Rapid Fiber-optic Raman Spectroscopy for Real-Time In Vivo Detection of Gastric Intestinal Metaplasia during Clinical Gastroscopy

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Abstract

We report a unique simultaneous fingerprint (FP) and high-wavenumber (HW) Raman spectroscopy technique coupled with a beveled fiber-optic Raman probe for improving in vivo detection of gastric intestinal metaplasia (IM)–precancerous lesions in real-time during clinical gastroscopy. A total of 4,520 high-quality in vivo FP/HW gastric Raman spectra (normal = 4,178; IM = 342) were acquired from 157 gastric patients undergoing endoscopic examination. Multivariate diagnostic algorithms based on principal components analysis and linear discriminant analysis together with the leave-one tissue site-out, cross-validation on in vivo tissue Raman spectra yield the diagnostic sensitivities of 89.3%, 89.3%, and 75.0%; specificities of 92.2%, 84.4%, and 82.0%; positive predictive values of 52.1%, 35.2%, and 28.4%; and negative predictive values of 98.9%, 98.8%, and 97.2%, respectively, by using the integrated FP/HW, FP, and HW Raman techniques for identifying IM from normal gastric tissue. Further, ROC curves generated show that the integrated FP/HW Raman technique gives the integration area under the ROC curve of 0.92 for IM classification, which is superior to either FP (0.89) or HW Raman (0.86) technique alone. This work demonstrates for the first time that the simultaneous FP/HW fiber-optic Raman spectroscopy has great potential to enhance early diagnosis of gastric precancer in vivo during routine endoscopic examination. Cancer Prev Res; 9(6); 476–83. ©2016 AACR.

Introduction

Gastric cancer is one of the most common malignancies in the world with particularly high incidence rates in East Asia, East Europe, and South America (1, 2). Gastric cancer is a multistep carcinogenesis process associated with the intermediate transformation from intestinal metaplasia (IM) phenotype before progression to dysplasia and invasive carcinoma (3). Gastric IM is recognized as a frequent precancerous lesion that can be very challenging to identify by using conventional white light reflectance (WLR) endoscopy with a limited diagnostic accuracy (~58.8%; ref. 4). Epidemiologic studies suggest that patients with IM have more than 10-fold increased risk of developing dysplasia into gastric cancer (5). Early detection and diagnosis of gastric IM together with effective therapeutic interventions is crucial to reducing the mortality rate of the patients associated with gastric precancer and cancer (6, 7). Currently, endoscopic examination with subsequent biopsies for histopathology remains the gold standard for gastric cancer diagnosis. However, it suffers from fundamental clinical limitations due to the lack of obvious morphologic changes of preneoplastic lesions on the tissue surface under conventional WLR endoscopy, leading to poor diagnostic accuracy (~15.7%; ref. 8). Therefore, it is of imperative clinical value to develop molecular-sensitive optical diagnostic technologies that can assist in guiding endoscopists for the targeted biopsies of gastric IM lesions for improving early gastric disease diagnosis during gastroscopic examination.

Raman spectroscopy is an optical vibrational technique based on the fundamental principle of inelastic light scattering (9). When incident laser light induces a polarization change of molecules, a small proportion of incident photons (∼1 in 106) is inelastically scattered with the frequency shifts corresponding to the specific Raman active vibrational modes of molecules in the sample. Hence, Raman spectroscopy is capable of revealing specific biochemical and biomolecular structures and conformation of tissue, providing the unique opportunity for label-free differentiation among different pathologic tissue types at the molecular level. However, there have been technical barriers hindering the translation of Raman spectroscopy into in vivo clinical endoscopic applications, such as intrinsically weak tissue Raman scattering, lengthy acquisition times (>5 seconds; ref. 10), and a necessity to develop miniaturized flexible fiber-optic Raman probe that is compatible with conventional endoscopes. Driven by technological advances including development of high-throughput imaging spectrometers, near-infrared (NIR) sensitive charge-coupled devices (CCD), compact diode lasers, and fiber optics, we have successfully developed a fiber-optic Raman endoscopic technique that can acquire in vivo gastrointestinal tissue Raman spectra within 0.5 seconds (11, 12), paving the way for real-time tissue Raman diagnosis in clinical endoscopic settings.

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To date, Raman spectroscopy studies of gastric tissue have mostly been focused on the so-called fingerprint (FP) spectral range (e.g., 800–1,800 cm$^{-1}$) with encouraging accuracies ranging from 85% to 95% (10–21). The unrivaled advantage of the FP Raman spectroscopy technique stems from its capability to uncover specific information about backbone structures of proteins, lipids, and nucleic acid assemblies in cells and tissue (12, 17). The efficiency of FP Raman spectroscopy technique is, however, compromised in certain organ sites (e.g., gastric antrum, liver, lung) owing to extremely weak tissue Raman signals but overwhelming tissue autofluorescence background. Recent attentions have been directed toward the use of high-wavenumber (HW) regime (e.g., 2,800–3,600 cm$^{-1}$), as the HW spectral range exhibits stronger tissue Raman signals with much reduced autofluorescence interference (22–25). The clinical rationales for combining both the FP and HW spectral ranges for in vivo gastric Raman measurements are therefore manifold: (i) For tissues that could exhibit intense autofluorescence overwhelming the tissue FP Raman signals, the HW range could still contain intense tissue Raman peaks with diagnostic information; (ii) The FP and HW Raman spectra offer complementary biomolecular information, and combining FP/HW Raman technique could further improve tissue characterization and diagnosis (26–29). Therefore, the primary aim of this study is to apply the simultaneous FP and HW fiber-optic Raman spectroscopic techniques developed for in vivo gastric tissue measurements at endoscopy (26–28). Briefly, the fiber-optic Raman spectroscopic system consists of a NIR diode laser ($\lambda_{ex}$ = 785 nm; maximum output: 300 mW; B&W TEK Inc.), a high-throughput reflective imaging spectrograph (Acton LS-785 f/2; Princeton Instruments Inc.) equipped with a gold-coated 830 gr/mm grating and a thermo electric-cooled, NIR-optimized CCD camera (PIXIS: 400BR-eXcelon; Princeton Instruments Inc.; refs. 26, 28, 29). A customized parabolic-aligned fiber bundle [64 × 100 μm fibers, numerical aperture (NA) = 0.22] was coupled into the entrance slit to compensate for the image aberration in the broad spectral range (800–3,600 cm$^{-1}$; refs. 11, 30), which significantly improves the signal-to-noise ratio (SNR; 20-fold improvement; ref. 11) as well as the spectral resolution of the Raman system as compared with a conventional straight slit imaging spectrograph. The FP/HW system acquires in vivo tissue Raman spectra in the spectral range from 400 to 3,600 cm$^{-1}$ with a resolution of approximately 9 cm$^{-1}$ in real time (<0.5 seconds; refs. 26, 28, 31). We have developed a 1.9-meter-long fiber-optic Raman probe (1.8 mm in outer diameter) for both laser light delivery and in vivo epithelial tissue Raman signal collection at endoscopy (31, 32). The compact fiber-optic Raman endoscopic probe designed for endoscopy comprises 18 × 200 μm beveled collection fibers (low-OH fused silica, NA = 0.22) surrounding the central light delivery fiber (low-OH fused silica, 200 μm in

**Materials and Methods**

**Clinical fiber-optic Raman instrumentation**

Figure 1 shows the simultaneous FP and HW fiber-optic Raman spectroscopy technique developed for in vivo tissue Raman measurements at endoscopy (26–28). Briefly, the fiber-optic Raman spectroscopic system consists of a NIR diode laser ($\lambda_{ex}$ = 785 nm; maximum output: 300 mW; B&W TEK Inc.), a high-throughput reflective imaging spectrograph (Acton LS-785 f/2; Princeton Instruments Inc.) equipped with a gold-coated 830 gr/mm grating and a thermo electric-cooled, NIR-optimized CCD camera (PIXIS: 400BR-eXcelon; Princeton Instruments Inc.; refs. 26, 28, 29). A customized parabolic-aligned fiber bundle [64 × 100 μm fibers, numerical aperture (NA) = 0.22] was coupled into the entrance slit to compensate for the image aberration in the broad spectral range (800–3,600 cm$^{-1}$; refs. 11, 30), which significantly improves the signal-to-noise ratio (SNR; 20-fold improvement; ref. 11) as well as the spectral resolution of the Raman system as compared with a conventional straight slit imaging spectrograph. The FP/HW system acquires in vivo tissue Raman spectra in the spectral range from 400 to 3,600 cm$^{-1}$ with a resolution of approximately 9 cm$^{-1}$ in real time (<0.5 seconds; refs. 26, 28, 31). We have developed a 1.9-meter-long fiber-optic Raman probe (1.8 mm in outer diameter) for both laser light delivery and in vivo epithelial tissue Raman signal collection at endoscopy (31, 32). The compact fiber-optic Raman endoscopic probe designed for endoscopy comprises 18 × 200 μm beveled collection fibers (low-OH fused silica, NA = 0.22) surrounding the central light delivery fiber (low-OH fused silica, 200 μm in
diameter, NA = 0.22). A 1.0-mm sapphire ball lens (NA = 1.78) is coupled to the fiber tip of the probe to tightly focus the excitation light onto the gastric tissue subsurface, enabling the effective Raman spectrum collection from the epithelial lining (31, 32). The depth-selective capability of the fiber-optic Raman spectroscopy technique ensures the shallower tissue interrogation (<300 μm) with tissue probing volume of <0.02 mm³ (31, 32), thereby reducing the interferences and signal dilution from deeper bulky tissues, while selectively interrogating the epithelium mucosa associated with neoplastic onset and progression. At the proximal ends of the Raman probe, the excitation and emission fibers were coupled into two separate in-line filter modules: one integrated with a narrow bandpass filter for suppressing laser noise, and the other integrated with an edge long-pass filter for further reduction of the scattered laser light while permitting the scattered tissue Raman signals to transmit into the Raman spectrometer. The atomic emission lines of mercury-argon spectral calibration lamps (HG-1 and AR-1; Ocean Optics, Inc.) are used for wavelength calibration. All wavelength-calibrated spectra were corrected for the wavelength dependence of the filter-optic Raman endoscopic system is controlled using a foot pedal in an intuitive software framework with auditory probabilistic feedback to the gastroenterologist in real time, pushing the frontier of Raman spectroscopy into routine clinical endoscopic diagnostics (19).

Clinical Raman study protocol

This work was approved by the Institutional Review Board of the National Healthcare Group of Singapore. Prior to Raman measurements, all patients signed an informed consent, permitting the in vivo Raman spectroscopic measurements during endoscopy examinations. The exclusion criteria for Raman examination included: (i) patients presenting with comorbid diseases, severe acute/chronic medical conditions, or bleeding disorders, in which biopsies may be contraindicated; (ii) patients who had undergone neoadjuvant therapy; and (iii) patients presenting with severe bleeding, food debris, and neoplasms with extensive ulcerous exudates to obscure light penetration into the tissue were also excluded to prevent erroneous interpretations. A total of 157 gastric patients (male, 89; female, 68; mean age, ~56) undergoing endoscopic examinations while fulfilling the above exclusion criteria for Raman examination were recruited and examined. The Raman probe passes down to the instrument channel of a medical endoscope (GIF Q260Z, Olympus Medical Systems) under the guidance of WLR endoscopic imaging (11). The Raman probe tip was visible approximately 0.5 cm in front of the endoscope camera and could precisely be placed in gentle contact with the suspected lesions. The positioning against the tissue sites was verified on the endoscopy monitor by the endoscopists in charge during gastroscopic examinations. The 785-nm laser power incident on the tissue surface is approximately 12 mW (equivalent to ~1.5 W/cm² within the spot size of ~500 μm) permissible by the American National Standards Institute (33). Tissue Raman measurements were performed on 1 to 5 suspicious lesion sites in the stomach for each patient through gently placing the fiber-optic Raman probe on the tissue surfaces. Multiple Raman spectra (~10–15) for each tissue site were measured, and each high-quality spectra can be acquired within 0.1 to 0.5 seconds, which permits a rapid survey of large tissue areas. Immediately after the tissue Raman acquisitions, each tissue site measured was biopsied and sent for histopathologic examinations by three senior gastrointestinal pathologists who were blinded to the Raman scans. The consensus histopathology assessments serve as the gold standard to determine the diagnostic capability of the simultaneous FP/HW fiber-optic Raman technique for identifying IM from normal gastric tissues. In total, 4,520 Raman spectra (4,178 normal and 342 IM) from 323 different tissue sites (295 normal and 28 IM) of 157 gastric patients were used for in vivo gastric tissue Raman diagnosis and classification.

Data preprocessing and statistical analysis

The raw FP/HW Raman spectra measured from in vivo gastric tissue represent a combination of weak tissue Raman signal, intense autofluorescence background, and noise. The raw spectra are preprocessed by a third-order Savitzky-Golay smoothing filter (a window width of 3 pixels) to remove the spectral noise (34). In the FP region (800–1,800 cm⁻¹), a fifth-order polynomial was found to be optimal for fitting the AF background in the noise-smoothed spectrum, and this polynomial was then subtracted from the measured FP spectrum to yield the FP tissue Raman spectrum alone. In the HW range (2,800–3,600 cm⁻¹), a first-order polynomial fit was used for removing the AF background (35). The FP/HW Raman spectra are then normalized over the integrated area under the FP and HW ranges to allow a better comparison of the spectral shapes and relative Raman band intensities between normal and IM gastric tissues. There are no Raman peaks observed in the wavenumber range of 1,800 to 2,800 cm⁻¹, hence this silent spectral range is excluded for data analysis. All raw spectral data were processed on-line with software developed in the Matlab environment (Mathworks Inc.). PCA and LDA were implemented to develop robust diagnostic algorithms for differentiation between normal and IM gastric tissues (36, 37). Leave-one tissue site-out, cross-validation was utilized to evaluate the diagnostic models developed in an unbiased manner (36, 37). One notes that multiple Raman spectra (10 to 15) were acquired from each tissue site within 0.5 seconds, and the majority voting strategy was applied for final classification. The diagnostic outcomes can be displayed on the computer screen in real time. ROC curves were also generated by successively changing the thresholds to determine correct and incorrect classifications for all tissues (38). All the spectra preprocessing and multivariate statistical analysis were performed online using in-house written scripts in the Matlab programming environment (19).

Results

A total of 4,520 in vivo Raman spectra [i.e., normal (n = 4,178 spectra) and IM (n = 342 spectra)] were successfully acquired in real time (<0.5 seconds) from 323 tissue sites [i.e., normal (n = 295 sites) and IM (n = 28 sites)] as confirmed by consensus histopathology examinations. Figure 2A shows the mean in vivo Raman spectra ± 1 SD (shaded light-gray) measured (i.e., normal and IM). Prominent tissue Raman peaks with tentative assignments are observed in the FP range at 875 cm⁻¹ (ν(C–C) proteins), 1,004 cm⁻¹ (ν(C–C) ring breathing of phenylalanine), 1,078 cm⁻¹ (ν(C–C) of lipids), 1,302 cm⁻¹ (CH₂ twisting and wagging of lipids), 1,445 cm⁻¹ (δ(CH₂) deformation of proteins and lipids), and 1,655 cm⁻¹ (amide I ν(C=O) of proteins; refs. 10–21, 27, 29). In addition, intense Raman peaks in the HW region are also observed at 2,885 cm⁻¹ (symmetric and asymmetric...
CH$_2$ stretching of lipids), 2,940 cm$^{-1}$ (CH$_3$ stretching of proteins), 3,300 cm$^{-1}$ (Amide A, NH stretching of proteins), and the broad Raman band of water (OH stretching vibrations peaking at $\sim$3,400 cm$^{-1}$) that are related to the local conformation and interactions of OH-bonds in the cellular and extracellular space of tissue (22–29). Figure 2B shows the difference spectra (i.e., IM-normal ± 1 SD) resolving the unique spectral features between normal and IM gastric tissues.

![Figure 2](image1)

**Figure 2.**

A, the mean FP and HW in vivo Raman spectra ± 1 SD of gastric IM ($n = 342$) and normal mucosa ($n = 4,178$) acquired from 157 patients during clinical endoscopic examination. Each tissue Raman spectrum is acquired within 0.5 seconds. The spectra have been normalized to the integrated area in the FP and HW ranges for comparison purpose. B, difference spectra (i.e., IM-normal ± 1 SD) resolving the unique spectral features between normal and IM gastric tissues.

CH$_2$ stretching of lipids), 2,940 cm$^{-1}$ (CH$_3$ stretching of proteins), 3,300 cm$^{-1}$ (Amide A, NH stretching of proteins), and the broad Raman band of water (OH stretching vibrations peaking at $\sim$3,400 cm$^{-1}$) that are related to the local conformation and interactions of OH-bonds in the cellular and extracellular space of tissue (22–29). Figure 2B shows the difference spectra (i.e., IM-normal ± 1 SD) resolving the unique spectral features (e.g., peak intensity, shifting, and band broadening) associated with IM transformation, confirming the potential of FP/HW Raman spectroscopy for early diagnosis of IM at endoscopy.

Figure 3 shows the representative hematoxylin and eosin (H&E) slides of the corresponding tissue sites using Raman endoscopy, which include (i) normal gastric mucosa (200X magnification) and (ii) extensive gastric IM whereby the gastric epithelium contains apparent goblet cells (200X magnification). Histopathology characterizations (Fig. 3) reveal the cellular and morphologic features of normal and IM lesions in the gastric tissue, whereas the simultaneous FP/HW Raman endoscopy uncovers the specific biochemical constituents (e.g., proteins, lipids, and water) of the epithelial tissue at the molecular level.

To develop sophisticated multivariate diagnostic algorithms and compare tissue diagnostic performance among the three different Raman techniques (i.e., FP, HW, and the integrated FP/HW), PCA-LDA together with Student t test are implemented on the in vivo tissue Raman spectra acquired (Fig. 2) to evaluate the elusive differences observed in the spectra of different tissue types. The leave-one tissue site-out, cross-validated PCA-LDA diagnostic algorithms were further developed based on the diagnostic significant PCs ($P < 0.01$) as shown in Fig. 4, accounting for 45.3% (PC1), 33.6% (PC2), 4.2% (PC3), 3.1% (PC4), and 1.2% (PC5) of Raman spectral variations, respectively. Note that the features of different significant PCs are distinct; in particular, some PC

![Figure 3](image2)

**Figure 3.** Photomicrographs of the H&E-stained sectioned slides of gastric tissues: A, normal gastric mucosa (magnification, 200X); (B) extensive IM (magnification, 200X).
features, such as the peaks, troughs, and spectral shapes in Fig. 4, are similar to those of tissue Raman spectral patterns (Fig. 2). The first significant PC accounts for the largest variance within the spectral datasets (i.e., 45.3%), whereas successive PCs describe features that contribute progressively smaller variances. All the five diagnostically significant PCs are then fed into the LDA model together with leave-one tissue-out, cross-validation technique for gastric tissue diagnosis and classification.

Figure 5 shows the cross-validation classification results (posterior probabilities) between normal and IM pathologies by PCA-LDA algorithm modeling as calculated for (i) FP, (ii) HW, and (iii) the integrated FP/HW Raman technique, respectively. The threshold lines (0.5) applied to the posterior probability scatter plots yield the diagnostic accuracies of 84.8% (274/323), 81.4% (263/323), and 92.0% (297/323); sensitivities of 89.3% (25/28), 75.0% (21/28), and 89.3% (272/295); and specificities of 84.4% (249/295), 82.0% (242/295), and 92.2% (272/295), respectively, for the FP, HW, and the integrated FP/HW Raman spectroscopic techniques. The result demonstrates that the simultaneous FP/HW Raman spectroscopy performs best for gastric IM diagnosis as compared with FP or HR Raman technique. The positive predictive value (PPV) and negative predictive value (NPV) using integrated FP/HW Raman spectroscopic techniques are 52.1% and 98.9, respectively, superior to using either FP (PPV: 35.2%, NPV: 98.8%) or HW (PPV: 28.4%, NPV: 97.2%) Raman technique. The ROC curves (Fig. 6) are also generated for the FP, HW, and the integrated FP/HW Raman techniques, revealing the relationships between the diagnostic sensitivities and specificities of gastric IM identification. The integration AUCs are 0.89, 0.86, and 0.92, respectively, for the FP, HW, and integrated FP/HW Raman techniques, reconfirming that the simultaneous FP/HW Raman technique provides the best diagnostic performance for in vivo gastric IM detection. We have also compared the diagnostic performance of Raman spectroscopy and conventional WLR endoscopy for the differentiation between normal gastric tissue and gastric IM. Conventional WLR endoscopy only yielded a diagnostic sensitivity of 85.7% (24/28) and specificity of 53.9% (159/295), reconfirming that FP/HW Raman spectroscopy...
for enhancing simultaneous FP/HW endoscopic examinations. Overall, the above results demonstrate

**Discussion**

In this study, we have explored the feasibility of applying the simultaneous FP/HW fiber-optic Raman spectroscopy technique for enhancing in vivo diagnosis of gastric precancerous lesions in vivo during endoscopic examination.

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... can significantly enhance the diagnosis of gastric IM during endoscopic examinations. Overall, the above results demonstrate the great potential of the simultaneous FP/HW Raman spectroscopic technique for enhancing early diagnosis of gastric precancerous lesions in vivo during endoscopic examination.

![Image](image_url)

**Figure 6.**
ROC curves of the classification results for distinguishing IM from normal gastric tissue for the integrated FP/HW, FP, and HW Raman, respectively, together with PCA-LDA algorithms with leave-one tissue site-out, cross-validation methods. The integration areas under the ROC curves are 0.92, 0.89, and 0.86, respectively, for the integrated FP/HW Raman, FP Raman, and HW Raman techniques.

We also applied PCA-LDA multivariate algorithms to compare tissue Raman diagnostic performance of different spectral regions (i.e., FP, HW, and the integrated FP/HW) for gastric IM diagnosis. The integrated FP/HW Raman technique could identify IM lesions from normal gastric tissues with a higher diagnostic accuracy of 92.0% as compared with FP (84.8%) or HW (81.4%) Raman modality, proving the advantage of the simultaneous FP/HW Raman endoscopy for improving gastric IM diagnosis in clinical settings. In contrast to our previous IM Raman studies using volume-type Raman probe (47) with a poorer diagnostic sensitivity (<50%) for gastric IM, the utilization of the beveled fiber-optic Raman probe coupled with a ball-lens in this work dramatically increased the diagnostic sensitivity of gastric IM (>90%). Our Monte Carlo simulation shows that approximately 85% of the total Raman signal collected by the beveled Raman endoscopic probe is arising from the top ~200 μm of the gastric mucosa (31, 32), indicating that the beveled Raman probe design is more efficient for selectively targeting IM that is largely confined to the mucosal epithelium (Fig. 3B).

Tissue Raman signals in the FP range contain specific information like proteins, lipids, and DNA conformations (e.g., 875, 1,004, 1,078, 1,302, 1,445, and 1,655 cm⁻¹). In contrast, HW Raman spectra contain additional information of the asymmetric and symmetric CH₃ stretching (~2,885 and ~2,940 cm⁻¹) molecules which are related to proteins and lipids, as well as the broad water bands (~3,250 and ~3,400 cm⁻¹) associated with the local conformation and interactions of hydrogen-bonds in the cellular and extracellular space of tissue which are not contained in the FP...
range (49). By back-tracing the misclassified spectra of each Raman spectral modality, the integrated FP/HW Raman spectral modalities could enhance the final tissue diagnosis. For instance, FP and HW modalities misclassified 49 and 60 lesions from 323 sites, respectively, of the total 157 patients recruited, in which 22 misclassified sites were identical in both the modalities. However, the integration of FP/HW Raman technique reduced the misclassified sites down to 26 (IM = 3; normal = 23). Furthermore, misclassified spectra by the FP modality (that were correctly classified by the integrated FP+HW modality) are comprised of weaker tissue Raman signals and relatively higher autofluorescence background; while the addition of the HW range containing stronger Raman peaks (e.g., 2,885, 2,940, 3,250, and 3,400 cm⁻¹) but much reduce autofluorescence could improve the overall SNR of integrated FP/HW Raman spectra with more diagnostic information, and hence enhances the performance of gastric IM classification. The diagnostic results in this study confirm that the simultaneous FP/HW Raman spectroscopy technique with complementary biomolecular information contained in both the FP and HW spectral ranges can significantly enhance the IM tissue diagnosis in the stomach. On the other hand, we have retrospectively traced back our Raman database including all the 323 tissue sites of the 157 patients recruited in the current study. The diagnosis results of the WLR endoscopy and the Raman spectroscopy were recorded. The biopsy numbers indicated by the diagnosis results of the conventional WLR endoscopy and the Raman spectroscopy are 160 and 48, respectively. Whereas among all the biopsies indicated, only 28 biopsies are positively indicated as gastric IM. The diagnostic yield by using Raman spectroscopy is 58.3% (28/48), superior to conventional WLR endoscopy [17.5% (28/160)], suggesting the promising potential of Raman spectroscopy for assisting the endoscopists in targeted biopsies of gastric precancerous lesions in real time during endoscopic examinations. One notes that the current in vivo FP/HW tissue Raman datasets acquired are unbalanced with a relatively larger number of true negatives but a smaller pathologic tissue (e.g., IM), which may give rise to a bias overall accuracy within the PCA-LDA models. No tissue sites with dysplasia were encountered in this study, which is probably due to the low incidence in this small gastric patient cohort recruited. Further clinical FP/HW Raman studies on a larger series of gastric patients for prospectively randomized multicenter clinical trials are underway for assessing true clinical diagnostic value of the real-time fiber-optic FP/HW Raman spectroscopy, as well for follow-up monitoring of gastric patients with gastric IM to progress into gastric dysplasia or early cancer during endoscopic examinations.

In summary, this study demonstrates that the simultaneous FP and HW fiber-optic Raman spectroscopy can be performed in real time during clinical screening of gastric patients in vivo at gastroscope. Significant FP/HW Raman spectral differences between normal and IM gastric tissue are observed. The use of complementary biomolecular information acquired from the integrated FP and HW Raman spectra significantly improves the detection of precancerous lesions (i.e., IM) as compared with using either the FP or HW Raman alone. This work illustrates that the unique simultaneous FP/HW fiber-optic Raman spectroscopy has great potential for improving in vivo diagnosis of gastric precancer during clinical endoscopy examination.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: K. Lin, J. Wang, K.G. Yeoh, Z. Huang
Development of methodology: K. Lin, J. Wang, Z. Huang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Lin, J. Wang, W. Zheng, K.Y. Ho, K.G. Yeoh, Z. Huang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Lin, J. Wang, Z. Huang
Writing, review, and/or revision of the manuscript: K. Lin, W. Zheng, Z. Huang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Lin, Z. Huang
Study supervision: K. Lin, Z. Huang
Other (histopathologic examination): M. Teh

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