

## Raman spectroscopy for early real-time endoscopic optical diagnosis based on biochemical changes during the carcinogenesis of Barrett's esophagus

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### Abstract

Raman spectroscopy is a spectroscopic technique based on the inelastic scattering of monochromatic light that represents the molecular composition of the interrogated volume to provide a direct molecular fingerprint. Several investigations have revealed that confocal Raman spectroscopy can differentiate non-dysplastic Barrett's esophagus from esophageal high-grade dysplasia and adenocarcinoma with high sensitivity and specificity. An automated on-line Raman spectral diagnostic system has made it possible to use Raman spectroscopy to guide accurate target biopsy instead of multiple random forceps-biopsies, this novel system is expected to improve *in vivo* precancerous diagnosis and tissue characterization of Barrett's esophagus.

**Key words:** Raman spectroscopy; Barrett's esophagus; Confocal; High-grade dysplasia; Diagnosis

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**Core tip:** Raman spectroscopy is a very sensitive tool to detect subtle biochemical and molecular changes, which is crucial for differentiating nondysplastic from high-grade dysplastic Barrett's esophagus. With an increased accuracy of updated algorithms and a real time automatic analysis system, Raman spectroscopy is expected to improve *in vivo* precancerous diagnosis and tissue characterization of Barrett's esophagus.

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## INTRODUCTION

Confirmed by the presence of intestinal metaplasia with or without goblet cells from a squamous to a columnar-lined esophageal epithelium<sup>[1,2]</sup>, Barrett's esophagus is a metaplastic precursor of esophageal adenocarcinoma. Given the poor prognosis that has remained relatively constant, with current 5-year survival rates of only 8% to 15%<sup>[3]</sup>, early identification of Barrett's esophagus associated with high-grade dysplasia followed by targeted endoscopic resection is the most critical measure to prevent progression to invasive esophageal malignancy<sup>[4]</sup>. According to the current diagnostic guidelines, patients with Barrett's esophagus are recommended to undergo strict biopsy samplings (typically 4-quadrant random samplings) for every 2 cm of Barrett's mucosa during endoscopic surveys at intervals of 3 to 5 years. This approach may produce a large number of negative biopsies and increase the risk of bleeding. Considering the elevated incidence of esophageal adenocarcinoma, the need for new advanced endoscopic technologies that can transition standard Barrett's esophagus surveillance from random biopsies to a real-time "optical biopsy" is imperative.

Optical spectroscopy is a technique that utilizes microstructural information contained in light-tissue interactions to enhance suspicious tissue recognition during standard endoscopy<sup>[5]</sup>, including fluorescence, elastic scattering, and inelastic (Raman) scattering.

### Principle of raman spectroscopy

Raman spectroscopy represents a unique optical vibrational technique based on the inelastic scattering of a monochromatic laser light source. Inelastic scattering is a phenomenon in which the frequency of the scattered photon is shifted up or down with respect to the incident excitation light depending on the specific vibrational motions of the molecules in the tissue being interrogated, which is called the Raman effect. This shift of frequency provides unique information on the scattering molecules.

Taking the unique advantage of the ability of Raman spectroscopy to harvest a wealth of direct molecular fingerprint information from inter and/or intracellular components such as proteins, lipids, carbohydrates and DNA in cells and tissue, Raman spectroscopy has shown great promise for histo-pathologic assessments at the biochemical and molecular levels<sup>[6]</sup>. Because the progression from non-dysplastic Barrett's esophagus to esophagus adenocarcinoma manifests a progressive series of molecular and biochemical changes, Raman spectroscopy may provide the capability to analyze the carcinogenesis process. Furthermore, the majority of biological molecules are Raman active, each with its own unique fingerprint. As a result, Raman spectroscopy is a very sensitive tool to detect subtle biochemical and molecular changes, which is crucial for differentiating nondysplastic from high-grade dysplastic Barrett's esophagus.

### Overall configuration of the raman spectroscopy system

Briefly, the Raman spectroscopy system consists of four major components<sup>[6]</sup>: A light generator (near-infrared diode laser); light collection optics; a wavelength selector (filter or spectrophotometer); and a detector (photodiode array, charge coupled device or photomultiplier tube). Compared with an ultraviolet ray illumination source, near infrared excitation not only minimizes spectral disruption from tissue fluorescence but also produces reduced mutagenic effects and deeper penetration capability.

The combination of Raman spectroscopy and an endoscopic system is realized by a Raman probe, which is coupled to an optical cable containing the excitation and collection fibers, with an outer diameter enabling easy passage through the instrument channel of an endoscope. Currently, the two novel confocal Raman probes<sup>[3,4]</sup> have the following advantages: They ensure the precise interrogation of the epithelium (with a volume of  $< 0.02 \text{ mm}^3$ ), which is closely related to early onset of Barrett's carcinogenesis, because the ratio of the epithelium to stromal Raman photons collected is 19-fold higher than that collected using previous volume-type Raman probes; and they provide the capacity for reproducible and objective Raman measurements achieved in a direct contact mode.

### Clinical application

Water molecules, the predominant constituents of living tissue, have a negligible influence on Raman signals due to the limited change in the polarity of the -OH bond, which enables Raman spectroscopic analysis of fresh, unprepared tissue, both *ex vivo* and *in vivo*.

Robles<sup>[5]</sup> summarized some clinical research on Raman spectroscopic technology for classification of malignant changes in Barrett's esophagus, carried out by two groups, from Gloucestershire Royal Hospital<sup>[3]</sup>, United Kingdom and the National University of Singapore<sup>[4]</sup>, Singapore. The latter demonstrated for the first time that confocal Raman spectroscopy can be used to target dysplasia identification and subsequent biopsy in Barrett's esophagus in real-time, which has also been used to diagnose gastric<sup>[7]</sup> and colorectal<sup>[8]</sup> lesions. The characteristics of the two abovementioned confocal probes were compared and listed in Table 1.

At present, most biomedical Raman research on pre-cancer and early cancer diagnosis remain focused on the fingerprint (FP) Raman spectra, which contain rich biochemical information regarding the tissue; however, some extremely weak tissue Raman signals in certain organ sites may be overwhelmed by the tissue autofluorescence (AF) background. Because the high-wavenumber (HW) Raman spectral range exhibits stronger tissue Raman signals with less AF interference, it has been integrated with the FP Raman spectra to improve the real-time *in vivo* diagnosis of esophageal squamous cell carcinoma (ESCC) during endoscopic examination, resulting in a predictive diagnostic sensitivity of 92.7% and specificity of 93.6% for ESCC

**Table 1 Comparison of two endoscopic confocal raman spectroscopic systems**

Technical parameters	Developed by Almond <i>et al.</i> <sup>[3]</sup>	Developed by Bergholt <i>et al.</i> <sup>[4]</sup>
λex	830 nm	785 nm
Diameter of probe	2.7 mm	1.8 mm
Range of Raman spectra	400-1850 cm <sup>-1</sup>	800-1800 cm <sup>-1</sup>
Acquisition times	1 s	0.2 s
Classification model	Principal component fed linear discriminant analysis	Partial least-squares discriminant analysis <sup>[9]</sup>
Diagnostic way	<i>Ex vivo</i>	Real-time <i>in vivo</i>
Sensitivity and specificity for detecting HGD in BE	86% and 88%	87.0% and 84.7%

HGD: High grade dysplasia; BE: Barrett's esophagus.

identification<sup>[10]</sup>.

## CONCLUSION

Despite some limitations, such as only identifying molecular features, susceptibility to interference of fluorescence from impurities or from the sample itself, and thermal damage to tissues, confocal Raman spectroscopy uncovers the biochemical and molecular changes occurring in the epithelium during Barrett's carcinogenesis. This technique is expected to improve *in vivo* precancerous diagnosis and tissue characterization of Barrett's esophagus with increased accuracy based upon updated algorithms and the on-line real time automatic analysis system.

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