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Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus

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Abstract

Esophageal cancer is increasing in frequency in the United States faster than any other cancer. Barrett's esophagus, an otherwise benign complication of esophageal reflux, affects approximately three million Americans and precedes almost all cases of esophageal cancer. If detected as high-grade dysplasia (HGD), most esophageal cancers can be prevented. Standard-of-care screening for dysplasia uses visual endoscopy and a prescribed pattern of biopsy. This procedure, in which a tiny fraction of the affected tissue is selected for pathological examination, has a low probability of detection because dysplasia is highly focal and visually indistinguishable. We developed a system called endoscopic polarized scanning spectroscopy (EPSS), which performs rapid optical scanning and multispectral imaging of the entire esophageal surface and provides diagnoses in near real time. By detecting and mapping suspicious sites, guided biopsy of invisible, precancerous dysplasia becomes practicable. Here we report the development of EPSS and its application in several clinical cases, one of which merits special consideration.

Previously we demonstrated that spectroscopic information in light scattered by nuclei could reveal precancer cellular changes[1]. The first application of light-scattering spectroscopy successfully detected dysplasia in Barrett's esophagus[1–5] using a fiber optic probe that illuminated 1 mm^2 of tissue. Searching the entire area of a diseased esophagus with a single-point probe is clinically impractical.

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Note: Supplementary information is available on the Nature Medicine website.

AUTHOR CONTRIBUTIONS

L.Q., E.V., M.D.M., E.B.H., I.I. and L.T.P. developed and evaluated the method; S.I., L.Q. and E.V. contributed codes for instrument control; D.K.P., R.C., J.D.G., J.L., N.O., L.G., L.Q. and A.S. performed clinical procedures; L.Q., D.K.P., R.C., E.B.H., I.I. and L.T.P. contributed to the writing of the manuscript; E.B.H., I.I., D.K.P., R.C. and L.T.P. designed and planned the project.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Since then, several new approaches have been explored using high-resolution endoscopy (HRE) combined with narrow band imaging (NBI)[6], autofluorescence imaging (AFI)[7], trimodal imaging[8], which is a combination of the previous three, and confocal laser endo-microscopy[9]. These techniques showed promise in increased detection of dysplasia in Barrett's esophagus, although none has as yet achieved clinical acceptance.

A clinically useful technique in the detection of dysplasia in Barrett's esophagus must rapidly survey a comparatively large area while simultaneously detecting changes on a cellular scale. We felt we could achieve both goals by combining an endoscopically compatible scanning instrument with polarized light-scattering spectroscopy (PLSS)[5], an optical technique that relates the spectroscopic properties of light elastically scattered by epithelial cell nuclei to their size, shape and refractive index. Polarization subtraction in PLSS cancels the contribution of the deeper tissues. Here we report the development and early tests in humans of the EPSS instrument, which achieves these goals.

Light-scattering spectra from tissues *in vivo* consist of a Rayleigh component due to scattering by small organelles, a large background from submucosal tissue[10] and a relatively small backscattered component due to epithelial cell nuclei. The shape of the backscattered spectrum over the wavelength range is a measure of nuclear size[2], and its amplitude is related to the number density of epithelial nuclei (nuclei per unit area), a measure of nuclear crowding. Enlarged, dense and crowded epithelial nuclei are the primary pathological indicators of cancer, dysplasia and cell regeneration in Barrett's esophagus[11].

Detection of the backscattered component from the epithelium can be enhanced by using polarized light. Light backscattered from the superficial epithelial layer retains its polarization and is polarized parallel to the incoming light. Light backscattered from the deeper tissues becomes depolarized, containing equal amounts of parallel and perpendicular polarizations; subtracting the two cancels the contribution of the deeper tissues. The result is proportional only to the signal from the superficial epithelial layer, which contains the information about early precancerous changes[5].

Although we use the polarization technique principally to extract diagnostic information about dysplasia from the epithelial layer, we also sum the two polarizations, which, by diffuse reflectance spectroscopy, provides additional information about early stages of adenocarcinoma[12].

The EPSS instrument (Fig. 1) is compatible with existing endoscopes, can scan any esophageal area chosen by the physician and has the software and algorithms necessary to obtain quantitative, objective data about tissue structure and composition. These data can be translated into diagnostic information in real time, thus providing the location of otherwise invisible HGD *in vivo* and serving as a guide for biopsy. This enables the physician to take confirming biopsies at suspicious sites, minimizes the number of biopsies taken at non-dysplastic sites, reduces the time and labor involved in screening and diagnosis, causes less discomfort for the subject and ensures reliable detection of precancerous lesions.

The EPSS instrument (Fig. 1) uses commercially available endoscopes, video processors, spectrometers and a standard PC for system control. The outer surface of the EPSS probe is a stainless steel, parylene-coated torque tube that provides rotary and linear scanning via a control box with two stepper motors. The probe consists of a delivery fiber and a receiver fiber that are polarized in parallel, and a second receiver fiber that is polarized orthogonally. A parabolic mirror at the distal tip of the probe (Supplementary Fig. 1) collimates the illumination beam and ensures maximum overlap of the three visual fields at around 11 mm from the probe axis, the radius of a typical adult human esophagus. Light is emitted approximately 70° proximal to the probe axis to avoid specular reflections.

RESULTS

We checked EPSS instrument performance using freshly resected bovine esophagi. We inserted an endoscope into a vertically mounted bovine esophagus, which was then scanned point by point, and recorded the data. We also took histological specimens at the EPSS data collection sites (Supplementary Methods). Comparing nuclear sizes in the H&E image with the EPSS result showed reasonable agreement (Supplementary Fig. 2).

We performed clinical measurements using EPSS during routine endoscopic procedures (Fig. 2 and Supplementary Video 1) for individuals with suspected dysplasia at the Beth Israel Deaconess Medical Center (BIDMC) Interventional Endoscopy Center (IEC). All studies used a state-of-the-art, high-resolution endoscope with NBI. Subjects reporting to the IEC at BIDMC underwent initial screening at other institutions and were referred with confirmed Barrett's esophagus and suspicion of dysplasia. We explained the procedure, indications, preparation and potential complications to the subjects, who indicated their understanding and signed the corresponding consent forms. Our protocol was reviewed by the BIDMC Institutional Review Board, and the requisite approvals were obtained.

Addressing unwanted background and peristaltic motion

Two observations support the clinical feasibility of this method. First, spectroscopic data collected during clinical procedures confirm that the polarization technique is effective in removing unwanted background signals. For example, the perpendicular polarization spectral component, originating in the deeper tissue layers, shows standard diffuse reflectance features, with hemoglobin absorption bands clearly observable in the 540–580-nm region. The parallel polarization spectral component, in addition to diffuse features, has a clear oscillatory structure, which is characteristic of diagnostically important nuclear scattering originating in the uppermost epithelial layer (Fig. 3a).

Second, the issue of peristaltic motion is addressed by EPSS. During a procedure, it is difficult to maintain a fixed distance between the optical probe head and the esophageal surface, owing to peristaltic motion and other factors. Therefore, a key feature of the EPSS instrument is its ability to collect spectra of epithelial tissue that are not affected by the orientation or distance of the distal probe tip to the mucosal surface. This is achieved with collimated illumination and collection optics. Analysis of parallel polarization spectra collected at ten locations during a standard clinical procedure (Fig. 3b) showed that, although amplitudes of the spectra differ from site to site, the spectral shape is practically unchanged. The fluctuation of the normalized difference of the perpendicular and parallel spectra in the 600–800-nm spectral range, which carries the diagnostic information, is substantially less than 10% for non-dysplastic sites, regardless of the distance of the probe from the esophageal wall.

Endoscopic studies

To date, we have collected a total of 10,800 EPSS spectra in eight procedures, covering the entire scanned regions of the esophagus in seven subjects. We validated the capabilities of the method by comparing EPSS data with subsequent pathology at each site where biopsies were taken. For the first two subjects, pathology was reported per quadrant, rather than per biopsy, and so we did not use the data.

For the other subjects, we collected 95 biopsies according to the standard-of-care protocol[13,14]. We recorded the locations of biopsied tissue sites by their distances from the upper incisors and their angles relative to the start of the EPSS scan (Fig. 4). Pathological examination revealed a total of 13 dysplastic sites, of which nine were classed as HGD and four as low-grade dysplasia (LGD). The rest of the sites were diagnosed as nondysplastic.

We extracted the diagnoses for each EPSS location from the residuals of the parallel and perpendicular backscattered spectral components collected by the EPSS instrument. The results are presented as pseudocolor maps (Fig. 4a).

Double-blind comparison of the EPSS maps with the biopsy reports revealed 11 true-positive sites, 3 false-positive sites, 80 true-negative sites and 1 false-negative site (Fig. 4a). Thus, EPSS measurements are characterized by a sensitivity of 92% and a specificity of 96%.

Our third Barrett's esophagus subject (the first from whom we took individually marked biopsies; subject A in Fig. 4a) underwent endoscopy and biopsy, concurrent with EPSS. Visual endoscopic examination using HRE with NBI did not reveal any areas suspicious for dysplasia. Pathology of tissue biopsies taken in the pattern prescribed by the standard-of-care protocol revealed no dysplasia, and we dismissed the subject. However, our EPSS scan indicated several probable sites of focal dysplasia, which were located in regions where biopsies were not taken. Therefore, we recalled subject A and took several biopsies in the vicinity of each site indicated by EPSS; we also repeated the standard-of-care protocol. A freeze frame of the endoscopic video image of a site identified by EPSS as suspicious for dysplasia demonstrates that the site is visually indistinguishable from the surrounding nondysplastic tissue, even under HRE with NBI. This site is marked by an arrow (Fig. 5). Pathology confirmed HGD at all three EPSS-directed sites and one additional HGD at a point located between two EPSS-indicated sites (Fig. 4b). The latter site, considered a false negative, is close to the sites indicated by EPSS and may arise from imperfect correspondence of the actual biopsy site with the EPSS-mapped site. This subject will now be given appropriate treatment. Standard-of-care procedures, even when diligently performed by highly skilled and experienced gastroenterologists, can miss focal dysplasias, because these procedures biopsy only a small fraction of esophageal tissue, blindly, according to the prescribed protocol[15,16]. The capability of EPSS to examine the entire esophageal epithelium millimeter by millimeter enables detection of dysplastic cells and guidance of confirmative biopsy, greatly increasing the probability of early detection and treatment and, in all likelihood, of saving lives.

Pathology found HGD in biopsies from subjects B and E, who will be treated. We found no suspicious sites in subject C (Fig. 4a). However, using EPSS, we identified a number of suspicious sites in subject D, whereas standard-of-care biopsies located no abnormal pathology. Subject D will now be recalled.

The frequency of dysplasia in our subject sample is consistent with that of the prescreened population referred to the BIDMC IEC for confirmation and treatment but is higher than would be expected in the general Barrett's esophagus subject population. In fact, the frequency of HGD detection in the general population of Barrett's esophagus subjects underscores the importance of having more comprehensive and effective methods for gastroesophageal cancer screening.

DISCUSSION

EPSS is a considerable advance for several reasons: (i) it scans the entire esophagus; (ii) it integrates data-analysis software with the instrument, providing the physician with real-time diagnostic information for guiding biopsy; (iii) it uses collimated illumination and collection optics, enabling multispectral mapping of epithelial tissue unaffected by peristaltic motion; and (iv) it combines PLSS information with diffuse reflectance spectroscopy information in the same instrument, thereby improving diagnostic assessment capability.

The EPSS technique can rapidly survey large areas of tissue to discover invisible dysplasia, which is a major advantage over single-point approaches, such as our original nonscanning techniques[1,3,4], or newer techniques that have a submillimeter field of view, such as confocal laser endomicroscopy[9]. Single-point methods without scanning or wide-field modalities are not suitable for guiding biopsy in realistic clinical settings. Compared to state-of-the-art wide-field techniques, such as the HRE with NBI used in these studies, or emerging methods such as AFI[7] and trimodal imaging (HRE with NBI and AFI)[8], EPSS is distinguished by its ability to locate dysplasia in tissue that shows no visible abnormalities or lesions when observed with white light or fluorescence. By elucidating microscopic subcellular structure with macroscopic spectral measurements (Supplementary Fig. 3), EPSS can locate dysplastic tissue independent of any visual cues.

We conclude that EPSS offers great promise for the early detection of dysplasia in Barrett's esophagus. If the EPSS technique were to be used to guide biopsy routinely, unnecessary biopsies would be avoided and focal dysplastic spots would be biopsied that otherwise would be missed.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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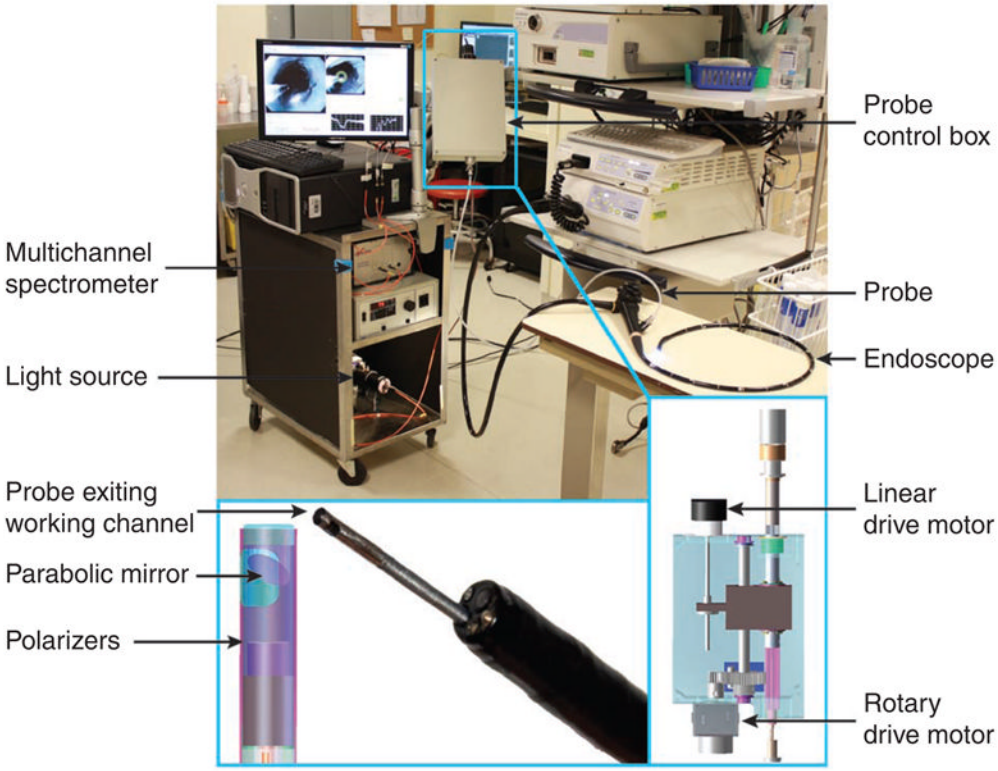

Figure 1.
Clinical EPSS instrument. The EPSS instrument is shown in the endoscopy suite before the clinical procedure, with the scanning probe inserted into the working channel of an endoscope. The insets show details of the scanning probe tip and the control box.

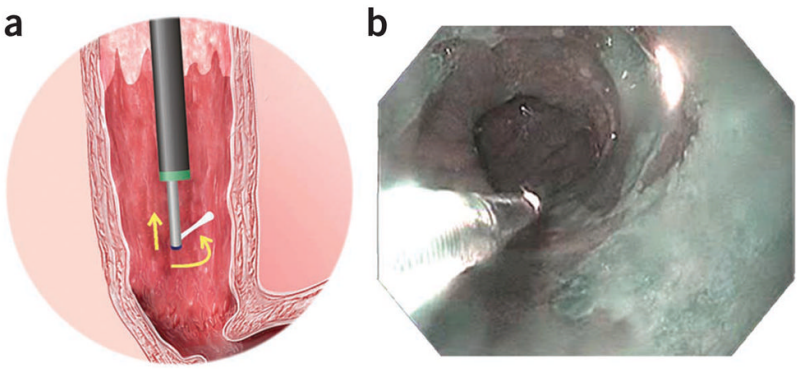

Figure 2.

EPSS scanning esophageal epithelium during screening endoscopy. (**a**) Illustration depicting the probe tip extended from the endoscope working channel during the scan; arrows indicate linear and rotary motions of the probe tip before and during each scan, respectively. (**b**) Frame capture, obtained and shown via the EPSS user interface, of an image acquired by the endoscope video channel showing the actual EPSS probe tip during scanning of the esophageal epithelium of a patient with Barrett's esophagus during a clinical procedure. The scanning illumination spot is seen on the esophagus wall at the upper right of the image. The EPSS probe tip diameter is 2.5 mm.

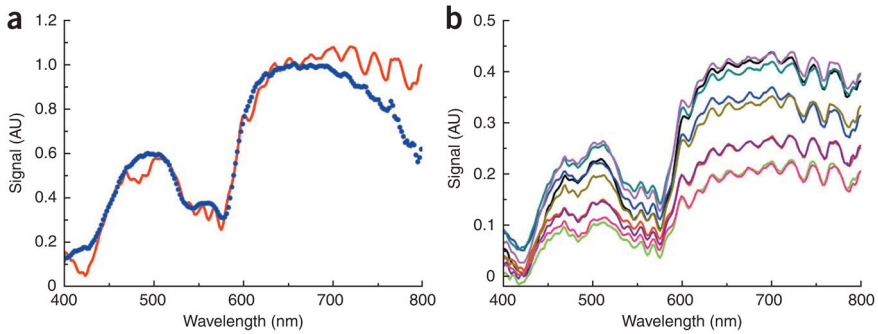

Figure 3.

EPSS spectra acquired during routine screening endoscopy. (**a**) Parallel (solid red line) and perpendicular (dotted blue line) polarization spectra collected with the EPSS instrument from a single spatial location in a subject with Barrett's esophagus. (**b**) Parallel polarization spectra from ten different locations in the same subject. AU, arbritary units.

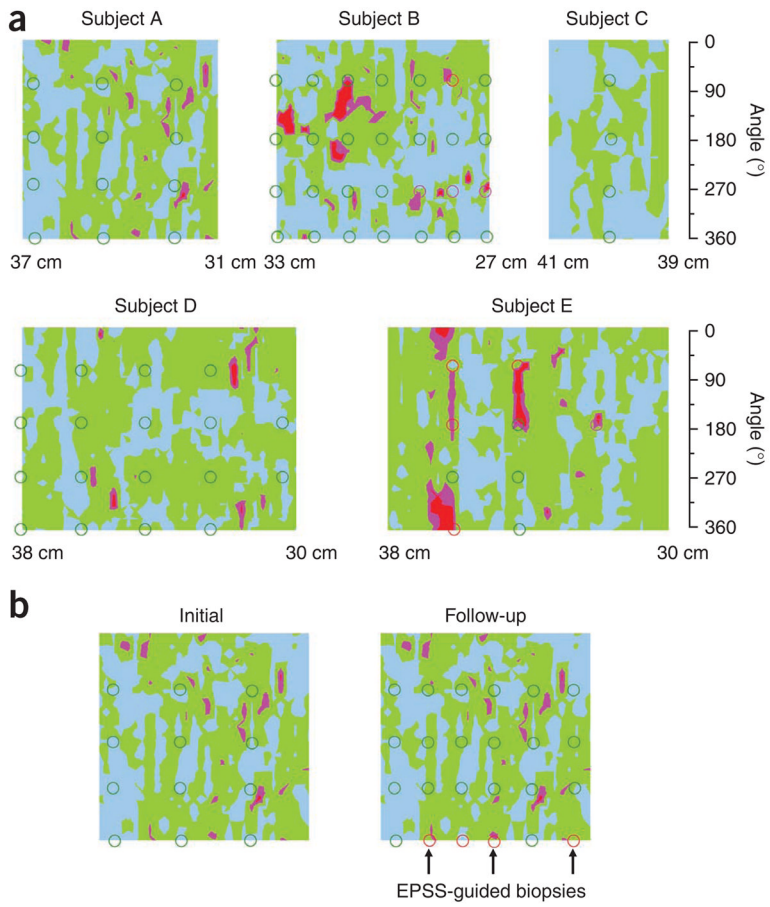

Figure 4.

Pseudo-color maps highlighting areas suspicious for dysplasia in five subjects. Maps produced from EPSS data are overlaid with circles indicating biopsy sites and confirmed pathology. The vertical axis indicates the angle of rotation (°) from the start of each rotary scan; the horizontal axis indicates the distance (cm) from upper incisors. Blue and green map areas and red and pink map areas represent epithelium unlikely for dysplasia and suspicious for dysplasia, respectively, as determined by EPSS. Red, pink and green circles indicate biopsy sites of HGD, LGD and nondysplastic Barrett's esophagus, respectively, as determined by pathology. (**a**) EPSS maps, biopsy sites and pathology for subjects A–E. (**b**) Biopsies taken during the initial and follow-up endoscopy procedures for subject A, overlaid on the EPSS map acquired during the initial procedure. Three follow-up biopsies were guided by the EPSS map, and pathology was confirmed HGD for each (indicated at 360°).

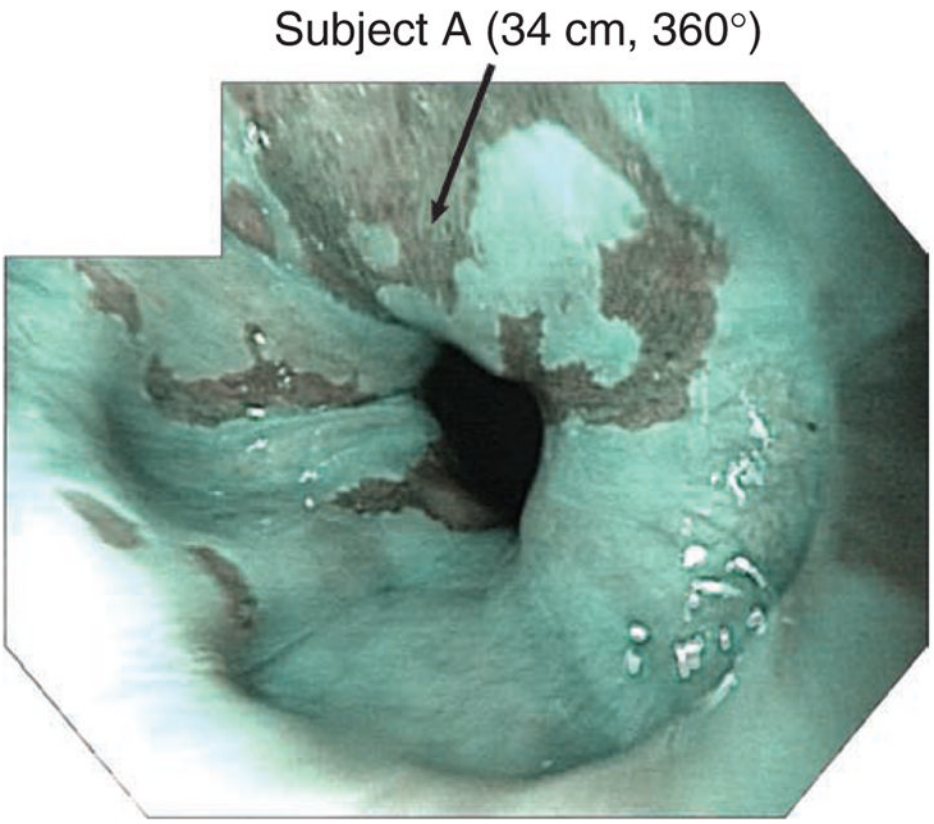

Figure 5.
HRE with NBI image of a location with invisible HGD. Video capture was acquired in subject A at one of the locations where invisible dysplasia was missed by visual examination by HRE with NBI, but located by EPSS, and later confirmed by pathology. The site is marked by an arrow. Note that the site is visually indistinguishable from the surrounding nondysplastic Barrett's esophagus tissue.