High resolution microendoscopy for classification of colorectal polyps

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Background and study aims: It can be difficult to distinguish adenomas from benign polyps during routine colonoscopy. High resolution microendoscopy (HRME) is a novel method for imaging colorectal mucosa with subcellular detail. HRME criteria for the classification of colorectal neoplasia have not been previously described. Study goals were to develop criteria to characterize HRME images of colorectal mucosa (normal, hyperplastic polyps, adenomas, cancer) and to determine the accuracy and interobserver variability for the discrimination of neoplastic from non-neoplastic polyps when these criteria were applied by novice and expert microendoscopists.

Methods: Two expert pathologists created consensus HRME image criteria using images from 68 patients with polyps who had undergone colonoscopy plus HRME. Using these criteria, HRME expert and novice microendoscopists were shown a set of training images and then tested to determine accuracy and interobserver variability.

Results: Expert microendoscopists identified neoplasia with sensitivity, specificity, and accuracy of 67% (95% confidence interval [CI] 58%–75%), 97% (94%–100%), and 87%, respectively. Nonexperts achieved sensitivity, specificity, and accuracy of 73% (66%–80%), 91% (80%–100%), and 85%, respectively. Overall, neoplasia were identified with sensitivity 70% (65%–76%), specificity 94% (87%–100%), and accuracy 85%. Kappa values were: experts 0.86; nonexperts 0.72; and overall 0.78.

Conclusions: Using the new criteria, observers achieved high specificity and substantial interobserver agreement for distinguishing benign polyps from neoplasia. Increased expertise in HRME imaging improves accuracy. This low-cost microendoscopic platform may be an alternative to confocal microendoscopy in lower-resource or community-based settings.

Introduction

Colorectal cancer is the third most common cancer for men and women in the United States, and has an overall 5-year mortality of 39% [1]. The United States Preventive Services Task Force currently recommends screening for colorectal cancer beginning at age 50, with high sensitivity fecal occult blood testing, flexible sigmoidoscopy, or colonoscopy [2]. Of these three screening tests, colonoscopy has emerged as the gold standard since it can be both diagnostic and therapeutic. More than 14 million patients are screened for colorectal cancer using colonoscopy each year in the United States [3]. Approximately 45% of patients have polyps detected during colonoscopy [4].

Adenomatous polyps are precursors of colorectal cancer. Hyperplastic polyps, on the other hand, are thought to have no potential for malignant development. On routine white-light colonoscopy, it is often difficult to distinguish adenomatous polyps from hyperplastic polyps. As a result, most polyps visualized during colonoscopy are removed endoscopically and sent for pathologic examination for tissue diagnosis. Among asymptomatic individuals undergoing screening colonoscopy, approximately 25% of polyps detected will be adenomatous [5].

As is the case with any medical procedure, polyp removal is not without risks and complications. The most common complication after polypectomy is immediate or delayed bleeding [6–8], which occurs in 1%–6% of polyp removals [6]. Polyp removal can also be time-consuming and expensive. Sending every polyp for pathologic examination increases costs without necessarily improving patient outcomes. Indeed, studies have shown that approximately half of all detected colorectal polyps are found to be normal or hyperplastic polyps [4, 9].

Considering the currently rising costs of healthcare, the idea of selectively sending polyps to pathology, the "resect and discard" strategy, deserves some attention. The adoption of a selective biopsy approach requires an accurate method for distinguishing non-neoplastic polyps from neoplastic polyps. Confocal laser endomicroscopy (CLE) in conjunction with narrow-band imaging (NBI) has high accuracy in the discrimination of neoplastic from non-neoplastic polyps (sensitivity 94%, specificity 97%) for diminutive and small colorectal polyps [10]. However, widespread utilization of CLE is limited by several factors including cost, learning curves, and the use of intravenous contrast (intravenous fluorescein).

High resolution microendoscopy (HRME) is a novel method for imaging colorectal mucosa at a subcellular level with × 1000 magnification and 4.4 µm resolution. First described by Muldoon et al., the HRME device is a flexible, portable, 1 mm diameter fiberoptic bundle containing 30 000 optical fibers with light-emitting diode (LED) illumination connected to a charge-coupled device (CCD) camera [11]. The HRME endoscope can be inserted through the accessory port of the colonoscope to visualize subcellular characteristics of polyp mucosa in vivo.

This is the first published study to describe the use of HRME in the colon. Criteria for the discrimination of colonic tissue types have not previously been created or evaluated using this portable system.

The goals of this study were twofold. The first goal was to develop HRME criteria for the characterization of colorectal mucosa (normal mucosa, hyperplastic polyps, adenomas, cancer). The second goal was to determine the accuracy and interobserver variability of expert and novice microendoscopists in distinguishing neoplastic (adenomatous, cancerous) from non-neoplastic (normal, hyperplastic) mucosa when using these criteria in still images obtained with HRME.

Methods

The HRME system

Technical details on the HRME design, assembly, and usage in endoscopy have been described in detail by Muldoon et al. and Pierce et al. [12,13]. Briefly, the system operates as a compact, battery-powered fluorescence microscope, coupled to a flexible, 1 mm diameter fiberoptic imaging probe. LED illumination (output spectrum centered at a wavelength of 455 nm) is delivered from the HRME unit, through the imaging probe, to the tissue surface. The fluorescent light returning to the bundle is directed to a scientific grade CCD that transmits real-time images to a personal computer at a rate of 12 frames per second. The probe used in the current study provides a 720 µm diameter field-of-view with 4.4 µm spatial resolution.

The probe can be used for an average of 60 to 75 insertions before the tip of the fiber bundle needs to be repolished. Disinfection of the probe is done by soaking in Cidex after each use.

HRME image acquisition

A total of 68 consecutive patients undergoing routine screening or surveillance colonoscopy were enrolled in an institutional review board (IRB)-approved protocol for HRME imaging, from October 2010 to August 2011 (clinical trial registration no. NCT01384240). A single endoscopist performed standard colonoscopic examination using a high definition white-light endoscope. All polyps visualized in white light that were biopsied and removed were targeted for HRME imaging. Prior to imaging, fluorescent contrast was applied using 1 – 4 ml topical proflavine (0.01%) administered through an endoscopic spray catheter. Proflavine, which is covered under an investigational new drug (IND 102,217) application from the US Food and Drug Administration (FDA), is a fluorescent contrast agent that selectively labels cell nuclei, with peak absorption and emission wavelengths of 445 nm and 515 nm, respectively. The HRME probe was inserted through the endoscope accessory channel and gently placed against the mucosa (**Fig. 1**). Images were acquired at a rate of 12 frames per second; video collection was initiated using a foot pedal. The imaged polyps were then subsequently removed by either forceps biopsy or snare polypectomy and then sent for histopathologic analysis according to standard of care. For each site imaged, one representative freeze-frame (jpg format) per video was subsequently extracted from the video files (avi format) for analysis. **Fig. 2** provides an example of use of an HRME image.

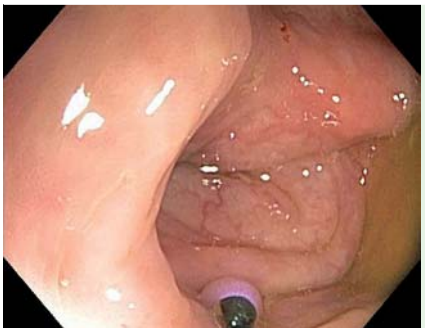


Fig. 1 High resolution microendoscopy (HRME): use of the HRME probe during colonoscopy.

All biopsies were interpreted by a single expert gastrointestinal pathologist who was blinded to the HRME interpretation. An HRME image database was created for the following histopathologic categories: normal colorectal mucosa, hyperplastic polyps, tubular adenomas, tubulovillous adenomas, and invasive adenocarcinoma.

Establishment of HRME classification criteria

Two expert gastrointestinal pathologists (A.D.P., N.H.) reviewed representative HRME images for each diagnostic category. In reviewing the HRME images, each pathologist noted the distinctive characteristics that corresponded to the histological results including, for each pathologic category, criteria that were glandular (size, shape, density), epithelial (thickness), and nuclear (size, arrangement). The pathologists then created consensus HRME imaging criteria descriptions based on the established World Health Organization histopathologic criteria applicable for each category (**Fig. 4**) [14].

Selection of images for training and test sets

To create a training set and a test set, image selection was performed by three of the authors (S.S.C., R.S., and R.R.K.) who were not endoscopists and were not involved in either image acquisition or testing. Images were included as "high quality" in the training and test sets if > 50% of the field was visible and not obscured by motion artifact or debris.

Using the pre-established microendoscopic criteria, a scripted, 5-minute training set was created that comprised 10 representative images of nondysplastic lesions (normal colorectal mucosa, hyperplastic polyps) and dysplastic/neoplastic lesions (tubular adenoma, tubulovillous adenoma, cancer) lesions.

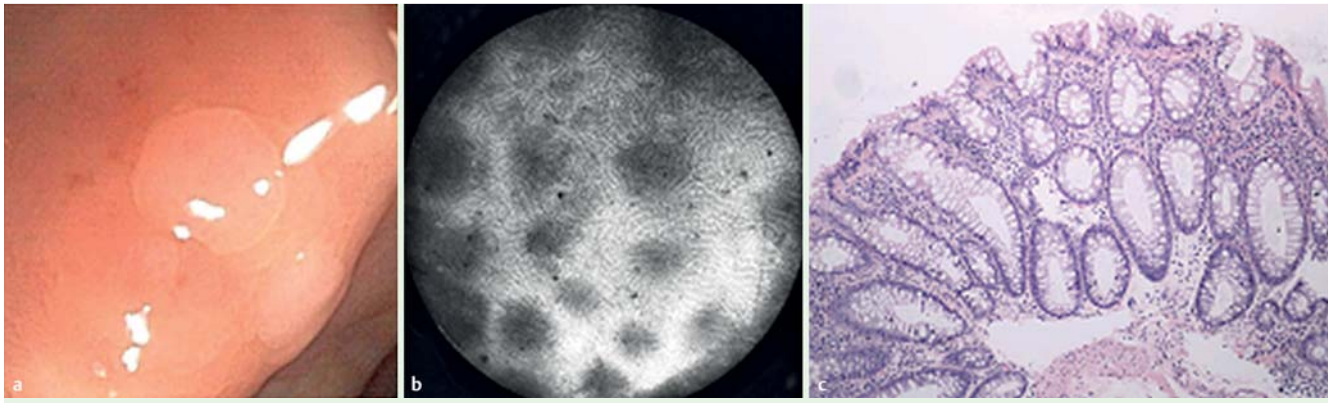


Fig. 2 High resolution microendoscopy (HRME) imaging. **a** The appearance under white light was "indeterminate." **b** The HRME evaluation was "no neoplasia." **c** The pathological finding was "benign: hyperplastic polyp."

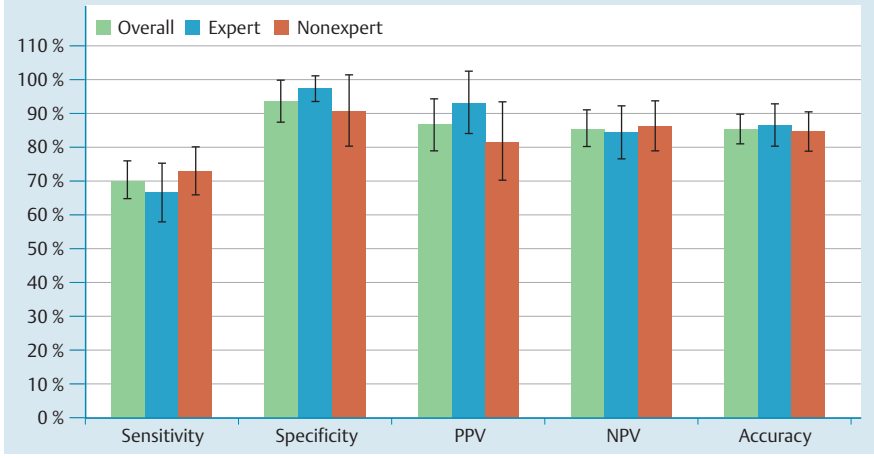


Fig. 3 Diagnostic performance measures of high resolution microendoscopy (HRME) in routine screening or surveillance colonoscopy, for classifying benign conditions (normal mucosa, hyperplastic lesions) versus adenomatous/malignant lesions (tubular adenoma, tubulovillous adenoma, carcinoma). Seven gastroenterologists (three HRME experts, four nonexperts in HRME) classified 37 representative images. PPV, positive predictive value; NPV, negative predictive value.

A test set of 37 images was also created (see power calculation in the Statistical analysis section). No images were duplicated in the training and test sets.

HRME training and testing

Four gastroenterologists with no prior experience in microendoscopy, along with three expert endoscopists with experience in over 50 HRME cases, completed the training and test sets in a blinded fashion. None of the seven observers tested had taken part in creation of the test set or training set. The three expert endoscopists were included in the study to serve as a reference for comparison with the gastroenterologists who had no prior microendoscopic experience.

Immediately upon finishing the training, the test-takers were given the 37-image test and asked to classify the image as either nondysplastic (normal colorectal mucosa, hyperplastic) or dysplastic/neoplastic (tubular adenoma, tubulovillous adenoma, cancer). Images were displayed in a random order and were shown only once for a duration of 10 seconds.

Statistical analysis

Interobserver variability among all endoscopists and within subgroups was assessed using the unweighted kappa statistic [15]. The calculations were performed using the MAGREE function macro in SAS software (Version 9.2; Cary, North Carolina, USA), modeled after a previously reported method [16]. Measures of diagnostic accuracy were calculated for each individual rater, and then averaged within each group (HRME-expert, HRME-novice, etc.). The kappa values were interpreted as follows: value of 1.00, perfect agreement; 0.81 – 0.99, almost perfect agreement; 0.61 – 0.80, substantial agreement; 0.41 – 0.60, moderate agreement; 0.21 – 0.40, fair agreement; 0.10 – 0.20, slight agreement; 0.00, less than chance agreement [17]. A two-sided P value of <0.05 was considered to demonstrate a statistically significant difference, using the normal z test.

A power calculation performed using nQuery Advisor v. 7.0 found that a sample size of n = 37 images would provide 80 % power to discriminate between kappa values of 0.25 ("fair agreement") and 0.70 ("substantial agreement") in the overall kappa measure of agreement, using a 2-sided type I error probability of 0.05 [18].

Results

Among 103 polyps imaged, 83 were selected as providing images of sufficiently high quality for inclusion in the training and test sets. Of the images agreed upon as high quality, ten representative images were selected for inclusion in the training set, and, on the basis of the power calculation described above, 37 representative images were placed in the test set (**Table 1**). The ten images in the training set were comprehensive and sufficient to teach observers the different types of pathology.

Based on the expert pathologists' comparisons of HRME images with histopathology, consensus criteria for interpretation of HRME images had been defined (**Fig. 4**).

Seven observers were trained and tested in the application of these new criteria. There were no missing data.

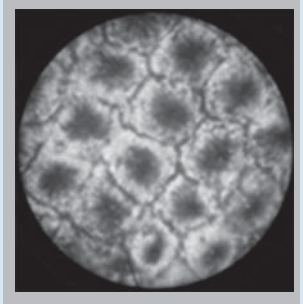
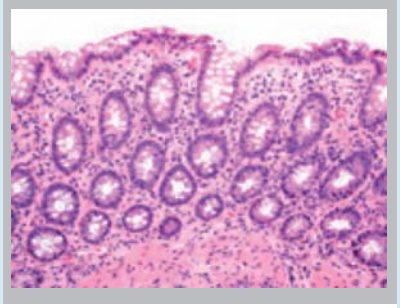
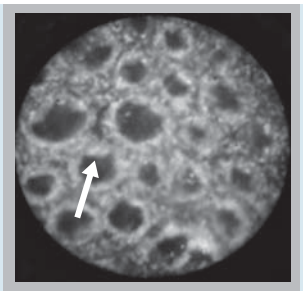
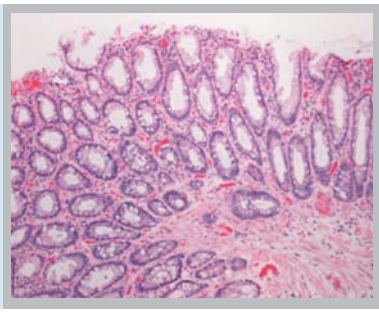
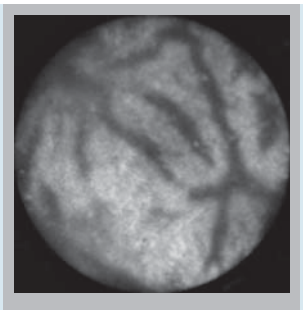
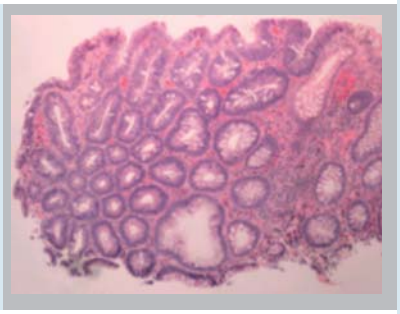
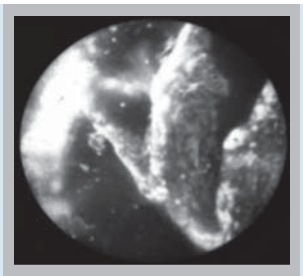
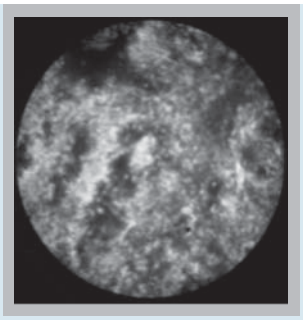
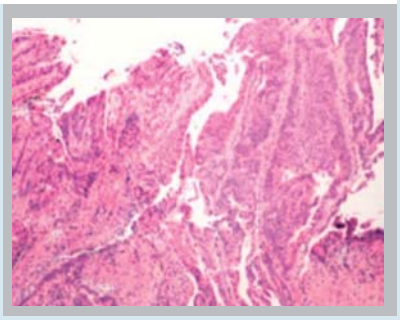
Tissue diagnosis	Description	Example images	
		HRME	Histopathology
(A) **Normal colorectal mucosa**	▪ Uniform glands, i.e., same size, shape, and luminal caliber throughout image. ▪ No expansion of epithelium or lamina propria.		
(B) **Hyperplastic polyp**	▪ Slightly distorted glands of varying size, shape, and luminal caliber. ▪ Serrated "sawtooth" architecture (arrow). ▪ Occasional expansion or widening of the epithelium.		
(C) **Tubular adenoma**	▪ More linear crypts. ▪ Elongated nuclei, occasionally visible and aligned in parallel. ▪ Increased epithelium to crypt ratio evidenced by expanded epithelium and crypt luminal narrowing.		
(D) **Tubulovillous adenoma**	▪ Same as tubular adenoma with additional widened or even open crypt lumens. ▪ Villiform structures visible.		
(E) **Colon cancer**	▪ Architectural disarray. ▪ Large, dense, overlapping, and pleomorphic nuclei.		

Fig. 4 High resolution microendoscopy (HRME) imaging. Consensus criteria created by two expert gastrointestinal pathologists, based on the established World Health Organization histopathologic criteria for each category [14].

Table 1 High resolution microendoscopy (HRME) image sets for training and testing. Distribution of image sites grouped by pathology.

	HRME images		
	Training set	Test set	Total
Pathologic category			
Normal colorectal mucosa	2	12	14
Hyperplastic polyp	3	12	15
Tubular adenoma/tubulovillous adenoma	4	12	16
Cancer	1	1	2
Total	10	37	47

Expert microendoscopists (n=3) were able to identify adenomatous and cancerous polyps with a sensitivity of 67% (95% confidence interval [CI] 58%–75%), specificity 97% (94%–100%), and overall accuracy 87%. The positive predictive value (PPV) was 93%, and the negative predictive value (NPV) was 84% (**Fig. 3**). Calculation of interobserver agreement among experts showed high agreement (k=.857).

Nonexperts (n=4) achieved a sensitivity of 73% (95%CI 66%–80%), specificity 91% (80%–100%), and accuracy 85%. The PPV was 82% and NPV was 86%. Calculation of interobserver agreement among nonexperts showed substantial agreement (k=.719). Comparisons between experts and nonexperts showed the following P values: sensitivity P=.06; specificity, P=.08; overall accuracy, P=.39; PPV, P=.03; NPV, P=.53.

Overall, participants were able to identify adenomas and carcinoma with a sensitivity of 70% (95%CI 65%–76%), specificity 94% (87%–100%), and accuracy 85%. The overall PPV was 87% and overall NPV was 84%. The kappa value for overall interobserver agreement showed substantial agreement (k=0.78) among all test-takers.

Experts had a greater specificity than nonexperts for identifying adenomas and cancer (97% versus 91%).

There were no adverse events or complications reported related to the act of HRME imaging or the use of proflavine dye.

Discussion

This study is the first to define a new classification system for HRME imaging in the colon. Using these new criteria, the interobserver agreement and accuracy for predicting histopathology were assessed in expert and nonexpert microendoscopists for select microendoscopic images obtained during routine colonoscopy and displayed without the clinical context of white-light appearance (no endoscopic image of the polyp was shown). Analysis showed that these HRME criteria provide high specificity (91%–97%) for identifying adenomas and cancer. Increased expertise in HRME imaging provides greater specificity and less interobserver variability. Interobserver agreement was substantial in novices (k=.719) and high in experts (k=.857). The sensitivity (67%–73%) and accuracy (85%–87%) were modest.

This form of "optical biopsy" technology has been used ex vivo and in vivo to study tissue of irregular appearance in other organs such as the esophagus. Lee et al. found that general gastroenterologists using HRME during esophagogastroduodenoscopy could identify Barrett's neoplasia with good diagnostic accuracy (sensitivity 81%, specificity 85%) [19]. Thus, the specificity for identifying colorectal neoplasia has been found to be greater than specificity in the esophagus.

Similar criteria for colorectal tissue have been published for confocal laser endomicroscopy (CLE) both probe-based (pCLE) and dedicated endoscope (eCLE) (**Table 2**) [20,21]. Kulper et al. [21] studied pCLE using a comparable number of observers (n=5) and found similarly modest sensitivities (66% to 80%) with correspondingly higher specificities (83% to 95%), depending on the number of observers in agreement. Accuracy was in approximately the same range as our study (81% to 94%). However, the interobserver agreement for pCLE in identifying neoplasia was only moderate (k=.56), as compared with our overall kappa value of .78. Similarly to our study, the observers were not shown the endoscopic white-light images.

Gomez et al. reported moderate interobserver agreement (k=.55) among three observers in identifying colorectal neoplasia using pCLE images [22]. The sensitivity, specificity, and accuracy were 76%, 72%, and 75%, respectively. The observers in this study were also not shown the endoscopic images.

A post hoc study by Kulper et al., with three observers, found that endoscope-based CLE (eCLE) could diagnose neoplasia with substantial interobserver agreement (k=.73 and.72) [23]. The accuracy with eCLE ranged from 85.6% to 95.6%. Overall sensitivity ranged from 77.1% to 94%, depending on the observer. Overall specificity ranged from 89.1% to 100%.

There is hope for improving accuracy by enhancing or adding technology. DePalma et al. combined conventional pCLE with video-mosaicing and reported substantial interobserver agreement (k=.85), sensitivity 100%, specificity 84%, and accuracy 92.3% [24]. Using eCLE images in conjunction with the macroscopic appearance of the lesion seen during colonoscopy, Kiesslich et al. reported excellent accuracy, sensitivity, and specificity (99.2%, 97.4%, 99.4%, respectively) [20].

There were several strengths of this study. HRME is a novel technique that has never previously been used in the colon to identify neoplasia, and this is the first ever description of HRME criteria for pathologic characterization in the colon. When interobserver variability and diagnostic performance measures were assessed, the HRME colon criteria performed comparably to or superior to colon criteria previously defined for pCLE and eCLE (**Table 2**).

If a new technology is to be adopted, it must be easy to learn. Test results for distinguishing benign tissue from precancerous adenomas and carcinoma did not show significant differences be-

Table 2 Comparison of diagnostic performance measures, including interobserver variability, obtained for different techniques for predicting colorectal neoplasia.

Study	Technique	Observers, n	Kappa value	Accuracy	Sensitivity	Specificity
Chang et al., present study	HRME	7	.719–.857	85%–87%	67%–73%	91%–97%
Kulper et al., 2011 [21]	pCLE	5	.56	81%–94%	66%–80%	83%–95%
Gomez et al., 2010 [22]	pCLE	3	.55	75%	76%	72%
Kulper et al., 2012 [23]	eCLE	3	.72–.73	85.6%–95.6%	77.1%–94%	89.1%–100%

HRME, high resolution endomicroscopy; pCLE, probe-based confocal laser endoscopy; eCLE, dedicated endoscope confocal laser endoscopy

tween HRME experts and novices for accuracy, sensitivity, or specificity. These results suggest that gastroenterologists who are new to HRME can be trained successfully, in short periods of time, to use this adjunct technology with results similar to those of experts. Of course, as can be predicted with most activities, more experienced HRME users in this study did have slightly better results in most diagnostic performance measures, though the differences were not statistically significant.

There were several limitations to this study. First, the HRME images were analyzed post hoc rather than as real-time video images. Secondly, endoscopists in this study were shown the HRME image without the benefit of the endoscopic image. For this study, our intent was solely to evaluate the accuracy of our preestablished classification criteria, and consequently the observers did not have the information provided by the clinical context or by the white-light appearance of the polyp. While this approach was being used, the lack of endoscopic view may have had a negative impact on overall accuracy and, in particular, on the sensitivity of the technique. Indeed, HRME is not intended to be a stand-alone screening tool but rather an adjunct technology that could be used in combination with white-light endoscopy or another 'red flag' technology such as narrow band imaging (NBI), chromoendoscopy, or autofluorescence imaging, with the intent of enhancing diagnostic specificity and preventing unnecessary biopsy or polypectomy. Shahid et al. combined NBI with pCLE and reported sensitivity greater than 94% [10]. Several studies have shown that chromoendoscopy with methylene blue dye improves the endoscopist's ability to detect intraepithelial neoplasia and colorectal cancer in patients with ulcerative colitis when compared with regular white-light colonoscopy [25, 26]. The major limiting factors with regard to combination of techniques will be the increased time commitment, clinical testing, and equipment acquisition.

Once high diagnostic specificity can be consistently achieved, gastroenterologists could become more selective about which polyps need to be biopsied. Hassan et al. conducted a cost-effectiveness study and reported that, for screening colonoscopy, a "resect and discard" policy (with only suspicious polyps being sent for pathologic examination) would save an average of $25 per person without meaningfully affecting screening efficacy [27]. Extrapolated over the entire United States population, this small $25 per person saving could result in yearly savings of $33 million. Continued improvements in optical biopsy techniques could lead to more selective polyp biopsies in the future.

In conclusion, this is the first study to establish HRME classification criteria for the distinguishing benign from neoplastic colonic mucosa. Since HRME uses a low cost (<$3500 to build), portable, battery-operated imaging device, use of this endoscopic technique may be more feasible than CLE in lower-resource settings and community-based practices. The topical contrast used with the device may also be an advantage in areas where unsedated examinations are performed without intravenous access and thus precluding use of intravenous fluorescein. This preliminary evaluation suggests that HRME can identify adenomas and cancer with high specificity, accuracy, NPV, and substantial interobserver agreement. A prospective trial evaluating the accuracy of high definition white-light endoscopy and HRME is currently under way.

Competing interests: Dr. Richards-Kortum serves as an unpaid scientific advisor to Remicalm LLC, holds patents related to optical diagnostic technologies that have been licensed to Remicalm LLC, and holds minority ownership in Remicalm LLC.

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