MINIREVIEWS

Ultra high magnification endoscopy: Is seeing really believing?

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Abstract

Endoscopy is an indispensable diagnostic and therapeutic instrument for gastrointestinal diseases. Endocytoscopy and confocal endomicroscopy are two types of ultra high magnification endoscopy techniques. Standard endoscopy allows for 50 × magnification, whereas endocytoscopy can magnify up to 1400 × and confocal endomicroscopy can magnify up to 1000 ×. These methods open the realm of real time microscopic evaluation of the GI tract, including cellular and subcellular structures. Confocal endomicroscopy has the additional advantage of being able to visualize subsurface structures. The use of high magnification endoscopy in conjunction with standard endoscopy allows for a real-time microscopic assessment of areas with macroscopic abnormalities, providing "virtual biopsies" with valuable information about cellular and subcellular changes. This can minimize the number of biopsies taken at the time of endoscopy. The use of this technology may assist in detecting pre-malignant or malignant changes at an earlier state, allowing for earlier intervention and treatment. High magnification endoscopy has shown promising results in clinical trials for Barrett’s esophagus, esophageal adenocarcinoma, esophageal squamous cell cancer, gastric cancer, celiac disease, colorectal cancer, and inflammatory bowel disease. As the use of high magnification endoscopy techniques increases, the clinical applications will increase as well. Of the two systems, only confocal endomicroscopy is currently commercially available. Like all new technologies there will be an initial learning curve before operators become proficient in obtaining high quality images and discerning abnormal from normal pathology. Validated criteria for the diagnosis of the various gastrointestinal diseases will need to be developed for each method. In this review, the basic principles of both modalities are discussed, along with their clinical applicability and limitations.

Key words: Endocytoscopy; Confocal endomicroscopy; Confocal laser endomicroscopy; High magnification endoscopy

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INTRODUCTION

Endoscopy is an essential tool for the diagnosis and treatment of upper and lower gastrointestinal diseases. From the humble beginnings of various tubes and catheters of the 1600 s, the technological development in endoscopic imaging has been astounding. The most significant developments in gastrointestinal imaging include...
fibre optic endoscopy with the first clinical publication on fibre optic gastroscopy in 1958, followed by the introduction of video endoscopy first showcased in 1983. Technological progress over the last 20 years has significantly improved the imaging capabilities of endoscopy. Ultra high magnification endoscopy is now possible and has allowed gastroenterologist to see the smallest of lesions for in vivo microscopic evaluation in real time. Two major modalities have been developed: endocytoscopy and confocal endomicroscopy.

Each modality has its own strengths and weaknesses, nevertheless each enable “virtual biopsies” and significantly reduce biopsy error by providing a direct microscopic target. High magnification endoscopy has shown promising results in trials for Barrett’s esophagus and esophageal adenocarcinoma, esophageal squamous cell cancer, gastric cancer, celiac disease and colorectal cancer. These techniques may allow for the earlier detection and treatment of neoplastic conditions as the earliest changes of malignancies take place at the cellular and subcellular levels, including changes in capillary architecture and the characteristics of the nucleus.

In this review, we discuss the basic principles of endocytoscopy and confocal endomicroscopy along with their clinical applications.

**BASIC PRINCIPLES OF HIGH MAGNIFICATION ENDOSCOPY**

**Endocytoscopy**

Endocytoscopy (ECS) is an ultra-high magnification modality that allows visualization of surface epithelial architecture at the cellular and subcellular level. It is a contact microscopy technique where physical contact with the mucosal surface is required to obtain the image[2]. ECS provides real-time in vivo images in a parallel section to the mucosal surface. Highly magnified images from a small sampling site (< 0.5 mm diameter) are obtained using a fixed-focus, high power objective lens. These images are then projected on a charged-coupled device[3]. The use of a contrast agent is necessary for the visualization of subcellular entities. The mucosa is pretreated with a mucolytic agent, such as N-acetylcysteine, and then directly stained with 0.25%-1% toluidine blue or 0.5%-1% methylene blue[3]. Endocytoscopy is limited by its ability to only image a superficial layer of the mucosa and is therefore not well suited for analyzing the depth of suspicious lesions.

There are two types of endocytoscopy instruments available: probe-based and endoscope-based. The probe-based devices are used through the working channel of a standard endoscope (Figure 1). There are 2 probe-based models, each 380 cm long with a diameter of 3.2 mm and both produced by Olympus (Tokyo, Japan; models XEC-300 and XEC-120). One model is able to provide magnification of 450 × representing a field of view of 300 µm × 300 µm, and the other model magnifies to 1125 ×, representing an area of tissue 120 µm × 120 µm[4,5]. Using a 19-inch monitor, these two models magnify the image to 570 × and 1400 × respectively. The larger the monitor, the greater the magnification, but at the cost of decrease in resolution depending on the resolution capabilities of the probe and monitor. The endoscope-based devices have an integrated endocytoscope and endoscope. There are 2 models by Olympus (models XGIF-Q260EC1 and XCF-Q260EC1) which each provide a magnification of 580 × on a 19 inch monitor[5]. A soft plastic cap at the end of the endoscope allows for stabilization against the mucosal surface.

Figure 1  Probe-based endocytoscope being passed through the working channel of a traditional endoscope. Image from Sasajima et al[6] (used with permission).

**CEM**

CEM is a new imaging technique that allows in vivo microscopy and histology of the gastrointestinal tract during endoscopy. Confocal microscopy refers to the use of a fine laser beam that scans over a specimen through an objective lens. Reflected light from contrast-stained tissue is focused through a pinhole (confocal) to remove out-of-focus light[6]. By rejecting out of focus light, this technique is effective at producing high-resolution images. Standard endoscopy provides for 50 × optical magnification, whereas confocal endomicroscopy allows for 1000 × magnification[7]. It is able to demonstrate physiological activities such as the release of mucus from crypts and blood flow in capillaries[8]. Unlike endocytoscopy, confocal endomicroscopy is able to obtain images of the lamina propria down to a depth of 250 µm[9].

With the miniaturization of confocal devices it is...
now possible to use confocal endomicroscopy during routine endoscopy. Two types of confocal endomicroscopy systems are currently available: integrated and probe based systems. The confocal laser endomicroscope is an integrated laser and endoscope that allows for high-resolution images at variable depths below the surface (Figure 2). The integrated system obtains images of a section 475 µm × 475 µm, with variable imaging depth controlled by the user, to a maximum of 250 µm[7]. The maximum depth can be achieved through vertical increments of 7 µm. The lateral resolution is 0.7 µm, which represents the minimum detectable distance between two points. Images in the integrated system are obtained at a rate of 0.8 frames/second (1024 × 1024 pixels) or 1.6 frames/second (1024 × 512 pixels)[5]. The mini-probe system is used through the working channel of a standard endoscope, however only offers a fixed (rather than variable) imaging depth at a lower resolution. Each different probe allows for imaging to a specific fixed depth. The mini-probe system obtains images faster (12 frames/s) than the integrated system, however at the expense of resolution being limited by the number of fibers (30 000 single fibers = 30 000 pixels)[7]. The images in both systems are parallel sections to the mucosal surface[10].

CEM requires the use of a fluorescent contrast agent that is excitable and has emission spectra within the blue light range (excitation wavelength 488 nm). Most human studies have used intravenous fluorescein sodium[7,10]. Fluorescein is non-toxic, distributes throughout the tissue within seconds, and is safe for endomicroscopy[7,11]. Fluorescein is effective in demonstrating the structural design of vessels and cellular components but does not have good contrast for nuclei. Acriflavine is another common topical contrast agent, which allows effective visualization of nuclei. In practice, fluorescein and acriflavine may be used together. In animal studies there have been other contrast agents used as well as fluorescently labeled antibodies[2,10].

### CLINICAL APPLICATIONS

The first publications regarding ultra high magnification endoscopy were published in 2004 for both CEM and ECS[12,13]. Since then there have been further studies on a number of upper and lower gastrointestinal tract diseases. Ultra high magnification endoscopy allows for taking fewer targeted biopsies on areas of histological interest visualized by the endomicroscope compared to multiple random biopsy samples[19].

### UPPER GI TRACT

**Barrett’s esophagus and esophageal adenocarcinoma**

Preliminary studies assessed the ability of ECS and CEM for the detection of malignancy in Barrett’s esophagus, which was not evident endoscopically. In 2007, Pohl et al[16] compared ECS images in 16 patients undergoing Barrett’s surveillance with histology. One hundred and sixty-six biopsy sites with no macroscopic evidence of cancer were examined with ECS. Adenocarcinoma was diagnosed in 4.2% of biopsy sites, high-grade dysplasia in 16.9% and low-grade dysplasia in 12.1%. The major

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**Table 1** Comparison of endocytoscopy and confocal endomicroscopy

|                        | ECS                        | CEM                        |
|------------------------|---------------------------|----------------------------|
| Available systems      |                           |                            |
| Probe based            | Olympus (Japan)           | Optiscan (Australia) endomicroscope integrated into Pentax (Japan) endoscope |
| Integrated             | Olympus (Japan)           | Cellvizio, Mauna Kea Technologies (France) |
| Max resolution         | NA                        | 1024 × 1024 pixels with integrated system (lower with probe based system) |
| Max magnification      | 1400 × (probe) 1000 × (integrated) | 1000 ×                     |
| Field of view          | 300 µm × 300 µm (450 x magnification) 475 µm × 475 µm | 120 µm × 120 µm (1125 x magnification) |
| Depth of imaging       | Superficial mucosal layer only | Probe based: Different probes allow for different imaging depths |
|                        |                           | Integrated system: Variable, up to 250 µm below surface (lamina propria) |
| Contrast agents        | 0.5%-1% methylene blue Fluorescein sodium | 0.25% toluidine blue Acriflavine |
| Commercially available?| No                        | Yes                        |
| Advantages             | Higher magnification than CEM | Can visualize subsurface structures up to 250 µm |
|                        |                           | Commercially available Lower magnification than ECS |
| Disadvantages          | Cannot visualize subsurface structures Requires mucolytic preparation of tissue Two contrast agents required for optimal imaging |
|                        | Not commercially available |                           |

ECS: Endocytoscopy; CEM: Confocal endomicroscopy; NA: Not available.
issue in this early study was image quality: only 23% of images at 450 × magnification, and 41% of images at 1125 × magnification were of sufficient quality to visualize characteristics of neoplastic lesions. Therefore the authors concluded that ECS did not produce images of adequate quality to be useful in the malignancy surveillance for Barrett’s esophagus. A 2011 multicentre, randomized controlled trial demonstrated a significant improvement in the ability to detect malignancy in patients with Barrett’s esophagus using probe-based CEM in combination with high-definition white light endoscopy compared to white light endoscopy alone.  

CEM similarly can target suspicious areas of high-grade dysplasia and may identify abnormal neovascularization in esophageal adenocarcinoma. Dunbar et al performed a prospective, double blinded, randomized controlled trial to determine whether confocal endomicroscopy with targeted biopsies improves the diagnostic yield over standard endoscopy and random biopsy alone for unapparent Barrett’s associated neoplasms. Of 39 patients, 16 were suspected to have neoplasm, and 23 were for standard surveillance. All patients underwent standard endoscopy with random 4-quadrant biopsies according to Seattle Protocol, as well as confocal endomicroscopy with targeted biopsies. The order in which CEM and standard endoscopy were performed was randomized. The diagnostic yield of high-grade dysplasia or adenocarcinoma with the confocal endomicroscopy protocol was 33.7% vs 17.2% in the standard endoscopy arm, resulting in an absolute difference of 16.5% (95%CI: 5.2%-27.8%, P = 0.01). Significantly less biopsy samples were required using CEM (9.8 ± 23.8, P = 0.002).

Esophageal squamous cell cancer

Esophageal squamous cell cancer (SCC) is the most prevalent type of esophageal cancer worldwide, especially in Asia. Patients often have advanced stages at presentation resulting in a very poor prognosis. Squamous cell esophageal cancers can be easily diagnosed by ECS via 2 main criteria: an abnormal nucleus (abnormal staining, size and shape) and an increase in cell density (Figure 3). When assessed in ex vivo resected SCC specimens, cancer cells show an irregular heterogeneous cell distribution compared to normal cells arranged homogeneously with a uniform and low nucleucytic ratio. The cellular density is significantly increased with one study demonstrating a mean number of total nuclei per image of 550 ± 66.5 in the cancerous areas versus 129 ± 14.8 in the normal mucosa (P < 0.0001). Kumagai et al performed ex vivo and in vivo studies looking at endoscopictic observation for esophageal SCC. In 57 ex vivo specimens from 28 patients, the sensitivity of ECS for malignant lesions was 94.7%, with a specificity of 84.2%. The in vivo component had 71 lesions from 69 patients, each assessed by two endoscopists in consultation with a pathologist regarding nuclear abnormality and density. The 2 endoscopists diagnosed more than 90% of esophageal squamous cell carcinomas as cancers. The pathologist considered nuclear density to be increased in up to 98% of cases and saw nuclear abnormalities in up to 90% of cases. Specificity is very good (and even reported up to 100% but is reduced if only one criteria is present (either abnormal nucleus or increased cell density).

ECS may be used in early lesions to diagnose malignancy for consideration of local excision. In an in vivo study of 29 patients assessing for early cellular atypia, the ECS positive predictive value for malignancy was 94%, with a false positive rate of 6.3% and false negative rate of 16.7%. The accuracy of distinguishing malignant (Vienna 4 and 5) versus non-malignant (Vienna 1-3) was 82%.

Less data is available for CEM in diagnosing superficial invasion by SCC. Normal squamous epithelium has regular intraepithelial papillary capillary loops that are directed towards the luminal surface. With CEM, SCC demonstrates dilated intraepithelial papillary capillary loops in the upper layer of the squamous mucosa. A 2009 pilot study compared CEM done by 2 endoscopists to histology looking at abnormal cellular and vascular patterns for the diagnosis of SCC. Accuracies of 89% and 96% were obtained for abnormal cellular pattern, and 85% for abnormal vascular pattern.  

Table 2  High magnification endoscopy and esophageal squamous cell cancer

| Modality      | Findings          | Test characteristics |
|---------------|-------------------|----------------------|
| Endoscopy     | Nuclear atypia    | Sensitivity 81%-95%¹  |
|               | Increased nucleus: Cytoplasm ratio | Specificity 84%-100%¹ |
|               | Increase in cell density | Accuracy 82%-90%¹ |
|               | Irregular cellular distribution | |
| Confocal endomicroscopy | Dilated intraepithelial papillary capillary loops | Accuracy 85%-96% |
|               | Abnormal cellular structures | |

¹Determination of malignant vs non-malignant.
The findings seen by ECS and CEM in esophageal squamous cell cancer are summarized in Table 2.

**Gastric cancer**

CEM has been used to compare normal subsurface gastric mucosa with that of malignant lesions. Normal gastric body mucosa shows a honeycomb-like microvascular organization surrounding gastric pits, and a coiled-shaped regular microvascular arrangement surrounding the antral gastric pits. In contrast, undifferentiated gastric neoplasms showed decreased vascularity with irregular short branch vessels. Features of gastric neoplasm seen on CEM include cellular atypia with increased nuclear area and increased vascularity with irregularly shaped microvasculature of various diameters. Kitabatake et al. assessed the ability of pathologists to use CEM images as a “virtual biopsy” for the diagnosis of early gastric cancer. Using CEM still images obtained from 27 patients with early gastric cancer compared to standard histology as the gold standard, 2 blinded pathologists obtained accuracies of 94.2% and 96.2% for the diagnosis of malignancy when good quality images are obtained. Unfortunately, only 59% of images were deemed to be of good quality. The accuracy decreased significantly when lower quality images and inaccessible lesions were included, once again highlighting the limitation of being able to acquire high-quality images consistently. Interobserver variability between endoscopists is very good with a mean kappa value of 0.792 for the identification of neoplastic mucosa.

ECS has decreased sensitivity for neoplastic lesions in the stomach compared to esophageal or colonic lesions secondary to gastric mucous secretion. The sensitivity for gastric neoplasms compared to histology was 56%, with a specificity of 89%.

**Celiac disease**

High magnification endoscopy allows the opportunity for diagnosis of celiac disease in vivo as well as targeted biopsies of abnormal lesions, resulting in a higher diagnostic yield compared to random biopsies, in particular for patchy disease.

ECS has demonstrated three distinct patterns of in vivo histopathology with respect to celiac disease. The first pattern is normal duodenal mucosa showing the presence of normal-appearing, thin, long villi, lined with easily discernible surface epithelial cells. The second pattern of subtotal villous atrophy is demonstrated by thick, shortened villi. The third pattern corresponding to total villous atrophy is shown by the complete absence of villi and the presence of enlarged crypts. In a trial of 40 patients, (32 with known celiac disease, and 8 with suspected disease) 166 ECS recordings were prospectively obtained and compared to histopathology (Marsh classification). Endocytoscopy at 450 × magnification was accurate in predicting moderate to severe celiac disease (Marsh III), however was not reliable in detecting early disease pathology (Marsh I). The use of 1100 × endocytoscopic magnification provided no additional diagnostic value.

The CEM features of celiac disease were initially described in a pediatric trial of 9 patients with suspected celiac disease compared to 10 matched controls. Both endoscopists and pathologists were blinded to the diagnosis. A total of 1384 images were collected from the 19 patients, and 5 images per patient were selected and compared against a biopsy sample of the same site. With subtotal villous atrophy, the duodenal villi are broad and appeared to be folded onto themselves. There is a loss of the normal hexagonal pattern and decrease in goblet cells. With total villous atrophy, duodenal villi are completely absent, and crypts can be visualized with cellular infiltration (increased intraepithelial lymphocytes) in the surrounding stroma. The sensitivity of confocal endomicroscopy compared to histopathology was 100%, specificity was 80% and positive predictive value was 81%. In an adult trial of 30 celiac patients, including 6 with disease refractory to a gluten-free diet, sensitivities were good for intraepithelial lymphocytes (81%) but decreased for villous atrophy (74%) and crypt hyperplasia (52%). Thirty control patients in this study undergoing routine upper endoscopy demonstrated normal duodenal architecture on CEM and histology, resulting in a specificity of 100%. The largest study for CEM in celiac disease assessed 31 patients (17 with celiac disease, 14 controls) and compared over 7000 CEM images with 326 paired biopsy samples. The sensitivity for diagnosis of celiac disease was 94% with a specificity of 92%, with good correlation to the Marsh scoring system. By directing biopsies to microscopically abnormal regions, CEM may be a promising modality to investigate those with a suspected diagnosis of celiac disease but have negative pathology from traditional random biopsies due to patchy disease.

**LOWER GI TRACT**

**Colorectal cancer**

It is unlikely that virtual biopsies with ultrahigh magnification will replace standard histology for the diagnosis of colorectal cancer. It may however, help in certain situations where biopsies are not conclusive for invasive malignancy or when multiple biopsies pose problems with subsequent management, such as superficial rectal lesions amenable to local excision.

Using ECS, resolution can be so detailed that individual red blood cells can be seen circulating through the microvasculature and normal colonic mucosa can be seen and described. Cellular level and structural abnormalities can be observed and it is possible to differentiate between neoplastic and non-neoplastic lesions, as well as invasive malignancy versus adenoma. Aberrant crypt foci may represent the earliest pre-cancer stage of colorectal cancer. Using ECS, dysplastic aberrant crypt...
foci appear as polygonal instead of round, have elongated cell nuclei, and the crypt lumen is linear instead of circular (Figure 4). ECS provided 91.4% sensitivity for low-grade dysplasia and 100% specificity for absence of dysplasia. The interobserver kappa value between a trained endoscopist and the pathologist was 0.68 (95% CI: 0.59-0.78) [35]. A recent study looking at 52 polypoid and non-polypoid colorectal lesions in 49 patients showed that ECS provided good correlation with final histopathological diagnosis [36]. The positive predictive value (PPV) of endocytoscopy compared to pathology was 100% for normal mucosa, hyperplastic polyp and submucosal invasive cancer. The PPV was 93.1% for low-grade adenoma and 90.1% high-grade adenoma. In one case report, a synchronous microscopic lesion (confirmed with pathology) was found on ECS 7 cm away from a resected cancer of the transverse colon [37]. Overall sensitivity and specificity of ECS for the diagnosis of neoplasm ranges from 79%-91% and 90%-100% respectively [23,35].

CEM assessment of the colon is similar to ECS. In normal colonic mucosa, the crypts have regular lumens and are covered by a homogenous layer of epithelial and goblet cells (Figure 5). Normal vessel architecture is hexagonal with a honeycomb appearance, which represents capillaries surrounding the stroma of the crypts. Cancerous tissue shows irregular cellular organization and abnormal epithelial cells with a loss of the normal crypts and goblet cells. There is also decreased or complete absence of mucin. In cancer, the capillaries are distorted and dilated with increased leakage. The vessels have a sporadic organization with little or no orientation to the surrounding tissue [38]. A clinical trial of probe-based CEM versus virtual chromoendoscopy for the classification of colon polyps showed a higher sensitivity for CEM (91% vs 77%, P = 0.01) but no significant difference in specificity, when compared to histology as the gold standard [39]. Virtual chromoendoscopy diagnoses polyps based on the pit pattern seen during chromoendoscopy. CEM may also have a future role for neoplasia surveillance of an ileoanal pouch following proctocolectomy for familial adenomatous polyposis [40]. Table 3 provides a comparison of the colonic architecture in ECS and CEM.

**IBD**

Patients with longstanding inflammatory bowel disease involving the colon have a higher risk of developing colon cancer. Routine colonoscopy every 1-2 years with
multiple biopsies (> 32 biopsies) is recommended for those with pancolitis after 8 years of disease\(^44\). Chromoendoscopy further increases the sensitivity of detecting early neoplasm\(^3\). CEM may help perform targeted biopsies of suspicious areas has been associated with improved detection of intraepithelial neoplasia compared to the current standard of random biopsies with four tissues samples each 10 cm\(^3\). The combination of wide-field chromoendoscopy with narrow-field confocal endomicroscopy can result in a 5 times higher detection rate of neoplastic lesions. This combination technique can be especially helpful for flat lesions that can be otherwise difficult to detect with standard endoscopy\(^44\). There is still no data suggesting whether this early or increased detection confers any mortality benefit.

CEM can be used with high accuracy for the diagnosis of dysplasia-associated lesion or mass (DALM) or adenoma-like mass (ALM) in the setting of IBD. CEM was used in a study of 36 ulcerative colitis patients who had a DALMs or ALMs diagnosed within the previous 16 wk\(^44\). The kappa coefficient of agreement between traditional histopathology and confocal endomicroscopy images was 0.97 with 99% accuracy. This in vivo technique allowed for the differentiation between the two different types of masses, which provides an opportunity to safely determine which patients require immediate referral for total colectomy versus those patients who are suitable for endoscopic resection.

By providing high definition images, CEM may provide excellent insight into the in vivo process of inflammation\(^46\). A study of 31 patients, 17 with UC (12 active, 5 non-active) and 14 non-UC controls, compared histology of rectal biopsy samples with the images from confocal endomicroscopy\(^37\). The in vivo virtual biopsies from confocal endomicroscopy were congruent with traditional histology. In active inflammation goblet cells were not always visible and the crypts, as well as the lumens, were of various sizes and shapes, with an inconsistent arrangement. The capillaries were more visible in active inflammation and seen in all areas of the lamina propria.

### Other clinical applications

Bojarski et al\(^48\) demonstrated the in vivo diagnosis of acute intestinal graft-versus-host disease (GvHD) using confocal endomicroscopy. Nineteen out of 35 patients with acute diarrhea after stem cell transplant had histologic evidence of acute GvHD, with 14 of these 19 also showing confocal endomicroscopic evidence. The sensitivity of confocal endomicroscopy was 74% and specificity 100%. Patients with infectious colitis or ulcerative colitis served as controls and none of them showed any endoscopic evidence of GvHD. This modality may be especially helpful in the situation in which biopsies present a high risk, such as increased bleeding risk (from coagulopathy or low platelets) or increased infection risk (in the setting of severe leukopenia).

Venkatesh et al\(^49\) looked at the usefulness of confocal endomicroscopy in diagnosing pediatric gastrointestinal diseases. The trial involved 44 patients with a total of 36 upper endoscopies and 31 lower endoscopies using a confocal system. The confocal images were deemed to be comparable to traditional histopathology in both normal tissue and many disease states including esophagitis, H. pylori gastritis, celiac disease, inflammatory bowel disease, colonic heterotopia and graft versus host disease.

A variety of other case reports have been published using ultrahigh magnification endoscopy for the diagnosis of Helicobacter pylori\(^50\), collagenous colitis\(^51\), amoebic colitis\(^52\), intraoperative diagnosis of pancreatic cancer in the setting of chronic pancreatitis\(^53\), and intraoperative diagnosis of disseminated malignancy at time of laparoscopy\(^54\). Confocal endomicroscopy has been used in vivo during laparoscopy to analyze healthy and diseased human liver, which offers the possibility for targeted biopsies\(^55\).

### LIMITATIONS OF ENDOCYTOSCOPY AND CONFOCAL ENDOMICROSCOPY

Both confocal ECS and CEM are not effective for wide-field endoscopy, and are better used in conjunction with a wide-field technique. They are both useful for targeted images (optical biopsies) of abnormalities identified by a wide-field technique. As with any new technique, there is an initial learning curve. In this field, the learning curve is not only the technical aspects of attaining high quality images, it also includes learning in vivo pathology. While most endoscopists will likely be able to learn the technical aspects, identifying normal and abnormal pathology correctly is more challenging. Both ECS and CEM involve a time-consuming process with multiple steps including washing, staining and imaging\(^56\). ECS and CEM for diagnostic purposes are also limited by the current lack of validated criteria for diagnosis\(^57\). Finally, the economics of ultra high magnification endoscopy may
be limiting. The endocytoscopy system is currently not available for commercial use. The current cost effectiveness is uncertain. In the long-term both techniques may be economical if a significant number of biopsies taken per patient is reduced or abandoned altogether. However, at least until methods are validated in prospective studies with very high accuracies, histology will remain the gold standard for diagnosis.

CONCLUSION AND FUTURE DIRECTIONS

Endoscopy is invaluable in gastroenterology for the diagnosis and treatment of upper and lower gastrointestinal disorders. Endocytoscopy and confocal endomicroscopy are emerging endoscopic tools that allow for ultra-high magnification and “virtual biopsies” of tissue deemed atypical by standard endoscopy. Both ECS and CEM can come integrated into the end of an endoscope or as probes that can be used through the working channel of a standard endoscope, however only CEM is currently commercially available.

The benefit of high magnification endoscopy is that it provides for the first time a new opportunity to visualize cellular and subcellular pathology in vivo. This allows us to see and understand in real time normal physiologic functions of the GI tract. By knowing this, we can then understand the real time, in vivo pathological changes related to disease. Our knowledge of disease can significantly be expanded by this capability. Real-time inflammation can be analyzed and explored to better our knowledge of the pathophysiology (and therefore treatment) of inflammatory bowel disease. Cellular and vascular changes related to malignancy can be studied in vivo perhaps leading to new therapeutic targets. Early microscopic changes can be visualized without having to wait for larger, later stage macroscopic changes to be evident. As a result, ultra-high magnification endoscopy may conceivably have applications in cancer resections to look for clear resection margins.

Similar to other new technologies developed through the decades, including endoscopic retrograde cholangiopancreatography and endoscopic ultrasound, as the use of high magnification endoscopy increases, clinical applications will expand. Opportunities for research using these techniques are numerous. Further research is required to standardize classification systems for both ECS and CEM in the diagnosis of different malignancies. The current data suggests a promising future for ultra-high magnification endoscopy, and future larger scale research will help clarify the role and indications for endocytoscopy and confocal endomicroscopy.

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