 2003; **9**: 1897-1899

9 **Peng Q**, Soloway RD, Wu JG, Zhou XS, Zhao DW, Xu DF, Fu W, Wang SF. Infrared spectroscopic features of colon cancer cells differ from those of normal colonic cells from the same patient. *Gastroenterology* 1996; **110**: A576

10 **Li WH**, Peng Q, Soloway RD, De La Peña O, Sun B, Weng SF, Xu Z, Zhao DW, Zhou XS, Wu JG. Surface proteins of cancer and normal epithelium from the same patients differ as assessed by their primary and second structure determined by Fourier transform infrared (FTIR) spectroscopy. *Gastroenterology* 1997; **112**: A604

11 **Wang JS**, Shi JS, Xu YZ, Duan XY, Zhang L, Zhu AJ, Weng SF, Wu JG. A study on different tissues of gallbladder by Fourier transform infrared spectroscopy. *Zhonghua Gandan Waike Zazhi* 2003; **9**: 657-660

12 **Ren Y**, Xu YZ, Wang J, Zhang YF, Wang F, Shi JS, Wu JG. FTIR spectroscopic and statistical studies on the lung tissues. *Guangpuxue Yu Guangpu Fenxi* 2003; **23**: 681-684

13 **Zhang L**, Wang JS, Yang ZL, Xu YZ, Weng SF, Shi JS, Wu JG. The difference of FTIR spectroscopic studies on normal and malignant tissues of lung. *Gaodeng Xuexiao Huaxue Xuebao* 2003; **24**: 2173- 2176

14 **Ling XF**, Xu YZ, Weng SF, Li WH, Zhi X, Hammaker RM, Fateley WG, Wang F, Zhou XS, Soloway RD, Ferraro JR, Wu JG. Investigation of normal and malignant tissue samples from the human stomach using Fourier transform Raman spectroscopy. *Appl Spectrosc* 2002; **56**: 570-573

15 **Yang LM**, Xu Z, Zhang YF, Xu YZ, Zhou S, Zhang NW, Li QB, Liu Z, Wang LX, Zhou WJ, Xu DF, Song ZF, Weng SF, Wu JG. The application of mid-IR fiber optics in diagnosis of colon cancer *in vivo* and *in situ*. *Guangpuxue Yu Guangpu Fenxi* 2003; **23**: 883-884

16 **Xu YZ**, Zhang YF, Yang LM, Peng X, Sun KH, Li QB, Zhou WJ, Wu HZ, Yu GY, Xu YZ, Liu Z, Weng SF, Wang J, Zhao Y, Xu DF, Wu JG. *In vivo* and rapid detection of the malignancy tumor of oral tissues using Mid-FTIR fiber optics spectroscopy. *Gaodeng Xuexiao Huaxue Xuebao* 2004; **25**: 348-350

17 **Wu JG**, Xu YZ, Sun CW, Soloway RD, Xu DF, Wu QG, Sun KH, Weng SF, Xu GX. Distinguishing malignant from normal oral tissues using FTIR fiber-optic techniques. *Biopolymers* 2001; **62**: 185-192

18 **Sukuta S**, Bruch R. Factor analysis of cancer Fourier transform infrared evanescent wave fiberoptical (FTIR-FEW) spectra. *Lasers Surg Med* 1999; **24**: 382-388

19 **Blum HE.** Colorectal cancer: future population screening for early colorectal cancer. *Eur J Cancer* 1995; **31A**: 1369-1372

20 **Wang F**, Ling XF , Yang ZL , Xu Z, Ren Y, Li WH , Weng SF, Wu JG. FTIR spectroscopic study of normal and malignant tissues of rectum. *Guangpuxue Yu Guangpu Fenxi* 2003; **23**: 498-501

21 **Weng SF**, Lin XF, Yang LM, Soloway RD, Xu YZ, Tian W, Yang ZL, Kang N, Yan WF, Hu XB, Fately WG, Wu JG. Use of mid-infrared fiber optics to determine the extent of spread of gastric and colonic cancer. *Gastroenterology* 2000; **118**: A6436

22 **Xu YZ**, Soloway RD, Lin XF, Zhi X, Weng SF, Wu QG, Shi JS, Sun WX, Zhang TX, Wu JG, Xu DF, Xu GX. Fourier transform infrared (FTIR) mid-IR spectroscopy separates normal and malignant tissue from the colon and stomach. *Gastroenterology* 2000; **118**: A6438

23 **Sun XJ**, Su YL, Soloway RD, Zhang L, Wang JS, Ren Y, Yang LM, Zheng AG, Zhang YF, Xu YZ, Weng SF, Shi JS, Xu DF, Wu JG. Rapid, intraoperative detection of malignancy using attenuated total reflectance (ATR) and mobile Fourier transform infrared (FTIR) spectroscopy. *Gastroenterology* 2003; **124**: A420-A421

24 **Peng Q**, Xu Y, Li W, Wu J, Zhou X. FTIR study on the normal and tumor gastrointestinal tissues. *Guangpuxue Yu Guangpu Fenxi* 1998; **18**: 528-531

25 **Zhang L**, Sun KH, Soloway RD, Ling XF, Xu YZ, Wu QG, Weng SF, Yang ZL, Zhang TL, Yao GQ, Chen HH, Zhou XS, Xu DF, Wu JG. Intraoperative Fourier transform infrared spectroscopy can guide individual resections in patients with gastric cancer. *Gastroenterology* 2004; **126**: A626

26 **Wong PTT**, Lacelle S, Fung Kee Fung M, Senterman M, Mikhael NZ. Characterization of exfoliated cells and tissues from human endocervix and ectocervix by FTIR and ATR/FTIR spectroscopy. *Biospectroscopy* 1995; **1**: 357-364

Assistant Editor Guo SY **Edited by** Kumar M and Wang XL