In vivo early diagnosis of gastric dysplasia using narrow-band image-guided Raman endoscopy

Zhiwei Huang
Mads Sylvest Bergholt
Wei Zheng
Kan Lin
National University of Singapore
Department of Bioengineering
Faculty of Engineering
Optical Bioimaging Laboratory
Singapore 117576

Khek Yu Ho
National University of Singapore
and
National University Hospital
Yong Loo Lin School of Medicine
Department of Medicine
Singapore 119260

Ming Teh
National University of Singapore
and
National University Hospital
Yong Loo Lin School of Medicine
Department of Pathology
Singapore 119074

Khay Guan Yeoh
National University of Singapore
and
National University Hospital
Yong Loo Lin School of Medicine
Department of Medicine
Singapore 119260

1 Introduction

Gastric cancer is the second leading cause of cancer-associated death, accounting for approximately 600,000 annual deaths worldwide.1 Early diagnosis and localization with appropriate curative treatments (e.g., endoscopic submucosal dissection and gastrectomy) is critical to decreasing mortality.2 However, identification of early cancer and precancer can be difficult, as conventional white-light reflectance (WLR) endoscopy heavily relies on visual identification of morphological tissue changes. Thus, subtle changes of gastric precancer (i.e., dysplasia) and early cancer may not be apparent, limiting diagnostic accuracy. Positive endoscopic biopsy is the standard criterion for gastric precancer and cancer diagnosis, but it is invasive and impractical for screening high-risk patients who may have multiple suspicious lesions.1,2 Very recently, narrow-band imaging (NBI) that enhances visualization of irregular mucosal and vascular patterns has shown promise for improving in vivo diagnosis of intraepithelial neoplastic lesions in gastric tissue.3 Although the NBI technique provides good detection sensitivities, this wide-field endoscopic imaging modality still suffers from moderate diagnostic specificity due to the deficiency of revealing specific biochemical information of tissue. Hence, a noninvasive optical diagnostic technique providing a direct assessment of biochemical information of tissue, namely, in vivo Raman spectroscopy, has shown promise for improving in vivo diagnosis of intraepithelial neoplastic lesions in gastric tissue.4

Abstract. We first report on the implementation of a novel narrow-band image-guided Raman endoscopy technique for in vivo diagnosis of gastric dysplasia. High-quality in vivo Raman spectra can be acquired from normal and dysplastic gastric mucosal tissue within 0.5 sec under narrow-band image (NBI) guidance at gastroscopy. Significant differences are observed in in vivo Raman spectra between normal (n=54) and dysplastic (n=18) gastric tissue from 30 gastric patients, particularly in the spectral ranges of 825 to 950, 1000 to 1100, 1250 to 1500, and 1600 to 1800 cm⁻¹, which primarily contain signals related to proteins, nucleic acids, and lipids. The multivariate analysis [i.e., principal components analysis (PCA) and linear discriminant analysis (LDA)], together with the leave-one tissue site-out, cross validation on in vivo gastric Raman spectra yields a diagnostic sensitivity of 94.4% (17/18) and specificity of 96.3% (52/54) for distinction of gastric dysplastic tissue. This study suggests that narrow-band image-guided Raman endoscopy associated with PCA-LDA diagnostic algorithms has potential for the noninvasive, in vivo early diagnosis and detection of gastric precancer during clinical gastroscopic examination. © 2010 Society of Photo-Optical Instrumentation Engineers.

Keywords: gastric dysplasia; in vivo diagnosis; Raman endoscopy; narrow-band imaging; gastroscopy; precancer.

Paper 10011RR received Jan. 4, 2010; revised manuscript received Mar. 27, 2010; accepted for publication Apr. 1, 2010; published online Jun. 1, 2010.
formation. Diagnostic sensitivities of ~85 to 95% and specificities of ~90 to 98% have been reported for differentiation between normal and pathological (e.g., dysplasia, adenocarcinoma) gastric tissues in vitro using Raman spectroscopic technique.\textsuperscript{12–14} However, to date, in vivo Raman studies for early diagnosis of gastric precancer and cancer have not been reported. In vivo Raman endoscopic applications have been limited not only by the difficulty of capturing inherently weak tissue Raman signals, but also by the relatively high speed of spectral acquisitions required in clinical settings.\textsuperscript{4–6} The fabrication of a millimeter-scaled or even smaller flexible fiber optic Raman probe with abilities of efficient fiber evaporation in tissue\textsuperscript{6} is found to be optimal for fitting the broad autofluorescence background in the noise-smoothed spectrum, and this polynomial is then subtracted from the raw spectrum to yield the tissue Raman spectrum alone. Each background-subtracted Raman spectrum is also normalized to the integrated area under the curve from 800 to 1800 cm\(^{-1}\), enabling better comparison of the spectral shapes and relative peak intensities among different gastric tissues.\textsuperscript{5} All of the spectra preprocessing is completed online and the Raman spectrum can be displayed in real time during clinical Raman measurements at gastroscopy. The tridimensional endoscope imaging system primarily comprises a 300-W dedicated short-arc xenon light source, a gastrointestinal (GI) video-endoscope (GIF-FQ260Z, Olympus), and a video system processor (CV-260SL, Olympus) for white-light reflectance (WLR) imaging, autofluorescence imaging (AFI), and narrow-band imaging (NBI) during gastrointestinal examination. Both the wide-field endoscopic image (WLR/NBI/AFP) and the point-wise in vivo Raman spectra of the tissue imaged can be simultaneously acquired, stored, and displayed in real time on a color video monitor and computer screen, respectively.

In this work, all patients signed an informed consent permitting the in vivo Raman endoscopic measurements of gastric tissue in the Endoscope Centre at the National University Hospital (NUH), Singapore. This study was approved by the Institutional Review Board (IRB) of the National Healthcare Group (NHG) of Singapore. We have acquired in vivo Raman spectra [Fig. 1(a)] of 72 gastric mucosal tissue sites in 30 gastric patients (16 men and 14 women, with a median age of 67) under the guidance of wide-field endoscopic imaging [e.g., NBI and WLR imaging in Fig. 1(b)] during gastrointestinal examination. The Raman endoscopic probe was placed in gentle contact with the gastric mucosa surface, and the positioning against the tissue sites was verified on the endoscopy monitor by the endoscopists in charge during gastrointestinal examinations. Immediately after all Raman acquisitions, the biopsy samples were taken from the tissue sites measured (with suction markings) and fixed in 10% formalin solution for histopathological examinations by a senior gastrointestinal pathologist. For the assessment of diagnostic sensitivity and specificity of Raman endoscopy for gastric tissue classification, histopathological results served as the golden standard.

### 3 Results and Discussion

Figure 1(a) shows the in vivo mean Raman spectra \( \pm 1 \) standard deviations (SD) and the corresponding Raman difference spectrum of normal \((n=54)\) and dysplastic \((n=18)\) gastric tissue. The representative NBI and WLR image of dysplastic...
Raman spectra are observed in both normal and dysplastic gastric tissue acquired under Raman endoscopic measurements are shown in Fig. 1(b). All in vivo tissue Raman spectra are acquired within 0.1 to 0.5 sec (depending on the autofluorescence background level of different gastric tissues) with the 785-nm light irradiance power of 1.5 W/cm². Prominent Raman bands are observed in both normal and dysplastic gastric tissue at the following peak positions with tentative biochemical assignments: 875 cm⁻¹ [v(C=O)] hydroxyproline, 1004 cm⁻¹ [v(C-C)] ring breathing of phenylalanine, 1078 cm⁻¹ [v(CC) or v(CO) of phospholipids], 1265 cm⁻¹ [amide III v(CN) and δ(NH) of proteins], 1302 cm⁻¹ [CH₂CH₂ twisting of proteins and nucleic acids], 1450 cm⁻¹ [δ(CH₃) of proteins and lipids], 1655 cm⁻¹ [amide I v(C-O) of proteins], and 1745 cm⁻¹ [v(C=O) of phospholipids]. The difference spectrum [Fig. 1(a)] reveals the changes of relative percentages of distinctive biomolecules in dysplastic tissue, particularly in the spectral ranges of 825 to 950, 1000 to 1120, 1250 to 1500, and 1600 to 1800 cm⁻¹, associated with dysplastic transformation. Note that in vivo Raman spectra of both normal and dysplastic gastric tissues are vertically shifted for better visualization. The shaded areas in tissue Raman spectra stand for the respective standard deviations. (b) Narrow-band image (NBI) and white-light reflectance (WLR) image of dysplastic gastric lesions in the antrum acquired during clinical gastroscopy. NBI enhances the observation of the irregular mucosal glandular patterns and vascular patterns of dysplastic gastric mucosal lesions.

Fig. 1 In vivo mean Raman spectra±1 standard deviations (SD) of normal (n=54) and dysplastic (n=18) gastric tissue. The difference spectrum (i.e., the mean Raman spectrum of dysplastic tissue minus the mean Raman spectrum of normal tissue) reveals the Raman spectral changes, particularly in the regions of 825 to 950, 1000 to 1120, 1250 to 1500, and 1600 to 1800 cm⁻¹, associated with dysplastic transformation. Note that in vivo Raman spectra of both normal and dysplastic gastric tissues are vertically shifted for better visualization. The shaded areas in tissue Raman spectra stand for the respective standard deviations. (b) Narrow-band image (NBI) and white-light reflectance (WLR) image of dysplastic gastric lesions in the antrum acquired during clinical gastroscopy. NBI enhances the observation of the irregular mucosal glandular patterns and vascular patterns of dysplastic gastric mucosal lesions.

Fig. 2 The three significant principal components (PCs) (PC1 ~52.6%, PC2 ~13.2%, and PC4 ~4.7%) accounting for ~70.5% of the total variance calculated from in vivo gastric Raman spectra.
Raman spectra of normal and precancer gastric tissue using PCA-LDA together with the leave-one tissue site-out, cross-validation techniques. The PCA-LDA algorithm based on in vivo gastric Raman spectra provides a diagnostic sensitivity of 94.4% (17/18) and specificity of 96.3% (52/54) for distinguishing dysplasia from normal gastric tissue in vivo. To further evaluate the performance of the PCA-LDA-based diagnostic algorithms derived from all three significant PCs of in vivo gastric Raman datasets, the receiver operating characteristic (ROC) curve (Fig. 4) is also generated from the scatter plot in Fig. 3 at different threshold levels, displaying the discrimination results using PCA-LDA diagnostic algorithms together with the leave-one tissue site-out, cross-validation method. The integration area under the ROC curve is 0.997, further demonstrating the diagnostic efficacy of Raman endoscopy for in vivo diagnosis of gastric precancer during clinical gastroscopic examination.

In summary, we have acquired, for the first time, high-quality in vivo Raman spectra from normal and dysplastic gastric tissue within 0.5 sec under narrow-band imaging guidance during clinical gastroscopy. We have observed the significant differences in in vivo Raman spectra between normal and dysplastic gastric tissue. Good differentiation between normal and dysplastic gastric tissues can be achieved using PCA-LDA diagnostic algorithms, indicating the potential of Raman endoscopy for in vivo diagnosis of gastric precancer. Currently, we are conducting in vivo Raman measurements on a larger series of gastric patients at National University Hospital, Singapore, to further evaluate the clinical merits of Raman endoscopy techniques for prospective prediction of gastric precancer and early cancer in vivo during gastroscopic examination.

Acknowledgments
This research was supported by the National Medical Research Council, the Biomedical Research Council, and the Faculty Research Fund from the National University of Singapore.

References


