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Diagnostic Imaging of Esophageal Epithelium with Clinical Endoscopic Polarized Scanning Spectroscopy Instrument

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Abstract—**This letter reports the development of an endoscopic polarized scanning spectroscopy (EPSS) instrument compatible with existing endoscopes. This instrument uses light scattering spectroscopy (LSS). In proof-of-principle studies using a single-point instrument, LSS has successfully demonstrated the ability to identify pre-cancer in the epithelial tissues of five different organs, including Barrett's esophagus (BE). The EPSS instrument can provide real time in vivo information on the location of otherwise invisible high grade dysplasia (HGD), a predictor of adenocarcinoma, and thus can serve as a guide for biopsy. It should greatly reduce the time and labor involved in performing screening and obtaining diagnoses, cause less patient discomfort and ensure that fewer biopsies are required for the reliable location of pre-cancerous lesions.**

I. Introduction

THE incidence of adenocarcinoma of the esophagus is increasing more rapidly than any other type of carcinoma in the United States [1]. Almost 100% of cases occur in patients with Barrett's Esophagus (BE), a benign condition in which metaplastic columnar epithelium replaces the normal squamous epithelium of the esophagus. Although the prognosis of patients diagnosed with adenocarcinoma is poor, the chances of successful treatment increase significantly if the disease is detected at the dysplastic stage. The surveillance of patients with BE for dysplasia is challenging in three respects. First, dysplasia is not visible during routine endoscopy [2]. Thus, numerous random biopsy specimens are required. Second, the histopathologic diagnosis of dysplasia is problematic because there is poor interobserver agreement on the classification of a particular specimen, even among expert gastrointestinal pathologists [3, 4]. Third, reliance on histology imposes a time delay between endoscopy and diagnosis, limiting the diagnostic utility of the endoscopic procedure.

Optical spectroscopy based techniques offer great promise in the early detection of dysplasia in BE since they can greatly reduce the time and labor involved in performing screening and obtaining diagnoses, will cause less patient discomfort, require fewer biopsies, and can help the pathologist to base his diagnosis on uniform quantitative criteria, making the diagnosis more consistent. Thus, Barrett's Esophagus has been widely studied optically, using laser-induced fluorescence (LIF) spectroscopy [5, 6] light scattering spectroscopy (LSS) [7-9], diffuse reflectance spectroscopy [10], Raman spectroscopy [11] and optical coherence tomography (OCT) [12].

II. Polarized Light Scattering Spectroscopy

Enlarged, dense and crowded epithelial nuclei are the primary indicators of cancer, dysplasia and cell regeneration in BE [13]. Light scattering spectra from tissues *in vivo* consist of a small backscattered component that is oscillatory in wavelength because of scattering by those nuclei, superimposed on a large background from submucosal tissue. The shape of the backscattered component over the wavelength range is related to nuclear size. The amplitude of this component is related to the number density of epithelial nuclei (number of nuclei per unit area), a measure of nuclear crowding.

When tissue is illuminated with polarized light, the light backscattered from the superficial epithelial layer retains its polarization, i.e. it is polarized parallel to the incoming light. The light backscattered from the deeper tissues becomes depolarized and contains equal amounts of parallel and perpendicular polarizations. By subtracting the two polarizations we can cancel out the contribution of the deeper tissues and the resulting signal is proportional only to the signal from the superficial epithelial layer, which contains the information about early precancerous changes. This has been verified in our previous work [14].

III. Clinical Endoscopic Polarized Scanning Spectroscopy Instrument

LSS-based detection of dysplasia in BE has been demonstrated successfully using a simple proof-of-principle single-point instrument [7-9]. This instrument was capable of

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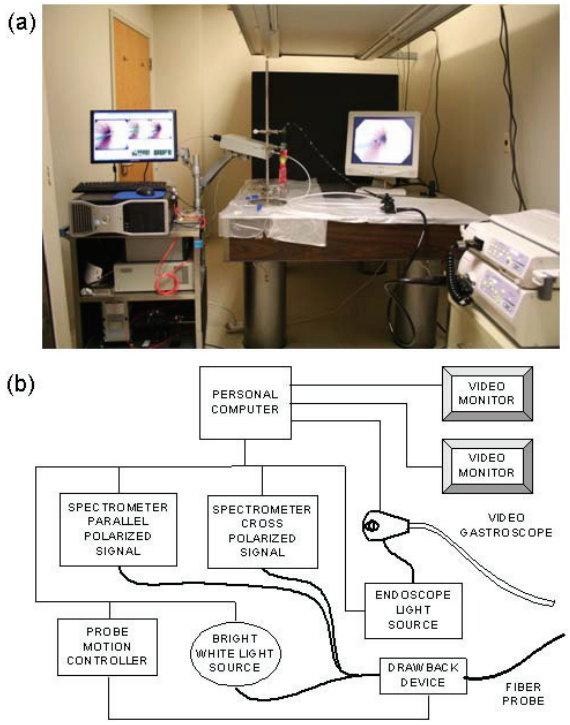

Fig. 1. Clinical EPSS instrument. (a) EPSS instrument performing measurements in *ex vivo* bovine esophagus. During the procedure the fiberoptic probe is inserted into the gastroscope's instrument channel; (b) instrument system block diagram.

collecting data at randomly selected sites, which were then biopsied. The data was processed off-line, and a comparison with biopsy results was made at a later time. The high correlation between spectroscopic results and pathology was sufficiently promising to justify the development of the clinical device, which is described herein.

Our recently developed clinical endoscopic polarized scanning spectroscopy (EPSS) instrument (Fig. 1a) is compatible with existing endoscopes. It scans large areas of the esophagus chosen by the physician, and has the software and algorithms necessary to obtain quantitative, objective data about tissue structure and composition, which can be translated into diagnostic information in real time. This enables the physician to take confirming biopsies at suspicious sites and minimize the number of biopsies taken at non-dysplastic sites.

The instrument detects polarized light coming primarily from the epithelial layer. Although principally using the polarization technique to extract diagnostic information about dysplasia, we also sum the two polarizations to permit the use of diffuse reflectance spectroscopy, which also can provide information about early stages of adenocarcinoma [10].

The EPSS instrument is a significant advance over the single-point fiber-optic instrument in that: (1) it scans the esophagus and has the software and algorithms necessary to obtain quantitative, objective data about tissue structure and composition, which can be translated into diagnostic information and guide biopsy in real time; (2) it employs collimated illumination and collection optics, which enables the instrument to generate maps of epithelial tissue not affected by the distance between the probe tip and the mucosal surface, making it dramatically less sensitive to peristaltic motion; (3) it incorporates both the polarization technique for removing the unwanted background in the LSS signal, and single backscattering in the diffuse reflectance spectroscopy signal; (4) it integrates the data analysis software with the instrument in order to provide the physician with real time diagnostic information; (5) it combines LSS information with diffuse reflectance spectroscopy information measured by the same instrument, thereby improving the diagnostic assessment capability.

A block diagram of the EPSS instrument is shown in Fig. 1b. The instrument uses commercially available gastroscopes and video processors. A standard PC is adapted to control the system. Commercially available spectrometers are also employed.

The polarized LSS (PLSS) scanning fiber probe consists of a delivery and two receiver fibers. A polarizer is mounted at the ends of the fibers so that the delivery fiber and one receiver fiber are polarized in the same direction, and the other receiver fiber is polarized in the orthogonal direction. A parabolic mirror above the fibers collimates the illumination beam and ensures that three fibers have the maximum overlap at around 11 mm from the probe axis which is the radius of a typical adult human esophagus in its natural state. Light coming out of the probe is pointing backwards at approximately 20 degrees from the normal to the probe axis to avoid specular reflections.

The rotation and linear motion of the distal tip of the probe is transmitted via a stainless steel, parylene-coated torque tube forming the outer surface of the probe. The torque tube is inserted into a control box which provides rotary and linear motions using two individual stepper motors. .

Custom designed software which can perform both scanning and data collection in imaging and single point modes were developed within a LabView 8.5 (National Instrument) interface. Video from the endoscope camera is acquired via the S-video output port of the video processor. It is overlaid with a pseudo-color map representing PLSS data. Currently the instrument collects 30 data points for each rotary scan and performs ten steps during a linear scan (2 mm per step) collecting 300 data points in 2 minutes for each 2 cm segment of BE.

For rapid extraction of histological and biochemical tissue parameters, we developed an inverse algorithm based on least–squares minimization, incorporating the advances from [15]. Since there can be multiple minima, we determined the biologically relevant intervals for each of the model parameters, and use those as constraints in the minimization procedure to improve data extraction. The algorithm was tested on phantoms and has been used in clinical data analysis.

IV. Experiments and Results

We checked the performance of the instrument in experiments using three freshly resected bovine esophagi from an abattoir. The intact bovine esophagus was mounted vertically and an Olympus GIF-H180 clinical endoscope was

inserted into the esophagus (Fig. 1a). The esophagus was scanned point-by-point and the PLSS data recorded.

We then performed histological examination of the sites where the PLSS data were collected. The bovine esophagus was cut and opened along its longitudinal axis. Esophageal tissues were laid flat and fixed with 10% formalin for at least 24 hours, then processed and embedded in paraffin. Five micrometer thick serial sections were stained with hematoxylin and eosin (H&E) and photographed with a Zeiss Axio microscope.

The esophageal wall consists of four layers: mucosa, submucosa, muscularis propria and adventitia. Mucosa is the top layer and consists of epithelium, lamina propria and muscle tissue. As shown in Fig. 2a, the esophagus has stratified squamous epithelia to around 200 □m depth, whose purpose is to protect and lubricate during swallowing. Cells become flattened toward the surface. Lamina propria is a thin layer of connective tissue right under the epithelium layer, which contains collagen, reticular and sometimes elastic fibers. The muscularis mucosa, a thin band of longitudinal smooth muscle, extends throughout the entire esophagus.

As we can see in Fig. 2a, the cells are very concentrated at the bottom of the epithelium layer where they originate. There, nuclei occupy almost the whole cell. The distance between cells increases as they move to the surface and the nuclei become elongated.

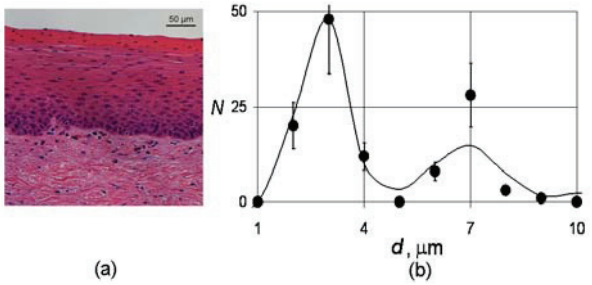

Fig. 2. Bovine lower-portion esophagus epithelium. (a) H&E staining. (b) Comparison of the nuclear size distributions extracted from the EPSS instrument measurements on intact epithelium (solid curve) and histological examination of the corresponding H&E stained sections (black dots).

LSS backscattering spectra depend mainly on the dimension of the scatterers along the direction of light propagation. Thus, short axis diameters should contribute most in the measurements described here. The nuclei in the upper part of the epithelium have short axes of approximately 3 μm. In the bottom part of the epithelium, nuclear sizes vary from 5 μm to 8 μm and are randomly oriented. By measuring nuclear sizes from the H&E stained image and comparing with the PLSS result, we observe reasonable agreement for the nuclear size distribution measurement along the light propagation direction (Fig. 2b).

We then performed clinical measurements using the EPSS instrument during a routine endoscopic procedure at the Interventional Endoscopy Center at Beth Israel Deaconess Medical Center for a patient with suspected dysplasia. The protocol was reviewed by the IRB of Beth Israel Deaconess Medical Center and the requisite approvals were obtained. The procedure, indications, preparation and potential complications were explained to the patient, who indicated his understanding and signed the corresponding consent forms. The patient was administered conscious sedation. The patient was placed in the left lateral decubitus position and an endoscope was introduced through the mouth and advanced under direct visualization until the second part of the duodenum was reached. Careful visualization of the upper GI tract was performed. The procedure was not difficult. The patient tolerated the procedure well. There were no complications.

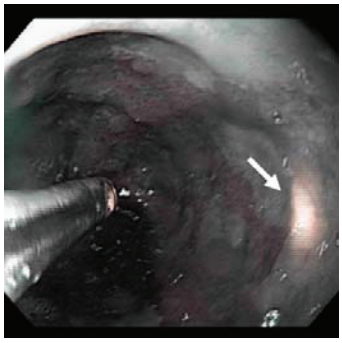

Fig. 3. Photograph of the PLSS probe performing scanning of BE during a clinical procedure obtained via the endoscope video channel. The "flying spot" is clearly seen on the esophagus wall to the right.

A salmon colored mucosa distributed in a diffuse pattern, suggestive of BE was found. Spectroscopy of the entire Barrett's segment was performed by scanning three adjacent 2 cm sections of BE with the PLSS probe. Narrow band imaging available with the Olympus EVIS EXERA II, CV 180 video controller was used simultaneously with spectroscopy recording and no interference from the endoscopic light source is observed in the PLSS spectra in the band from 600 nm to 800 nm. After scanning the 6 cm section of BE, four quadrant biopsies were performed every centimeter, the current standard of care. In addition, point spectroscopy was performed at several individual locations of BE and subsequently cold forceps biopsies were performed for histology at those sites as well.

V. Discussion

Spectroscopic data collected during the clinical procedures confirms that the polarization technique is very effective in removing the unwanted background signals. For example, data from a random spatial location presented in Fig. 4a shows that the perpendicular polarization spectral component exhibits standard diffuse reflectance features originating in the deeper tissue layers, with the hemoglobin absorption bands clearly observable in the 540-580 nm region. At the same time the parallel polarization spectral component, in addition to diffuse features, exhibits a very clear oscillatory structure, characteristic of the diagnostically important nuclear scattering originating in the uppermost epithelial layer.

Another important observation comes from the fact that spectra collected from multiple spatial locations inside the BE

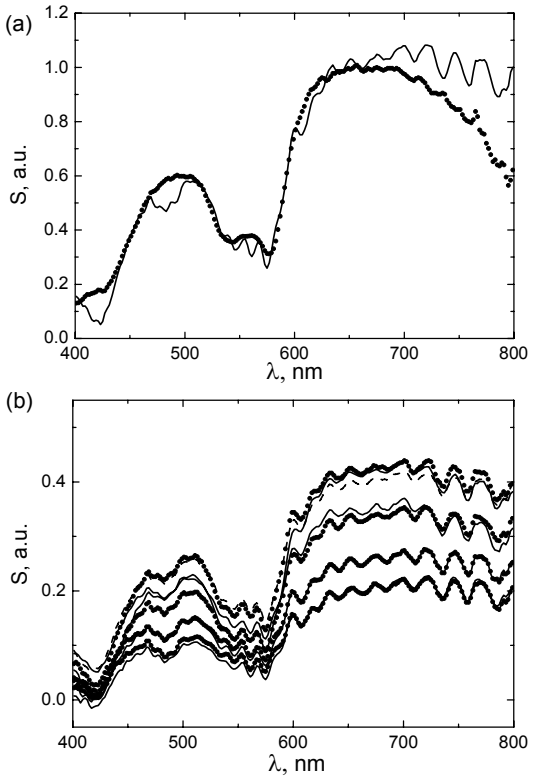

Fig. 4. (a) Parallel (solid line) and perpendicular (dotted line) polarization spectra collected with the EPSS instrument from a single spatial location in a patient with BE; (b) parallel polarization spectra from 10 different locations in the same patient.

are insensitive to the effects of motion. During a procedure, it is difficult to maintain a fixed distance between the optical probe head and the esophageal surface, due to peristaltic motion and other factors. Therefore, an important feature of the EPSS instrument is its ability to collect spectra of epithelial tissue that are not affected by the orientation and the distance between the distal tip of the probe and the mucosal surface. This is achieved with collimated illumination and collection optics. By analyzing parallel polarization spectra collected at multiple locations inside a BE during a standard clinical procedure (see the 10 spectra in Fig. 4b) we found that though the amplitudes of the spectra differ from point to point, the spectral shape is practically unchanged and, what is even more important, the oscillatory structure containing the diagnostically significant information is intact. Thus the issue of peristaltic motion appears to be addressed in the EPSS instrument.

The small number of patients measured to date does not provide us with sufficient statistics to make firm conclusions about diagnostic characteristics of the EPSS instrument. This information will be provided in a future publication.

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