Michael B. Wallace, Section Editor

# Probe-Based Confocal Laser Endomicroscopy

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Confocal laser endomicroscopy is a new field of endoluminal imaging that offers extremely high magnification and resolution, approximating white light microscopy. This has the potential to fundamentally change the current algorithms of gastroenterologic diagnosis. A recent consensus conference (International Conference of Cellvizo Users, Miami, FL, Feb 22–23, 2009) on probe-based confocal laser endomicroscopy (pCLE) held in February 2009, established basic indications, techniques, research priorities and standards for image interpretation. This article summarizes the findings of that meeting.

Since the inception of flexible endoscopy, the endoscope has been used as both a diagnostic and therapeutic instrument. The diagnostic component has relied heavily on endoscopically directed biopsy, with histology and all of its subforms serving as the gold standard. Although highly accurate, histology has major limitations including: incremental cost, risk, time delay, lack of in vivo information such as blood flow, and limited ability to predict disease course. In the case of bile duct cancers, biopsy is particularly prone to false-negative results. On the other hand, most endoscopic imaging tools, such as high-definition endoscopes, with or without optical enhancement, are useful for "guiding" biopsy, but are rarely able to make specific diagnoses of normal or abnormal tissue sufficient to replace biopsy. This paradigm seems likely to change.

CLE can be performed currently with 1 of 2 FDA approved devices: 1 integrated into an endoscope (Pentax, Ft Wayne, NJ; herein termed eCLE) and 1 as a stand-alone probe (herein termed pCLE) capable of passage through the accessory channel of most endoscopes (Cellvizio, Mauna Kea Technologies, Paris, France). This review focuses on the pCLE system (Figure 1). A previous column in this section of GASTROENTEROLOGY has discussed the eCLE system.[1] pCLE has several advantages and disadvantages compared with eCLE. Advantages include the greater versatility of pCLE probes, which can be used in conjunction with virtually any endoscope (or cholangioscope, bronchoscope, ureteroscope etc), ad hoc usage, such as when a lesion is detected with a normal endoscope, and acquisition at video frame rate of 12 frames/sec allowing in vivo imaging of capillary flow (Video). Disadvantages include a slightly lower resolution (approximately 1 $\mu$m compared with 0.7 $\mu$m for eCLE) and smaller field of view (240–600 $\mu$m). The fiber probes consist of a bundle of 30,000 optical fibers with a distal lens, and proximal precision connector. The fluorescent signal returning from the tissue is converted into an image using a detector (Avalanche Photo Diode), and software/hardware systems for image correction, stabilization, and display.

Clinical image acquisition is optimized by use of a contrast agent. Although many previously published images with the eCLE system have used topical acriflavine dye, concerns about DNA damage[2] by this and other nuclear stains have reduced its use. Most pCLE imaging is performed with intravenous fluorescein, an agent FDA approved for diagnostic fluorescein angiography or angioscopy of the retina andiris vasculature. Fluorescein is a highly safe agent whose major side effects are short term (1–2 hours) and include yellowish skin discoloration and 1–2 days of bright yellow-colored urine. In a safety analysis of IV fluorescein for pCLE imaging, no serious complications were observed in 410 consecutive cases.[3]

The current potential indications for pCLE imaging are broad and include almost all current applications of endoscopic biopsy. Early data suggest that the major capabilities of pCLE will be to distinguish non-neoplastic tissue from neoplasia, such as surveillance of nondysplastic Barrett's esophagus (BE), chronic inflammatory bowel disease (IBD), small colorectal polyps, and indeterminate bile duct strictures. Other novel applications include detection of early rejection in small bowel transplantation, detection of residual neoplasia after endoscopic mucosal resection of large flat colorectal polyps, detection of microscopic colitis, and detection of celiac sprue. In most of these, the key role will be to detect nondiseased tissue,

*Abbreviations used in this paper:* BE, Barrett's esophagus; eCLE, endoscope-based confocal laser endomicroscopy; IBD, inflammatory bowel disease; IEN, intraepithelial neoplasia; NPV, negative predictive value; pCLE, probe-based confocal laser endomicroscopy.



0016-5085/09/$36.00
doi:10.1053/j.gastro.2009.03.034

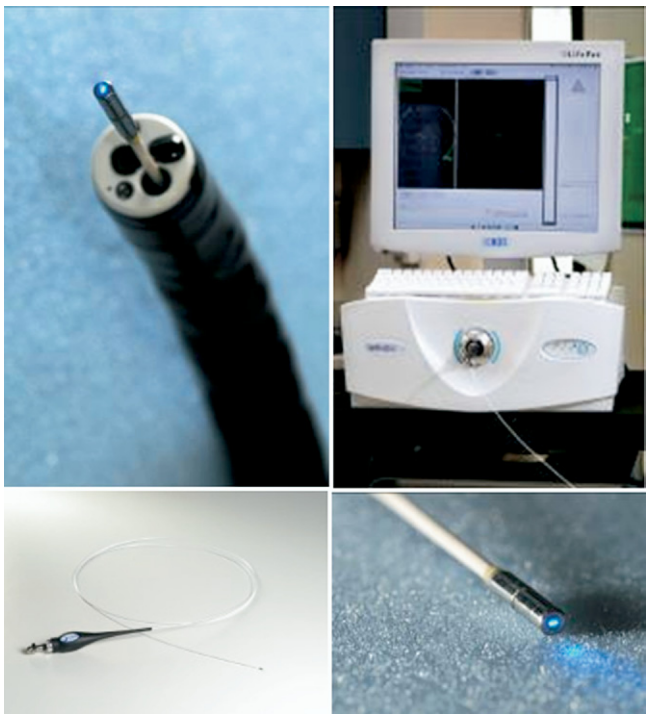


**Figure 1.** The probe-based confocal laser endomicroscopy (pCLE) imaging system showing the fiber probe within a standard endoscopic accessory channel, and the laser scanning unit and software interface.

and thus eliminate the large proportion of biopsies done, which yield no disease.

In all of these applications, pCLE will likely need to be used in conjunction with a "red flag" technology. pCLE is a small-field imaging system, and thus is only appropriate for classification of tissue at a site already detected by standard or optically enhanced endoscopy. An example would be use of narrow-band imaging to detect regions of suspicion in BE, followed by pCLE to confirm intraepithelial neoplasia (IEN), and guide immediate therapy.

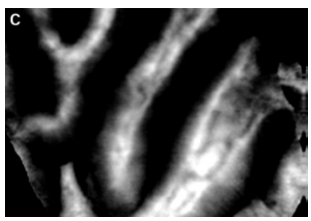
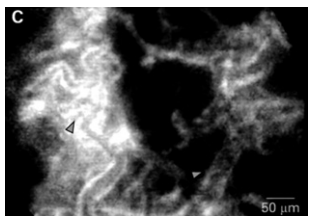
**Barrett's Esophagus.** Current surveillance guidelines for BE call for 4 quadrant random biopsies every 1–2 cm throughout the length of columnar epithelium in the esophagus. In patients without IEN, the annual incidence of high grade IEN or cancer is <1 in 200 per year. The pathology cost to Medicare alone for a single jar of 4 biopsies is substantial. Thus, a technology that could reliably exclude neoplasia has the potential to dramatically reduce the need for, and cost of, random biopsies.

Early evidence using pCLE for BE has identified key features of neoplasia, and was able to detect IEN with a per-biopsy sensitivity for 2 independent investigators of 75%, and specificity of 89%–91% with good interobserver agreement ($\kappa = 0.6$).[4] In the low-risk population studied, this led to a 98.8% negative predictive value (NPV), thus allowing nearly risk-free elimination of the random biopsy when pCLE was negative. The features and example images indicative of neoplasia are shown in Table 1. A prospective, multicenter trial is now underway to evaluate the accuracy of pCLE in comparison with high-definition white light and narrow-band imaging endoscopy.

Potential future applications of pCLE take advantage of the unique aspect of real-time in vivo imaging in living tissue. These include the potential for novel biomarkers of risk and prognosis such as angiogenesis, and the ability to image fluorescent-tagged molecular agents. Fundamentally, the field of Barrett's will need to move beyond reliance on histologic intestinal neoplasia alone as a biomarker of risk. Whether this will be accomplished by in vivo imaging markers, genomic biomarkers, proteomic biomarkers, or other means is not yet known.

**Colorectal Disease.** Colorectal cancer screening with colonoscopy and polypectomy remains the gold standard for disease prevention. Despite advantages, there are major limitations to the current paradigm including the large number of benign (small distal hyperplastic) polyps, and increased risks and costs associated with polypectomy. Recent studies have shown that polypectomy is the single greatest risk factor for major complications of colonoscopy.[5]

**Table 1.**

| | | |
|---|---|---|
| Nondysplastic Barrett's (with permission from Pohl et al[4]) |   | Absence of criteria below. |
| IEN |   | Irregular epithelial lining; variable width of the epithelial lining; fusion of glands; presence of "dark areas" (decreased uptake of fluorescein); irregular vascular pattern. |