Diagnosis of Gastric Inflammation and Malignancy in Endoscopic Biopsies Based on Fourier Transform Infrared Spectroscopy

Qing-Bo Li,1 Xue-Jun Sun,2 Yi-Zhuang Xu,1 Li-Min Yang,1 Yuan-Fu Zhang,1 Shi-Fu Weng,1 Jing-Sen Shi,2 and Jin-Guang Wu1*

Background: Fourier transform infrared (FT-IR) spectroscopy is an effective tool for investigation of chemical changes at the molecular level. We previously demonstrated that FT-IR spectroscopy can reliably distinguish multiple types of carcinoma from healthy tissue. Because various stomach diseases are common, it is important to explore a noninvasive and rapid method to detect malignancy and gastritis in endoscopic biopsies. Our aim was to classify endoscopic biopsies into healthy, gastritis, and malignancy through the use of FT-IR spectroscopy.

Methods: A total of 103 endoscopic samples, including 19 cases of cancer, 35 cases of chronic atrophic gastritis, 29 cases of chronic superficial gastritis, and 20 healthy tissue samples, were obtained at the First Hospital of Xi’an Jiaotong University, China. A modified attenuated total reflectance accessory was linked to a WQD-500 FT-IR spectrometer for biopsy measurement. The spectral characteristics for different types of tissues were correlated with the corresponding pathology results. The gastric biopsies were classified by FT-IR spectroscopy and a discriminant analysis method.

Results: There were significant differences in the FT-IR spectra of four types of gastric biopsies. The discriminant analysis results demonstrated that the sensitivity of FT-IR detection for healthy, superficial gastritis, atrophic gastritis, and gastric cancer was 90%, 90%, 66%, 74%, respectively, which could help satisfy clinical diagnostic requirements.

Conclusion: FT-IR spectroscopy can distinguish disease processes in gastric endoscopic biopsies.

© 2005 American Association for Clinical Chemistry

Chronic gastric diseases are common in China, and this may lead to a higher incidence of stomach carcinoma than is found in Western countries. Gastric endoscopy and biopsy analysis are performed frequently. Patients may suffer from gastric carcinoma, chronic superficial gastritis, or chronic atrophic gastritis. Chronic superficial gastritis is a mild inflammation with glandular preservation. Chronic atrophic gastritis may have pathologic characteristics such as gland dropout and precancerous changes. This is important because this kind of gastritis could develop into carcinoma if the disease is not closely monitored. It therefore is important to classify biopsies after endoscopy because this categorization could help determine proper medical treatment.

Fourier transform infrared (FT-IR) spectroscopy can effectively provide chemical information concerning the structure and composition of biological materials at the molecular level; the application of vibrational spectroscopy is therefore expanding (1–5). This noninvasive, convenient, and rapid technique has been applied to the study of various types of healthy and malignant tissues and is a powerful tool in researching the biochemistry of cancer (6–11). The technique also possesses a promising potential for detecting early cancer. Our research group has successfully used FT-IR spectroscopy to diagnose multiple kinds of carcinoma, such as stomach, colon, liver, esophagus, lung, gallbladder, breast, and parotid gland (12–24).
We previously performed FT-IR analysis on a large number of samples obtained at surgery from paired carcinomas and adjacent healthy tissues (diameter, 1–2 cm). The results showed that there were significant differences between the spectra of malignant and corresponding healthy tissues and that a series of discrimination regularities existed (25). In addition, our previous results revealed that FT-IR spectroscopy could detect molecular abnormalities that occurred before the change in morphology seen under the light microscope (25). FT-IR technology thus makes it possible to detect inflammatory and precancerous stages. The FT-IR method has the possibility of developing into a new technique for gastric endoscopic examination. We believe that noninvasive, rapid, accurate, and convenient analysis of gastric tissue can be performed with Fourier-transform mid-infrared spectroscopy if the mid-infrared fiber optics and stomach endoscopy technologies can be combined successfully, but a flexible mid-infrared optical fiber and miniprobe are not yet available. The aim of this study, therefore, was to explore the use of FT-IR spectroscopy, combined with multivariate analysis method, in the classification of gastric endoscopic biopsies.

**Materials and Methods**

**Patients**
A total of 103 gastric tissue specimens were obtained by the Medical Division of the First Hospital of Xi’an Jiaotong University, China. Informed consent was obtained from each patient before the study. One endoscopic pinch biopsy (diameter, 1–3 mm) was obtained from each patient. According to the results from the pathology review, the studied samples consisted of 19 cases of cancer, 35 cases of chronic atrophic gastritis, 29 cases of chronic superficial gastritis, and 20 samples of healthy stomach tissue.

**Instrument**
Because the endoscopic samples are small and it is hard to obtain a high-quality IR spectrum, a modified attenuated total reflectance (ATR) accessory was linked to a WQD-500 FT-IR spectrometer (Beijing No. 2 Optical Instrument Factory). The modified ATR accessory was made with a ZnSe crystal. The improvement in the ATR accessory involves reducing the number of internal reflectances to five, which can decrease the attenuation of light energy and provide higher light throughput, thus increasing the signal-to-noise ratio. The FT-IR spectrometer was equipped with a mercury cadmium telluride detector cooled by liquid nitrogen, which has higher stability and sensitivity than the traditional DTGS detector.

**Spectral Measurements**
The fresh surgically resected specimens were immediately noninvasively analyzed with the mobile WQD-500 FT-IR spectrometer. Each sample was placed on the ATR accessory for analysis. To collect the data for each spectrum, we performed 32 coadded scans at a resolution of 4 cm\(^{-1}\), with a typical range from 800 to 4000 cm\(^{-1}\). It took ~1–2 min to obtain the spectrum noninvasively. For comparison, after the spectra for the samples were recorded, the fixed samples were stored in liquid nitrogen and sent for histologic examination for comparison with the spectral analysis. Each IR result was compared with a biopsy from the same tissue sample.

**Multivariate Statistical Analysis**
After FT-IR spectra were collected, and in parallel, a pathology-based diagnosis was made using the routine method of hematoxylin/eosin staining for the property of interest. In the second step, we statistically analyzed the obtained FT-IR spectra to determine differences and convert the spectroscopic data into clinically useful information.

We used OMNIC5.0 as the data-processing software. The five-point moving average smoothing method was adopted for each spectrum to reduce the random noise in the data. To achieve more accurate differences in the ratio of peak intensities, we first performed baseline corrections of the spectra. For each spectral parameter, such as the relative intensities of peaks A and B, we carried baseline correction for these two absorption bands to sharpen the spectral differences among different kinds of tissues. For the purpose of multiple group classifications, we performed a supervised linear discriminant analysis (LDA), using the SPSS 10.0 statistical package (26) for the LDA algorithm.

For a supervised LDA, the class identity was known and used in the calculation. The LDA algorithm was derived from an equation (27):

\[
Z = w_1x_1 + w_2x_2 + w_3x_3 + \ldots + w_ix_i
\]

where \(Z\) is the discriminant score, and \(w_i\) is the discriminant weight for the \(i\)th independent variable \(x_i\). In this study, the four classifications of gastric endoscopic biopsies were identified by LDA algorithms with seven independent variables, which were the intensity ratios of ~1640 cm\(^{-1}\) (peak A) vs ~1550 cm\(^{-1}\) (peak B), ~1460 cm\(^{-1}\) (peak C) vs ~1400 cm\(^{-1}\) (peak D), ~1310 cm\(^{-1}\) (peak E) vs ~1240 cm\(^{-1}\) (peak F), and ~1160 cm\(^{-1}\) (peak G) vs ~1120 cm\(^{-1}\) (peak H), and the peak positions of the amide I band plus water (peak A), amide II band (peak B), and ~1310 cm\(^{-1}\) band (peak E), respectively. Because the number of samples for each group was not large enough, all spectra were split into a training set to train the LDA models, and a leave-one-out cross-validation was used to assess the quality of the discriminant model. The class assignment of any given spectrum was derived by computing its distance from all class centroids and allotting it to the class whose centroid was nearest.

**Results**
In the endoscopic biopsies, the majority of spectral peaks were in the 1000–1800 cm\(^{-1}\) region. The mean FT-IR
absorption spectra for the gastric endoscopic biopsies in the regions 1800–1480 cm$^{-1}$ and 1480–1000 cm$^{-1}$ are shown in Fig. 1. Similar to previous results, there was distinctive absorption at peaks A through H, which were selected as the seven independent variables for multivariate discriminant analysis.

The statistical results for the intensity ratios [mean (SD)] for peaks A/B, C/D, E/F, and G/H and the mean (SD) peak positions for A, B, and E in the FT-IR spectra for different types of gastric endoscopic samples are listed in Table 1. We observed that the change in the regularities of the spectral characteristics agreed with the type of disease. Therefore, all of the characteristics listed above needed to be considered simultaneously for the classification of gastric tissues obtained from endoscopic examination.

In this study, we first used three discriminant functions in the analysis and used three-dimensional scores for each sample for classification. The classification results for all four groups of gastric specimens in the training set and the cross-validation study are shown in Table 2. The results based on the FT-IR spectra with the LDA analysis, with reference to the histologic diagnoses, are shown in Table 3. The LDA-supervised classification was very promising, with 84% overall accuracy for the training set. For the cross-validation, each case was classified by the first three discriminant functions derived from all cases other than that individual case, with an overall accuracy of 77% correctly classified for the cross-validated grouped cases. The sensitivities for FT-IR spectroscopic classification of gastric cancer, chronic atrophic gastritis, superficial gastritis, and healthy tissue were 74%, 66%, 90%, and 90%, respectively.

**Discussion**

The spectral characteristics at peaks A-H in the FT-IR spectra differed among healthy, chronic superficial gastritis, atrophic gastritis, and cancerous gastric specimens. Significant shifts of the peak frequencies and changes in the intensity ratios occurred, as shown in Table 1 and Fig. 1. The spectral characteristics of the small gastric biopsies were similar to those of block gastric tissues, which were

<table>
<thead>
<tr>
<th>Peak frequency, cm$^{-1}$</th>
<th>Healthy tissue (n = 20)</th>
<th>Chronic superficial gastritis (n = 29)</th>
<th>Chronic atrophic gastritis (n = 35)</th>
<th>Cancer (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak frequency, cm$^{-1}$</td>
<td>1646 (2)</td>
<td>1642 (3)</td>
<td>1640 (3)</td>
<td>1641 (2)</td>
</tr>
<tr>
<td></td>
<td>1553 (2)</td>
<td>1556 (2)</td>
<td>1547 (2)</td>
<td>1549 (4)</td>
</tr>
<tr>
<td></td>
<td>1317 (6)</td>
<td>1316 (5)</td>
<td>1306 (8)</td>
<td>1313 (3)</td>
</tr>
<tr>
<td>Intensity ratio</td>
<td>1.65 (0.19)</td>
<td>2.12 (0.26)</td>
<td>2.33 (0.31)</td>
<td>2.48 (0.30)</td>
</tr>
<tr>
<td>A/B</td>
<td>1.10 (0.04)</td>
<td>1.40 (0.67)</td>
<td>0.91 (0.34)</td>
<td>0.86 (0.10)</td>
</tr>
<tr>
<td>C/D</td>
<td>0.67 (0.09)</td>
<td>0.34 (0.24)</td>
<td>0.52 (0.38)</td>
<td>0.79 (0.32)</td>
</tr>
<tr>
<td>G/H</td>
<td>1.22 (0.25)</td>
<td>0.91 (0.06)</td>
<td>0.85 (0.12)</td>
<td>0.89 (0.13)</td>
</tr>
</tbody>
</table>
that of healthy gastric tissue. The prominent band at shape of the spectrum for this condition was similar to some of the same characteristic bands as cancer. Chronic shape of spectra for chronic atrophic gastritis exhibited proteins, nucleic acids, and fats. As shown in Fig. 1, the structure and composition of biological molecules such as healthy tissue, as shown in Fig. 1.

from chronic atrophic gastritis to superficial gastritis to the strength of the amide II bands gradually increased the higher water content in cancerous tissue. In addition, because the intensity of peak A was stronger as a result of peak A to peak B were higher in the malignant tissue of gastric cancer biopsies, the ratios of the intensities of gastric tissues were in good agreement with the criteria obtained during surgery and measured in our previous studies. The spectral features of malignant and healthy gastric tissues were in good agreement with the criteria established in our previous work (28).

These spectral features are related to the changes in structure and composition of biological molecules such as proteins, nucleic acids, and fats. As shown in Fig. 1, the shape of spectra for chronic atrophic gastritis exhibited some of the same characteristic bands as cancer. Chronic superficial gastritis is a milder inflammation, and the shape of the spectrum for this condition was similar to that of healthy gastric tissue. The prominent band at $\sim 1640 \text{ cm}^{-1}$ belongs to the amide I band of protein and the H—O—H deformation vibration of water; the $\sim 1550 \text{ cm}^{-1}$ absorption peak arises from N—H bending and C—N stretching (amide II band) in proteins. In the spectra of gastric cancer biopsies, the ratios of the intensities of peak A to peak B were higher in the malignant tissue because the intensity of peak A was stronger as a result of the higher water content in cancerous tissue. In addition, the strength of the amide II bands gradually increased from chronic atrophic gastritis to superficial gastritis to healthy tissue, as shown in Fig. 1.

The intensity of the $\sim 1400 \text{ cm}^{-1}$ peak was stronger than that of the $\sim 1460 \text{ cm}^{-1}$ peak in the spectra of cancerous samples. For chronic atrophic gastritis, the decrease in intensity near 1460 cm$^{-1}$ was not significant, i.e., the intensity of the band at $\sim 1460 \text{ cm}^{-1}$ became slightly less than, or approximately equal to, that of the peak at 1400 cm$^{-1}$. In the spectra of superficial gastritis samples, the intensity of the peak at 1460 cm$^{-1}$ was slightly stronger than that of the peak at 1400 cm$^{-1}$, and the intensity of the peak at 1460 cm$^{-1}$ was stronger than that of the peak at 1400 cm$^{-1}$ in healthy tissue.

In the spectra for cancer, the intensity of absorption peak at $\sim 1310 \text{ cm}^{-1}$ increased, and the peak position shifted to a lower wavenumber. Compared with the spectra of the malignant tissues, the absorption peak near 1310 cm$^{-1}$ was weaker in the spectra of atrophic gastritis samples. Similar to the gastric cancer tissues, the position of peak E often shifted to a lower wavenumber, which are different from chronic superficial gastritis. In healthy tissues, the peak at $\sim 1240 \text{ cm}^{-1}$ was stronger, and the band near 1318 cm$^{-1}$ became weak and sometimes disappeared. In addition, the position of this band often shifted to a higher wavenumber.

The intensity of the band at $\sim 1160 \text{ cm}^{-1}$ was often less than that of the band at $\sim 1120 \text{ cm}^{-1}$ in the spectra of stomach cancer samples. In the healthy gastric tissues, the intensity of the peak near 1160 cm$^{-1}$ increased and was often stronger than that of band at $\sim 1120 \text{ cm}^{-1}$.

Our results indicate that FT-IR spectroscopy, combined with appropriate pattern recognition algorithms, can distinguish benign from malignant disease for endoscopic gastric biopsies. There are several advantages of FT-IR spectroscopy: it is inexpensive, less time-consuming, does not require special sample preparation or any biochemical reagents, and uses only small amounts of sample, leaving sufficient material for other clinical tests. Moreover, it is a computer-operated system, which helps standardize interpretation of results. Real-time FT-IR spectroscopy of biopsy samples taken at the time of endoscopy can provide accurate, rapid diagnostic differentiation between healthy tissue, superficial and atrophic inflammation, and cancer, using a technique that is currently available. If a miniprobe can be developed for IR detection that can pass through the endoscopic biopsy channel and

Table 2. Comparison of the FT-IR results with histologic examination.

<table>
<thead>
<tr>
<th>Histogram examination</th>
<th>FT-IR results</th>
<th>Chronic atrophic gastritis</th>
<th>Chronic superficial gastritis</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training set</td>
<td>Cancer</td>
<td>16</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chronic atrophic gastritis</td>
<td>1</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Chronic superficial gastritis</td>
<td>2</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cross-validation</td>
<td>Cancer</td>
<td>14</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chronic atrophic gastritis</td>
<td>2</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chronic superficial gastritis</td>
<td>3</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Results of statistical analysis of detection of endoscopic gastric samples by FT-IR spectroscopy.

<table>
<thead>
<tr>
<th>Training set</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Predictive value of a positive test, %</th>
<th>Predictive value of a negative test, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>84</td>
<td>88</td>
<td>62</td>
<td>96</td>
</tr>
<tr>
<td>Chronic atrophic gastritis</td>
<td>71</td>
<td>98</td>
<td>96</td>
<td>87</td>
</tr>
<tr>
<td>Chronic superficial gastritis</td>
<td>97</td>
<td>93</td>
<td>85</td>
<td>99</td>
</tr>
<tr>
<td>Healthy</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Cross-validation</td>
<td>Cancer</td>
<td>74</td>
<td>86</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Chronic atrophic gastritis</td>
<td>66</td>
<td>96</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Chronic superficial gastritis</td>
<td>90</td>
<td>92</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Healthy</td>
<td>90</td>
<td>99</td>
<td>95</td>
</tr>
</tbody>
</table>
be coupled with fiber optics, clinicians would have a 
noninvasive real-time technique for directed instead of 
random biopsy analysis.

We thank the National Natural Science Foundation of 
China (Grant 30371604), the State Key Project for Funda-
mental Research of China (Grant 2002CCA01900), and 
the Doctoral Project of the High Education Ministry of China 
for supporting this work. We gratefully acknowledge 
Beijing No. 2 Optical Instrument Factory (Beijing, China) 
for providing the FT-IR spectrometer and for excellent 
technical assistance.

References
spectrophotometry for measuring body water spaces. Clin Chem 
determination of fecal fat by Fourier transform infrared analysis 
(FTIR) with partial least-squares regression and an attenuated 
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 (FT-IR) spectroscopy. 
Gastroenterology 2002;122(Suppl):A303.
Glucose and other constituents of blood determined by ATR-FTIR-
5. Christopher VE, Arnold MA. Near-infrared spectroscopy for mea-
6. Rigas B, Morgello S, Goldman IS, Wong PT. Human colorectal 
cancers display abnormal Fourier-transform infrared spectra. Proc 
Kollias N, et al. Spectroscopic measurement of diffuse reflectance 
for enhanced detection of bladder carcinoma. Urology 1998;51: 
342–5.
8. Wang HP, Wang HC, Huang YJ. Microscopic FTIR studies of lung 
Mordechai S. Novel spectral method for the study of viral carcinoma-
10. Fung Kee Fung M, Senterman M, Eid P, Faught W, Mikhail NZ, 
Wong PT. Comparison of Fourier-transform infrared spectroscopic 
screening of exfoliated cervical cells with standard Papanicolaou 
H, et al. Diagnostic potential of Fourier-transform infrared mi-
scroproscopy and advanced computational methods in colon 
spectroscopic analysis of normal and cancerous tissues of esoph-
Infrared spectroscopic features of colon cancer cells differ from 
those of normal colonic cells from the same patient. Gastroentero-
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 (FT-IR) spectroscopy. 
Gastroenterology 1997;112(Suppl):A604.
spectroscopic analysis of gallbladder tissues. Chin J Hepatobili-
spectroscopic and statistical studies on the lung tissues. Spec-
application of mid-IR fiber optics in diagnosis of colon cancer in 
and rapid detection of the malignancy tumor of oral tissues using 
Mid-FIR fiber optics spectroscopy. Chem J Chin Uni 2004;25: 
348–50.
Distinguishing malignant from normal oral tissues using FTIR 
spectroscopic study of normal and malignant tissues of rectum. 
Fourier transform infrared (FT-IR) mid-IR spectroscopy separates 
normal and malignant tissue from the colon and stomach. Gas-
troenterology 2000;118:A6438.
intraoperative detection of malignancy using attenuated total 
reflectance (ATR) and mobile Fourier transform infrared (FT-IR) 
23. Peng Q, Xu YZ, Li WH, Zhou XS, Wu JG. FTIR study on the normal 
and tumor gastrointestinal tissues. Spectrosc Spect Anal 1998; 
the diagnosis of salivary gland tumors by means of mid infrared 
Intraoperative Fourier transform infrared spectroscopy can guide 
individual resections in patients with gastric cancer. Gastroenter-
ology 2004;126:A626.
26. Liu RX, Kuang J, Gong Q, Hou XL. Principal component regression 
analysis with SPSS. Comput Methods Programs Biomed 2003; 
27. Fujikawa N, Morimoto Y, Arai T, Ikikuchi M. Discrimination between 
normal and malignant human gastric tissues by Fourier transform 
28. Liu KZ, Schultz CP, Salamon EA, Man A, Mantsch HH. Infrared 
spectroscopic diagnosis of thyroid tumors. J Mol Struct 2003; 
661:397–404.