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Endoscopic Mucosal Imaging of Gastrointestinal Neoplasia in 2013

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Abstract The holy grail of gastrointestinal endoscopy consists of the detection, in vivo characterization, and endoscopic removal of early or premalignant mucosal lesions. While our ability to achieve this goal has improved substantially since the development of the modern video-endoscope, inadequate visual inspection, errors of interpretation, and lesion subtlety all contribute to the continued suboptimal detection and assessment of early neoplasia. A myriad of new technologies has thus emerged that may help resolve these shortcomings; high magnification endoscopes, as well as the techniques of dye-based and virtual chromoendoscopy, are now widely available, while confocal laser endomicroscopy and endocystoscopy, optical coherence tomography, and autofluorescence imaging are generally applicable only in a research setting. Such technologies can be broadly categorized according to whether they potentially afford endoscopists improved *detection*, or real-time *characterization,* of mucosal lesions. Enhanced detection of otherwise "invisible" lesions, such as a flat area of intramucosal adenocarcinoma within Barrett's esophagus, carries the potential of an endoscopic cure prior to the development into a more advanced or metastatic disease. The ability to characterize a lesion to achieve an in vivo diagnosis, such as a colonic polyp, potentially affords endoscopists the ability to decide which lesions require removal and which can be safely left behind or discarded without histological assessment. Furthermore targeted biopsies, such as in the surveillance of chronic colitis, may prove to be more accurate and efficacious than the current protocol of random biopsies. An important caveat in the discussion of developing technologies in early cancer detection is the fundamental importance of a health-care system that promotes screening programs to recruit at-risk individuals. The ideal tool to optimize the use of endoscopy in population screening would be a panel of reliable biomarkers (blood, stool, or urine) that could effectively select a high-risk group, thus reducing the indiscriminate use of an expensive technology. The following review summarizes the current endoscopic imaging techniques available, and in development, for the early identification of gastrointestinal neoplasia.

Keywords Endoscopic · Imaging · Gastrointestinal · Mucosa · Advanced

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Introduction: High-Definition Endoscopy

The evolution of flexible endoscopes, from early fiber-optic devices in the 1960s to the latest high-definition (HD) endoscopes of today, has exponentially increased our ability to inspect and visualize subtle mucosal details. While standard endoscopes use approximately 300,000 pixels to construct an image, high-definition endoscopes contain charge-couple chips with over 1,000,000 pixels. In addition, the use of HD monitors with 1,080 effective scanning lines of picture information delivers an image that is twice as good as with conventional monitors. The additional benefit of HD scopes over standard definition endoscopes has been examined in several studies. While early studies assessing polyp detection in the colon found no overall difference in adenoma or hyperplastic polyp detection rates [1–3], several recent studies [4, 5] have reported higher detection rates with HD endoscopes. A meta-analysis concluded that the incremental benefit of HD for the detection of any polyp was 3.8 % [6]. Despite the improvements in image quality, the fundamentals of good endoscopy—namely, the careful and thorough inspection

from "an educated eye"—remain the most important tools for dysplasia detection. The adage "looking but not seeing" may well account for many of the subtle lesions that are overlooked by endoscopists pressured by the time accept change of clinical practice. While well established for colonoscopic examinations [7••], the importance of longer inspection time was highlighted in a recent post hoc analysis of a trial evaluating Barrett's esophagus (BE) surveillance. They reported that patients with longer inspection times were more likely to have endoscopically suspicious lesions identified and to receive a diagnosis of high dysplasia or early adenocarcinoma [8••].

Chromoendoscopy

The application of contrast agents or stains to mucosal surfaces within the gastrointestinal (GI) tract to enhance visualization of mucosal detail has been extensively studied. A variety of stains have been utilized clinically (Table 1) which can be broadly categorized as (1) *absorptive* (Lugol's iodine, methylene blue, toluidine blue,) (2) *reactive* (Congo red, phenol red), and (3) *contrast* (indigo carmine [IC]) [9]. Application is generally topical through the use of a dedicated spraying catheter, although oral ingestion and rectal enema instillation have been used. Mucosal inspection is usually performed with white light endoscopy (WLE), but additional modalities, such as magnification endoscopy, optical image enhancement (e.g., narrow-band imaging), and confocal endomicroscopy, can also be employed for the evaluation of suspicious abnormalities. While chromoendoscopy is described as an "advanced" imaging technique, the use of stains is distinctly "low-tech," with most of the dyes inexpensive and widely available.

Squamous Cell Neoplasia

Lugol's iodine is a solution of potassium iodide and iodine that binds to glycogen in nonkeratinized squamous tissue, staining the normal mucosa a dark brown color. Dysplastic and malignant squamous mucosa, as well as columnar mucosa, have low concentrations of glycogen and are, therefore, seen as pale yellow "Lugol's negative" islands. Inflammation likewise causes nonstaining. Lugol's has been used principally in the detection and characterization of early squamous carcinoma of the esophagus. In a study of 225 patients in China, the use of Lugol's identified an additional 17 of 31 patients with moderate dysplasia (55 %) and 8 of 35 patients with severe dysplasia (23 %) not initially seen with standard WLE. Furthermore, 88 % of HGD and carcinomatous lesions were larger or more clearly identified after staining [10]. Similarly, in a study of 158 high-risk individuals where Lugol's only moderately improved the diagnostic accuracy of WLE, the extent of the lesion estimated by the dye-free surfaces were significantly larger than the endoscopic patterns observed initially on WLE (11.6 ± 9.2 cm^2 vs 1.4 ± 1.7 cm^2; $p<.02$) [11]. Severe retrosternal discomfort has been reported in up to 30 % of patients following the application of this Lugol's iodine. This adverse effect can be somewhat ameliorated by the application of sodium thiosulphate solution to the esophagus at the end of the procedure [12].

Barrett's Esophagus

Acetic acid (AA) is a weak acid that aids mucosal contrast enhancement when applied to surface epithelium and has been used with both conventional WLE and magnification endoscopy for the detection of BE and associated dysplasia. Conflicting studies regarding its utility have been published [13–16]. Balsamic vinegar, an agent that combines the advantages of chromoendoscopy with the structural enhancement of AA, has also been studied in the esophagus in a recent feasibility study that found the accuracy, sensitivity, and specificity for detecting BE to be 90 %, 100 %, and 82 %, respectively [17].

Methylene blue (MB) is an absorptive stain that is actively taken up by intestinal and colonic mucosa. Its use in the esophagus has been studied extensively due to its ability to positively stain the intestinal metaplasia characteristic of BE, while sparing the normal gastric and squamous esophageal mucosa. While the evidence to date is conflicting, several of the largest studies have suggested that MB enhances the detection of intestinal metaplasia with fewer biopsies, as compared with traditional surveillance schedules [18–20]. A recent meta-analysis of nine studies, however, concluded that MB chromoendoscopy has only a comparable yield to random biopsies for the detection of intestinal metaplasia and dysplasia [21]. Further limiting the potential use of MB in the esophagus is the somewhat laborious application process involving a need to prespray the mucosa with a mucolytic and, also, irrigate extensively following stain application. While IC has also demonstrated utility in Barrett's surveillance when combined with magnification endoscopy [22], further studies are needed to determine its efficacy in clinical practice.

Gastric Neoplasia

The use of chromoendoscopy in early gastric cancer has generally been limited to defining the margins of lesions considered suitable for endoscopic resection [23, 24]. Even this application may become obsolete, however, following a recent study demonstrating that narrow-band imaging (NBI) was able to successfully define the margins of 72.6 % (45/62) of lesions not able to be adequately characterized by CE [25].

Colonic Dysplasia and Inflammatory Bowel Disease

Commonly used dyes in the colon include both MB and IC. MB is absorbed by colonic mucosa and has been used in

Table 1 Tissue stains used during gastrointestinal endoscopy

Stain Type	What Is Stained	Mechanism of Staining	Positive Staining	Clinical Uses in GI
Vital stains				
Lugol's solution (iodine + potassium iodide)	Normal glycogen containing squamous cells	Binds iodine in nonkeratinized cells	Dark brown	1) Squamous cell esophageal cancer (nonstaining) 2) Columnar epithelium in the esophagus, including residual Barrett's esophagus following mucosal ablation (nonstaining) 3) Reflux esophagitis (nonstaining)
Methylene blue (methylthionine chloride)	Small or large intestinal cells or intestinal metaplasia	Active absorption into cells	Blue	1) Specialized epithelium (intestinal metaplasia) in Barrett's esophagus* 2) Intestinal metaplasia in the stomach 3) Early gastric cancer• 4) Gastric metaplasia in the duodenum (nonstaining) 5) Celiac and tropical sprue
Toluidine blue (tolonium chloride or dimethylamino-toluphenazothioni-chloride)	Nuclei of columnar (gastric and intestinal-type) cells	Diffuses into cell	Blue	1) Squamous cell carcinoma of the esophagus 2) Gastric or intestinal metaplasia in Barrett's esophagus
Reactive stains				
Congo red (biphenylene-napthadene sulfornic acid)	Acid-containing gastric cells	Acid pH <3.0 results in color change	Turns red to dark blue or black	1) Acid-secreting gastric mucosa (including ectopic locations) 2) Gastric cancer (nonstaining) (may be combined with methylene blue to outline intestinal metaplasia)
Phenol red (phenolsulfonphthalein)	H. pylori-infected gastric cells	Alkaline pH (from hydrolysis of urea to NH3 and CO2 by urease) results in color change	Turns yellow to red	Diagnose Helicobacter pylori infection (positive color change) and map its distribution in the stomach
Contrast stain				
Indigo carmineΔ	Cells are not stained	Pools in crevices and valleys between mucosal projections	Blue (indigo)	1) Colon, gastric, duodenal, esophageal lesions 2) Barrett's esophagus

* Methylene blue does not stain nonspecialized or gastric metaplasia; specialized columnar epithelium stains blue, but highly dysplastic or malignant specialized columnar epithelium in Barrett's esophagus generally takes up little to no dye; low grade dysplasia in Barrett's esophagus may or may not take up stain

• With or without Congo red

Δ Also used in combination with high-resolution or high-magnification endoscopy; may be used with or without crystal violet (for early colorectal cancers)

multiple settings, including polyp screening and dysplasia surveillance, and to better define sessile lesions during endoscopic mucosal resection (EMR). IC is a nonabsorbable "contrast" dye that enhances mucosal inspection by outlining epithelial topography. It facilitates detection of superficial irregularities, particularly sessile lesions, and aids in the recognition of pit patterns used to characterize colonic polyps. A Cochrane review published in 2010 evaluating the utility of chromoendoscopy, as compared with conventional endoscopy, included 1,059 participants from five clinical trials. They found that chromoendoscopy yielded significantly more patients with at least one neoplastic lesion (OR 1.67 [CI1.29–2.15]) and significantly more patients with three or more neoplastic lesions (OR 2.55 [CI 1.49-4.36]) [26]. A recent study of 1,008 patients utilizing 0.4 % IC sprayed continuously during extubation also yielded positive results. CE increased the overall detection rate for adenomas (0.95 vs. 0.66 per patient), flat adenomas (0.56 vs. 0.28 per patient), and serrated lesions (1.19 vs. 0.49 per patient) ($p<.001$) [27]. While these data support the efficacy of routine CE, endoscopist inexperience coupled with the additional time required to employ this

technique has resulted in limited uptake in real-world clinical practice. The use of MB-containing compounds within a timed-release matrix, taken orally with bowel preparation, is being evaluated [28].

Long-standing Crohn's and ulcerative colitis carries an excess risk of developing dysplasia and subsequent neoplasia. Prior to the use of chromoendoscopy, random quadratic biopsies every 10 cm was considered the standard of care. Two recent meta-analyses, however, have summarized the evidence to date regarding the diagnostic yield of chromoendoscopy with targeted biopsies, as compared with the random biopsy protocol [29, 30•]. Wu et al. included six randomized controlled trials using either MB or IC dye spray, which yielded a pooled sensitivity of 83.3 %, a specificity of 91.3 %, and a diagnostic odds ratio of 17.5 [29]. Subramanian et al. pooled the results from six studies with a total of 1,277 patients and found a difference in yield of dysplasia favoring chromoendoscopy of 7 % on a per patient analysis [30•].

Virtual Chromoendoscopy

The evolution of digital endoscopes has facilitated the recent development of a type of "digitally enhanced" imaging analogous to traditional chromoendoscopy but achieved with optical filters or the use of selective wavelengths of light. Known colloquially as "virtual chromoendoscopy" (VC), this type of imaging is based on the principle that light penetrates tissues to a variable depth on the basis of its wavelength, with blue light (shorter wavelength) penetrating less than red light (longer wavelength). NBI (Olympus, Japan) thus uses blue(415 nm) and green (540 nm) light to construct an endoscopic image that highlights superficial mucosal details such as capillaries and pit patterns. The related technologies of I-scan (Pentax) and FUJI Intelligent Chromo Endoscopy (FICE) use the same concept but achieve a similar result through the use of digital filters following image acquisition with white light. VC has several advantages over traditional chromoendoscopy, including its widespread availability on most new endoscopes, the ability to toggle from the normal to enhanced image repeatedly with the press of a button, and an avoidance of the laborious and often nonuniform application of dye using a spraying catheter. It has thus gained popularity among practicing endoscopists for a range of clinical uses.

Esophagueal Squamous Cell Carcinoma

While Lugol's iodine remains the gold standard for detecting squamous dysplasia of the esophagus, several studies have demonstrated the utility of NBI in this setting [31–34]. A comparative study of 202 patients reported that NBI detected 28/31 (90.3 %) lesions confirmed as high-grade dysplasia or carcinoma, as compared with 31/31 (100 %) by Lugol's (p=.08). Specificity of suspicious areas was higher for NBI (74.5 %), as compared with Lugol's (2.4 %) (p<.01) [31]. The routine use of NBI in this setting, however, remains to be confirmed.

Barrett's Esophagus

The ability of NBI to enhance the detection of BE and associated dysplasia has been studied in several prospective trials [35•, 36]. A randomized crossover trial of 123 patients found that NBI without magnification detected a higher proportion of patients with dysplasia (30 % vs. 21 %, p=.01) and a comparable number of patients with intestinal metaplasia but with fewer biopsies (3.6 vs. 7.6, p<.0001) [35•]. Likewise, a tandem study of 65 patients yielded higher rates of HGD (18 % vs. 0 %) and LGD (57 % vs. 43 %) with few biopsies taken (8.5 vs. 4.7 per patient, p<.01) [36]. Supporting these finding is a meta-analysis of eight studies involving 446 patients comparing the NBI-based diagnosis (with magnification) of HGD and intestinal metaplasia with histopathology as the gold standard. It reported high rates of sensitivity for both HGD (96 %) and SIM (95 %). Specificity was better for HGD (94 %) than for SIM (65 %) [37]. Complicating the implementation of NBI and targeted biopsies into clinical practice has been the lack of uniformity with regard to classification systems for mucosal and vascular patterns seen on NBI. Additionally, interobserver agreement for NBI images of intestinal metaplasia and dysplasia has been only moderate among both expert and nonexpert endoscopists [38]. Targeted NBI-guided biopsies have thus not supplanted random biopsies for routine surveillance in BE, and careful inspection with WLE remains a key factor in detecting subtle lesions.

Stomach

Analogous to the use of chromoendoscopy in the stomach, the use of NBI has generally been limited to preresection assessment of lesions identified during WLE. A study of patients with 40 suspicious lesions identified on WLE randomized them to additional inspection with either WLE or NBI. The combination of techniques increased the accuracy of diagnosis from 64.8 % to 96.6 % (p≤.001) [39]. Kato et al. compared NBI with WLE for defining the margin of early lesions and found it to have better sensitivity and specificity (92.9 % and 94.7 % vs. 42.9 % and 61 %, respectively, p<.0001) [40], while Kiyotoki evaluated its performance against chromoendoscopy and found it to more accurate (97.4 % vs 77.8 %, p=.009) [41]. A feasability study assessing NBI as part of a trimodal (TM) platform combined with WLE and autofluorescence (AF) also demonstrated the potential of NBI. They reported a higher diagnostic sensitivity and specificity per lesion with TM imaging (89.4 % and 98 %) than with

either WLE (76.6 % and 84.3 %) or AFI (68.1 % and 23.5 %) alone [42]. NBI may also have a role in the posttreatment assessment of residual dysplasia [43].

Colonic Polyps and Dysplasia

NBI has been applied to both polyp detection and characterization in the colon. The results from studies comparing NBI with WLE for the *detection* of adenomas have been summarily disappointing. A Cochrane review included eight randomized trials with 3,673 participants and concluded that NBI might be better than standard-definition WLE but equal to high-definition WLE for the detection of adenomas [44]. Two recent meta-analyses have also been performed comparing NBI with high-definition WLE [45•] and NBI with standard WLE [46], with comparable results. They both found that NBI did not increase the yield of colonic polyps, adenomas, or flat adenomas.

While the performance of NBI for polyp detection has been disappointing, its use in the *characterization* of already-identified lesions has yielded more promising results. The possibility of endoscopists achieving a "real-time" histological diagnosis has raised the concept that small benign polyps can be either left in situ or removed, but not sent for histological examination. Multiple recent studies have assessed the performance of this "resect and discard" concept [47–51]. An observational study of 235 rectosigmoid polyps reported NBI to have an accuracy of 97.7 % for a "high-confidence" prediction. Sensitivity for adenomatous histology was 93.9 %, specificity was 98.4 %, negative predictive value was 97.9 %, and positive predictive value was 75.6 %. The authors concluded that NBI was sufficiently accurate to allow distal hyperplastic polyps to be left in place and small, distal adenomas to be discarded without pathological assessment [48]. A meta-analysis of 11 studies found the sensitivity and specificity of NBI to accurately predict polyp histology to be 92 % and 81 %, respectively, with magnification and 91 % and 86 % without magnification [49]. A study by Kuiper et al. performed in a nonacademic setting, however, yielded an overall sensitivity of 77 % and a specificity of 78.8 %. As a consequence, they found that 19 % of on-site recommendations for a surveillance interval proved to be inaccurate [50]. One solution to this diminished effectiveness of NBI outside of academic centers may be with the use of computer-based evaluation systems, which, in two studies, demonstrated an accuracy equivalent to that of expert endoscopists [52, 53].

Colitis Surveillance

Given the beneficial detection of colitis-associated dysplasia afforded by traditional chromoendoscopy, multiple studies have assessed NBI in this setting [54–57]. The results have been uniformly disappointing, however, with NBI found to be no more efficacious at detecting dysplasia than either standard or high-definition WLE with random biopsies.

Additional Applications

While capsule endoscopy has revolutionized the study of the small bowel, the inability to modify image acquisition has been a significant limitation. Recent evidence, however, suggests that using postimage manipulation to achieve VC with FICE can enhance detection of mucosa lesions [58–60].

Investigational Modalities

Confocal Endomicroscopy

Confocal laser endomicroscopy (CLE) is a developing technology that enables high-resolution "in vivo" imaging of tissue microstructure at or near the level of histopathology without the need for tissue excision, akin to obtaining an "optical biopsy." Adapted from light microscopy, CLE uses depth-specific tissue illumination and pinhole-limited detection to create an image from fluorescent light reflected back from a very thin focal plane. Tissue fluorescence is achieved through the use of intravenous or topically applied contrast agents, with IV fluorescein the most popular. There are currently two commercially available devices: an endoscope-based system (eCLE) that is fully integrated into the tip of a conventional endoscopy (Optiscan, Australia; Pentax, Japan) and a probe-based system (pCLE) that can be passed down the working channel of a range of standard endoscopes (Cellvizio, France). The potential of CLE to enhance the detection of dysplasia while decreasing the number of required biopsies is a concept that has generated significant academic interest.

Esophagus

While rudimentary work has been undertaken defining the neoplastic changes visible by CLE in early squamous cell cancer [61, 62], the majority of clinical studies have focused on the detection of dysplasia in BE.

The initial report of 63 patients with BE found that CLE predicted BE and associated neoplasia with a sensitivity of 98.1 % and 92.9 % and a specificity of 94.1 % and 98.4 %, respectively [63]. The interobserver agreement was high (K= 0.843). Since then, multiple studies have evaluated CLE with promising results [64–68]. A multicenter study of 101 patients found that the addition of pCLE to HD-WLE significantly improved the detection of neoplasia [64]; the reported sensitivity and specificity of HD-WLE was 34.2 % and 92.7 %, respectively, as compared with 68.3 % and 87.8 % for pCLE and HD-WLE (p=.002 and p<.001). While several

preliminary studies were performed in patients referred with suspected HGD or neoplasia, a recent study in an unriched population undergoing surveillance for nondysplastic BE found that the use of pCLE in addition to WLE enhanced the detection of dysplasia (28 %), as compared with WLE alone (10 %, p=.04) [65]. The promising results of the probe-based system were recently replicated by Canto et al. using an endoscope-based platform. They demonstrated that the combination of WLE and eCLE resulted in a fourfold increase in the diagnostic yield of BE neoplasia, as compared with WLE alone [66]. Less impressive were the resultsfrom a trial of 68 patients across three centers that assessed the performance of pCLE against WLE. They found that while the specificity and negative predictive value of CLE for excluding neoplasia were high (95 % and 92 %, respectively), sensitivity and positive predictive value were both poor (12 % and 18 %, respectively) [67]. The use of CLE for the assessment of residual metaplasia after ablation or resection of BE has also been assessed. One hundred nineteen patients were interrogated with HD-WLE± CLE, with no difference in the number of patients "optimally treatment" between the two groups [68]. The ongoing refinement of the technical aspects of CLE was demonstrated in a study by Emmanuel et al., who used a new bioprobe on ex vivo specimens and found improved accuracy for a novel "fluorescence intensity" criterion [69].

Colonic Polyps

Several studies to date have demonstrated that CLE has a high degree of accuracy for differentiating between benign and neoplastic polyps in the colon [70–72]. Most have used ex vivo histology as the gold standard and evaluated the characteristics of CLE concurrently with NBI. A study by Buchner et al. of 119 polyps (81 neoplastic, 38 hyperplastic) revealed that pCLE had a higher sensitivity, as compared with NBI (91 % vs. 77 %; p=.010). Specificity between the groups was comparable [70]. A recent meta-analysis of 15 studies and 290 specimens yielded a pooled sensitivity of 94 % and a specificity of 95 % [71]. In contrast to these findings, however, was a subsequent study of 154 lesions that were interrogated by expert endoscopists using a combination of CLE, NBI, and chromoendoscopy. The results revealed poor accuracy of pCLE for both observers (66.7 % and 71.9 %), which was lower than that for both chromoendoscopy and NBI. In addition, they found the quality of video obtained by CLE to be poor, with only 40.5 % of recordings assessed as of sufficient quality [73]. Further studies are needed with in vivo estimation of histology, rather than evaluating CLE recordings.

Colitis Surveillance

A study of 161 patients with long-standing UC randomized them to conventional WLE with random biopsies or chromoendoscopy with CLE. By using chromoendoscopy and CLE, 4.75-fold more neoplasias were detected (p=.005) with 50 % fewer biopsies, as compared with conventional colonoscopy [74]. A study comparing the in vivo differentiation of dysplasia-associated lesional mass and adenoma-like mass was performed on 36 lesions. The kappa coefficient of agreement between CLE and ex vivo histopathology was 0.91, with an accuracy of 97 % [75]. Such findings have been supported by a more recent study of 51 UC patients undergoing routine surveillance. Chromoendoscopy and CLE were utilized in 27 % of patients with suspicious areas seen on WLE. In 5 out of 14 patients, the presence of dysplasia was confirmed with histology. The diagnostic accuracy of CLE for the detection of dysplasia, as compared with standard histology, was sensitivity 100 %, specificity 90 %, PPV 83 %, and NPV 100 % [76].

Miscellaneous

The utility of CLE in the evaluation of early gastric cancer and biliary strictures has also been studied [77, 78]. A Chinese group recently performed a large multiphase trial with interpretation of CLE images following, and then during, endoscopy. When a two-tiered classification was used, CLE yielded a sensitivity of 88.9 % and a specificity of 99.3 %, as compared with 72.2 % and 95.1 %, respectively, for WLE [78]. The diagnosis of indeterminate biliary strictures is a clinical challenge hampered by relative inaccessibility and poor tissue sampling. A probe-based version of CLE is able to pass through the channel of a regular cholangioscope and has been used in the differentiation between benign and malignant strictures. The largest study to date was performed across 5 centers and included 102 patients, of which 40 were proven to have cancer. The sensitivity, specificity, PPV, and NPV of pCLE for the detection of cancerous strictures were 98 %, 67 %, 71 %, and 97 %, respectively. The accuracy of combined ERCP and CLE was significantly higher, as compared with ERCP, for tissue acquisition (90 % vs. 73 %; p=.001) [79].

Endocytoscopy

Endocytoscopy involves high-level magnification endoscopy (up to × 1,400) that permits real-time microscopic inspection of the mucosa. Unlike confocal laser microscopy, it uses optical lenses alone to achieve the required magnification and is limited to visualizing the superficial mucosa. While not commercially available outside of Japan, both probe- and endoscope-based systems have been investigated. Mucosal staining is required and has generally been achieved with topical methylene blue and crystal violet.

Preliminary studies have been performed in the esophagus, stomach, and colon. In a pilot study of squamous neoplasia in

the esophagus, clear images were obtained in 25 patients with a PPV and NPV for malignancy of 94 % and 16.7 %, respectively [80]. A study evaluating patients with BE, however, found that adequate assessment of EC images was impossible in 49 % of sites at × 450 magnification and in 22 % of sites at × 1,125 magnification [81]. In the colon, a study of 48 lesions rated the quality of EC images as "good" in 81 % of cases. The sensitivity and specificity for diagnosing low-grade dysplasia (21patients) were 91.4 % and 100 %, respectively [82]. A larger study of 213 specimens assessing a novel classification system in the colon was able to differentiate hyperplastic from dysplastic lesions in all cases (sensitivity, 100 %; specificity, 100 %; $p<.05$). They were also able to differentiate superficial from advanced neoplasia with a sensitivity of 90.1 % and a specificity of 99.2 % [83].

Autofluorescence

The use of AF during endoscopy is based on the principle that tissues contain variable amounts of fluorophores (biological substances that emit fluorescent light when exposed to light of a short wavelength) and that the different fluorescence "signatures" or patterns can be used to discern normal from dysplasia. This is a wide-field imaging technique, analogous to chromoendoscopy, that has been evaluated in surveillance scenarios primarily in the esophagus, stomach, and colon.

Esophagus

An initial study evaluating AF in 60 patients with BE found that AF increased the detection of HGD/EC, as compared with WLE, but was associated with a high rate of false positives [84]. Several subsequent studies have thus combined the use of AF with WLE and NBI in an effort to improve specificity. In a prospective multicenter study assessing this "trimodality" approach, WLE alone identified only 59 % of the 27 patients with neoplasia found by AF. The use of NBI in addition to AF reduced the false positive rate from 81 % to 26 % [85]. Two further studies evaluated TM imaging in high- and intermediate-risk populations and concluded that it did not significantly increase the diagnosis of dysplasia, as compared with WLE, with random biopsies [86, 87]. A pilot study of AF for squamous cell carcinoma of the esophagus found both poor sensitivity and specificity and concluded that a randomized study was not justified [88].

Stomach

The use of AF in the diagnosis of early gastric cancer has similarly been limited by the constraints of poor specificity [89, 41]. An early study of 109 gastric lesions in 79 patients yielded a sensitivity of 96.4 % and a specificity of 49.1 % [89]. While lacking adequate specificity to be used alone, the potential of combining AF was studied by Kato et al., who found that the use of TM imaging (TMI) with WLE, NBI, and AF together yielded better sensitivity and specificity than did either WLE or AF when used alone [90].

Colon

AF has been used for both polyp detection and characterization. While two early studies [91, 92] demonstrated a lower polyp miss rate with AF, as compared with WLE, a trial of 234 patients evaluating TMI (WLE, AF, and NBI) found that detection rates for adenomas did not differ significantly between the two groups (TM 1.03 vs. WLE 0.97; $p=.360$) [93]. Additional studies have focused on the differentiation of hyperplastic from adenomatous polyps. A study evaluating 424 polyps found that AF and NBI could distinguish adenomatous from hyperplastic polyps with an accuracy of 84.9 % and 88.4 %, respectively, as compared with 75.9 % WLE [94]. In an analogous study, high-quality still images of 80 polyps < 1 cm were recorded using the three modalities and reviewed by nine experienced endoscopists. AFI was found to have worse accuracy than WLE, with NBI showing the best overall accuracy, as well as interobserver agreement [95].

AF has also been studied in colitis surveillance. In a small tandem colonoscopy study, Van den Broek et al. reported a neoplasia miss rate for AF and WLE of 0 % and 50 % ($p=.036$) [96]. An alternate study assessed the ability of AF to identify dysplasia in 48 patients with suspicious lesions already identified on WLE. One hundred twenty-six sites were classified as either low or high AF. While the positive rate of dysplasia in protruding lesions was significantly greater in low AF, as compared with high AF (45 % vs. 13.3 %), there was no apparent difference between low and high AF for flat lesions (8.2 % vs. 0 %) [97].

Despite the refinement of AF of the past 20 years, the issue of background fluorescence, frequently associated with inflammation, has adversely affected its specificity and seems to have limited its potential advantages over competing technologies.

Optical Coherence Tomography

Optical coherence tomography (OCT) is a novel technique that relies on the backscattering of light to obtain both cross-sectional and 3-D images of tissue microstructures. Such images are visually analogous to viewing a coarse "black and white" histological specimen. OCT uses reflected light to construct an image, just as ultrasound uses acoustic waves. Scanning in the gastrointestinal tract has thus far been achieved using a probe inserted through the working channel of a regular endoscope. While neither a water

interface nor tissue apposition is required, the depth of scanning achieved is generally limited to 1–2 mm due to scattering of light by tissue.

Preliminary studies of OCT have been performed throughout the GI tract. A prospective comparative study of OCT and EUS for tumor staging of superficial esophageal squamous cell carcinoma found that OCT (94.6 %) was more accurate at staging tumors confined to the epithelium or lamina propria, as compared with EUS (80.6 %) [98]. A study assessing for the presence of dysplasia in BE used 177 biopsy-correlated images to evaluate a novel "dysplasia index," giving a sensitivity and specificity for HGD/EC of 83 % and 75 %, respectively [99]. OCT may also prove useful following treatment to assess for residual Barrett's. Tsai et al. evaluated 33 patients with 3-D OCT pre- and-post radio-frequency ablation (RFA) and found that the thickness of Barrett's correlated with the likelihood of complete eradication and that the presence of persistent glands immediately following RFA predicted residual BE at follow-up [100]. Although the 3-D reconstruction capability is exciting, a current limitation is its inability to differentiate between the presence of dysplastic and nondysplastic glands in subepithelial Barrett's. If the resolution and depth of interrogation could be improved, OCT would have a major impact in following mucosal dysplasia after ablation.

The ability of the OCT probe to be inserted into the pancreatico-biliary tree means that it may be useful for the determination of indeterminate strictures. A study of 37 patients with biliary strictures (19 malignant) assessed two main criteria for the presence of malignancy. The sensitivity and specificity for the presence of at least one criterion were 79 % and 69 %, respectively, as compared with 53 % and 100 % if both criteria were met [101].

The Promise of the Future: Molecular Imaging

Our growing understanding of cancer biology of the GI tract at cellular and molecular levels has been a major driver of recent interest in exploiting *molecular imaging* in GI endoscopy [102]. In general, molecular imaging can be described as modalities that enable visualization of disease-specific morphologic, functional, cellular, and molecular changes in tissues on the basis of differences in specific molecular signatures of cells or whole tissues, beyond differences in glandular morphology, nuclear morphology, or vascular alterations associated with neoplasia. Identifying and characterizing lesions on the basis of molecular changes, rather than alterations in morphology or topography, has the inherent potential to increase the efficacy of endoscopic surveillance and screening programs. Many of the technological advancements in endoscopy outlined above provide a robust toolkit of imaging techniques with which to visualize and exploit molecular signatures of disease for enhanced diagnosis. Of these, fluorescence imaging is likely to have the greatest applicability in the GI tract, owing to its wide-field macroscopic imaging capability and its ability to provide multispectral imaging (e.g., imaging mucosal AF simultaneously with exogenous fluorescence contrast agents) [103]. Typically, exogenous molecular probes, including antibodies (Abs), their fragments, peptides, activated (molecular beacon) and nanoparticle probes, usually target disease-specific biomarkers [102]. Bioconjugation of such probes with bright fluorescent dyes (or nanoparticles) has been used to target commonly overexpressed epitopes in GI cancers, such as vascular endothelial growth factor and epidermal growth factor receptor [104, 105].

The highly specific binding affinity of Ab-based (or Ab fragment) probes to their respective biological targets provides an optimized signal-to-noise ratio, thereby providing visual contrast of the lesion against the normal mucosal background [106]. A barrier to Ab use, however, is the risk of allergic reaction following systemic delivery, as well as potential impeded diffusion and prolonged penetrance time to the target neoplastic epithelia due to their high molecular weight. Peptides, on the other hand, are of lower molecular weight, consisting of several amino acids in length, and can overcome some of these challenges. However, peptides must be designed carefully to retain high specificity and binding affinity for their targets, which has been an ongoing challenge. Nanoparticles, such as quantum dots and other metallic nanoparticles (with diameters tens of nanometers across), can produce very bright fluorescence signals and can be targeted using Abs and peptides and can be coated (e.g., with polyethylene glycol) to reduce nonspecific binding to proteins in the blood or sera. Such imaging probes can also be multiplexed to target individual biomarkers and emit separate fluorescence emission wavelengths for molecular multiplexing, although this is yet to be demonstrated either preclinical or clinically. If successful, this could translate into "*in situ* molecular pathology" that could be performed during endoscopy in real time, without the need for ex vivo biopsy, tissue processing, and staining by conventional immunohistochemistry. Importantly, however, concerns with pharmacotoxicity of nanoparticle probes have been a barrier to their widespread clinical validation. While the probes described above require direct binding to their biological targets and can be associated with suboptimal background signals due to unbound/nonspecific binding in vivo, another class of probes can be designed for activation by overexpressed endogenous enzymes (e.g., proteases) [107] or pH changes [108] in neoplastic tissues. The fluorescence activity of such molecular "beacons" is quenched in their native state, but after cleavage (e.g., of a peptide) by tumor-associated proteases, these probes produce a significant increase of fluorescence intensity in the tumor.

While molecular imaging in gastrointestinal endoscopy is in its nascent period and most of the studies reported to date have been preclinical in nature, some significant milestones have been achieved toward clinical translation. For example, Hsiung et al. (2008) reported the first use of a fluorescein-conjugated heptapeptide sequence, VRPMPLQ, in patients undergoing colonoscopy. The authors applied the fluorescent agents topically and, using fluorescence confocal microendoscope delivered through the instrument channel of a standard colonoscope, produced exquisite fluorescence images (12 frames/s with 2.5-micron (transverse) and 20-micron (axial) resolution. The fluorescein-conjugated peptide bound strongly to dysplastic colonocytes compared with adjacent normal cells with 81 % sensitivity and 82 % specificity [109]. In 2010, the same team reported the synthesis and clinical (ex vivo tissues) testing of an affinity peptide sequence SNFYMPL that binds specifically to dysplasia in BE and can be fluorescence labeled to target premalignant mucosa on imaging [110]. Li et al. (2013) reported more recently the use of a MG7 antigen-targeting fluorescent Ab agent used to detect human gastric cancer in ex vivo patient samples [111]. Similarly, Goetz et al. (2010) reported molecular imaging of EGFR expression in colorectal cancer using fluorescence confocal endomicroscopy of human specimens [105]. In 2012, Liu et a. reported the topical application of a fluorescent-labeled molecular probe against EGFR in 37 patients with colorectal cancer. Here, an EGFR-specific fluorescence signal was present in 18/19 CRC and 12/18 colorectal adenomas. No or only a weak fluorescence signal was observed in vivo in 10 cases of normal mucosa [112]. In 2012, Bird-Lieberman et al. reported the use of a fluorescently conjugated wheat germ agglutinin (a lectin) for endoscopic visualization of high-grade dysplastic lesions in patients with BE, which were not detectable by conventional endoscopy, with a high signal-to-background ratio of over five [113]. Collectively, studies like these, whether performed on ex vivo human samples or in vivo during fluorescence endoscopy, demonstrate the tremendous interest and potential of "molecular endoscopy." However, while a strong need exists for such molecular-level imaging in gastrointestinal endoscopic screening and surveillance, significant future challenges remain before such approaches are widely adopted by the practicing gastroenterologist.

A significant challenge in GI endoscopic imaging is the sheer size of the mucosal area that must be visualized, often in the setting of concurrent inflammation, in the search for microscopic signs of neoplasia. Molecular agents (e.g., fluorescence-based) must have exquisite target specificity and be able to differentiate normal and hyperplastic (benign) phenotypes from preneoplastic and neoplastic epithelial phenotypes. In the case of protease-activated probes, achieving this level of tissue-subtype specificity may be difficult to quantify. Most GI cancers arise in the epithelial lining, making molecular imaging a viable approach technically, since it could enable wide-field imaging. However, most studies using systemic application of Ab or molecular beacon probes currently require injection 24 h prior to imaging. Topical application of labeled Ab or peptide agents could be performed within a time frame compatible with standard colonoscopy [102]. Other important areas in need of further study and validation include pharmacokinetic and toxicology studies in patients, to define the best imaging time point and clearance for optimal tumor-to-background ratio while minimizing the potential for toxicity. Until such pivotal studies are performed—indeed, within the framework of large multicenter trials—limited studies on efficacy in specific GI disease sites, as well as regulatory hurdles, will continue to impede the wide use and clinical translation of such promising innovations. A cautious optimism toward the promises of molecular imaging in GI endoscopy is thus recommended.

Conclusion

The evolution of endoscopic imaging, from the use of early fiber-optic prototypes to the high-definition instruments currently available, has dramatically changed the paradigms of early cancer diagnosis and treatment. While a plethora of enhanced imaging modalities offer the exciting potential of early lesion detection and rapid in vivo diagnosis, further studies are needed to define the clinical capabilities and appropriate use of many of these techniques.

Despite the scientific and technological advances discussed in this review, the bottom line for detection of dysplasia continues to depend upon good endoscopic technique, careful examination by an "educated" eye, and conventional off-the-shelf high-definition endoscopes. In the colon, a thorough endoscopic examination for polyps or flat dysplasia is useless without a good bowel preparation. However, even with good endoscopic technology, subtle dysplasia and early cancers are still being missed. Dye spraying, which is "low tech," is still useful. Perhaps we just haven't come up with the right compounds or cocktail to detect subtle lesions—something that one can buy cheaply at the supermarket. The backbone of detection will always be the white light endoscopic image.

Considerable effort has gone into the development of the so called "optical biopsy." These devices have been available for almost 10 years (confocal endoscopes and, more recently, the confocal probe). Although they have shown great promise in expert hands and in trials with enriched lesions, they have not been a commercial success, since the great majority of endoscopists have not been convinced that the time and expense are worth the investment or that they

are better than conventional biopsy protocols. The role of molecular endoscopy is on even more tenuous ground as to effectiveness, cost, and biological safety, a great hurdle with regulatory officials.

Currently, the backbone will continue to be carefully examined with an educated eye and off-the-shelf high-quality chip endoscopes.

Compliance with Ethics Guidelines

Conflict of Interest P. Urquhart declares that he has no conflict of interest.

R. DaCosta declares that he has no conflict of interest.

N. Marcon declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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