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# Confocal Endomicroscopy

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Anjali Basil and Wahid Wassef

Additional information is available at the end of the chapter

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## 1. Introduction

Confocal endomicroscopy is a recently developed endoscopic technology that allows for histological analysis of tissue *in vivo*. Conventional endoscopy involves identifying lesions grossly followed by biopsy for histological analysis. Confocal endomicroscopy allows for the performance of real time biopsy during endoscopy by observation of the mucosal layer of the gastrointestinal tract at the cellular level. Images are displayed in real-time during the examinations.

Confocal laser endomicroscopy was developed in 2004 (Pentax Corporation, Montvale, New Jersey) and was cleared by the Food and Drug Administration in 2004 for use. Two systems are currently available and have been approved by the FDA, tip-integrated confocal laser endoscope and a flexible fiber-based confocal miniprobe [1].

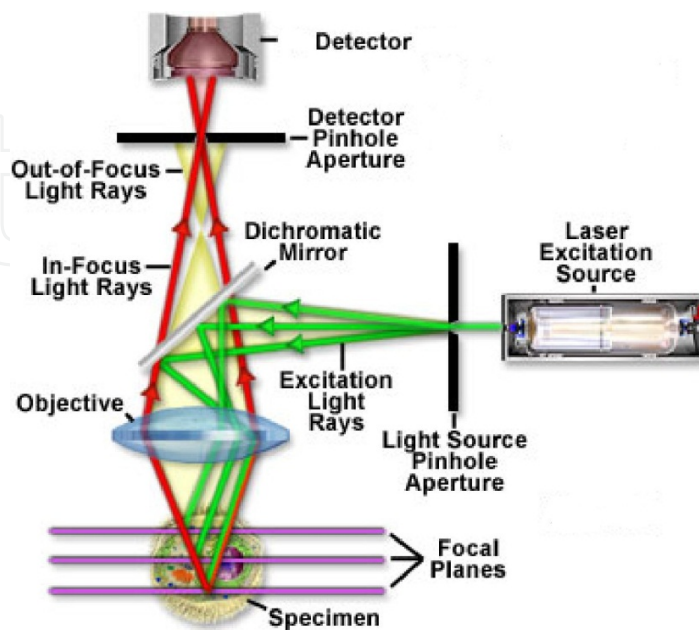
The technique of confocal endomicroscopy has been used for diagnosis of upper gastrointestinal disorders such as Barrett's esophagus, gastric carcinoma, Celiac disease. It also has application in diagnosis of lower gastrointestinal and biliary tract disorders such as colon polyps, Ulcerative colitis and pancreaticobiliary strictures.

## 2. Technical overview

### 2.1. Basic principle of confocal microscopy

CLE is based on low-power blue laser light based tissue exposure and fluorescence. The laser light is focused on an area of interest and back scattered light is then refocused onto the detection system by the lens. The back scattered light passes through a pinhole aperture which increases the resolution of the image. A dye is used to provide the contrast for adequate

visualization. Serial optical sections are obtained oriented parallel to the tissue surface. The confocal setup is shown in Figure 1 [2].



**Figure 1.** [2] Confocal microscope setup

## 2.2. Types of endoscopes

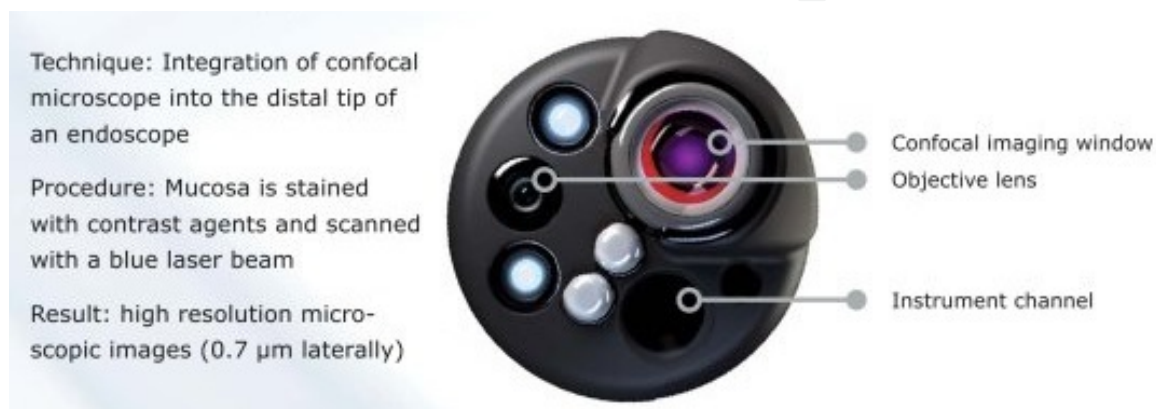
There are two clinically available CLE systems, the eCLE and the pCLE. The eCLE system is integrated into the Pentax endoscope with the confocal imaging window at the distal tip of the endoscope as shown in Figure 2 [3]. The pCLE system-the Cellvizio system (pCLE; Mauna Kea Technologies, Paris, France) is probe based and is inserted through the accessory channel of traditional endoscope. These fiber-bundle probes provide faster image acquisition. The pCLE system has a plane of visualization of up to 250 microns while for the probe based system it is 70 to 130 microns.

## 2.3. Staining techniques

Florescent dyes used for staining in confocal endomicroscopy include flourescein, acriflavine hydrochloride and cresyl violet. Flourescein is the most widely used dye. Acriflavine is not FDA approved as it is potentially mutagenic due to binding to nucleic acids. Acriflavine also has limited penetration and gives uneven staining. Cresyl violet has limitations due to the fact that it does not demonstrate well the vascular structure of the tissue being studied. Flourescein is used intravenously and is nontoxic. It quickly distributes throughout the body allowing for endoscopy to be performed immediately after injection. It also provides good contrast for visualization of the vasculature and subcellular structures. Flourescein's nontoxic nature, rapid visualization post injection and its ability to provide good contrast for visualization of vasculature make it the most widely used dye.

## 2.4. The confocal endoscopy system

There are two FDA approved confocal endomicroscopy systems. The eCLE system uses a fiber optic cable integrated at the tip of the endoscope to convey laser light. The confocal microscope in the eCLE system is integrated in the tip of the endoscope. The probe-based CLE (pCLE) uses a fiber optic probe bundle inserted into the port of a standard endoscope to convey light from a confocal microscope situated outside the patient. Laser light collected from the tissue in focus by the confocal microscope is transferred to the photodetector. The intensity of the photosignal is measured to create a two-dimensional image. The image depth can be adjusted by moving the microscope optics.



**Figure 2.** [3].Confocal Endoscope

## 2.5. Practical aspects of confocal endomicroscopy

Patient preparation for confocal endomicroscopy is the same as the preparation for conventional endoscopy. The procedure is performed under moderate sedation or general anesthesia depending on patient co-morbidities and the type of endoscopic procedure i.e. colonoscopy, upper endoscopy, endoscopic retrograde cholangiopancreatography or endoscopic ultrasound. Procedure times are similar to those for the conventional endoscopic procedures. In addition to the contraindications for conventional endoscopies, confocal endoscopy also has the additional contraindication of allergy to contrast agents.

# 3. Application of confocal endomicroscopy for various clinical modalities of the gastrointestinal tract

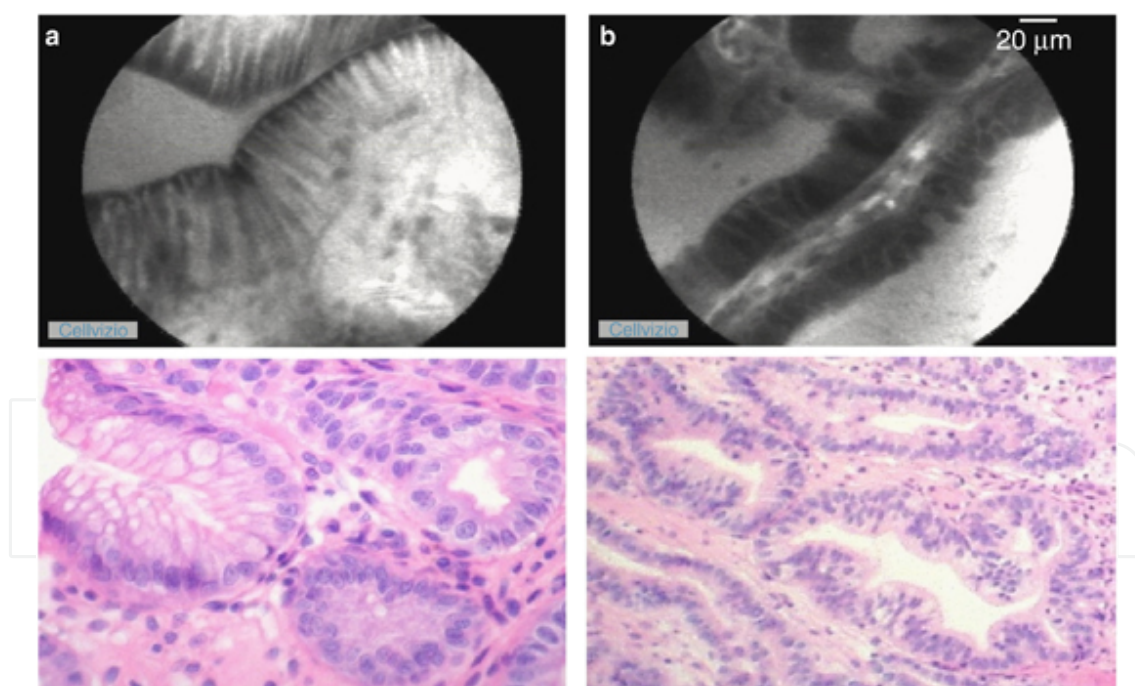
## 3.1. Barrett's esophagus

Barrett's esophagus is a premalignant condition in patients with long standing gastroesophageal reflux disease. Current guidelines for screening for Barrett's esophagus in patients with chronic gastroesophageal reflux disease recommend that random four-quadrant biopsies be

performed at 1cm or 2cm intervals based on suspicion of high grade intraepithelial neoplasia. The random biopsy method has a high likelihood of sampling error as only a fraction of the epithelium is biopsied.

Confocal endomicroscopy allows for real time biopsies of target tissues identified by fluorescein staining. Kiesslich et al. [4] studied confocal imaging as a method for diagnosis of Barrett's esophagus. They found it to be a reliable modality to identify different epithelial cell types at the squamocolumnar junction. In patients with neoplasia, the CLE image showed large black cells with loss of architecture and variable size of lumina. As per the Miami Classification – described by a consensus of pCLE users to standardize image criteria, the normal squamous epithelium appears as flat cells without crypts or villi and with bright vessels. Villiform structures and dark, irregularly thickened epithelial borders and dilated irregular vessels were seen in Barrett's esophagus with high grade intraepithelial neoplasia. Figure 3 [5] demonstrates a comparison of histopathological images and confocal images.

pCLE also has a role in treatment management of Barrett's esophagus. It aids in the appropriate localization of lesions and hence prediction of pathology with targeted biopsies [6]. A possible therapeutic role for pCLE has also been shown by providing real time information to guide therapy during endoscopy.



**Figure 3.** Comparison between pCLE images and histopathological images. (a) Non-dysplastic Barrett's mucosa seen on probe-based confocal laser endomicroscopy (pCLE): representative images are from different samples. Features include regular epithelial surface, easily identifiable goblet cells, equidistant glands, glands that are equal in size and shape, normal size cells without enlargement, and regular and equidistant cells. (b) Dysplastic Barrett's mucosa as seen on pCLE: representative images are from different samples. Features of dysplasia include saw-toothed epithelial surface, not easily identifiable goblet cells, non-equidistant glands, unequal size and shape of glands, enlarged cells, and irregular and non-equidistant cells.



3.2. Gastric cancer

In the stomach, confocal endomicroscopy can detect gastric cancer associated co-morbidities including *Helicobacter pylori*, intestinal metaplasia and gastritis. It can differentiate between these co-existing conditions and gastric cancer so that target biopsies can be performed. Confocal endomicroscopy can define villous and crypt architecture. Zhang et al. [7] devised a classification system based on gastric pit patterns as shown in Figure 4 [7]. They evaluated 137 patients with CLE based on gastric pit patterns. A sensitivity of 90% and specificity of 99% was found for detecting gastric cancer.

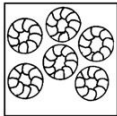
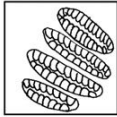
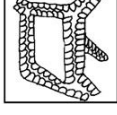
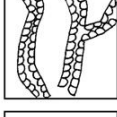
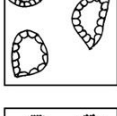


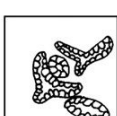
The classification of gastric pit patterns by confocal endomicroscopy			
Category	The appearance of pit patterns by confocal endomicroscopy	Distribution area	Diagram
Type A	Round pits with round opening	Normal mucosa with fundic gland	
Type B	Non-continuous short rod-like pits with short thread-like opening	Corporal mucosa with chronic inflammation	
Type C	Continuous short rod-like pits with slit-like opening	Normal mucosa with pyloric gland	
Type D	Elongated and tortuous branch-like pits	Antral mucosa with chronic inflammation	
Type E	The number of pits decreasing and pits prominently dilating	Chronic atrophy gastritis	
Type F	Villus-like appearance, interstitium in the centre and goblet cells appearing	Intestinal metaplastic mucosa	
Type G			
Type G <sub>1</sub>	Normal pits disappearing, with the appearance of diffusely atypical cells	Signet-ring cell carcinoma and poorly differentiated tubular adenocarcinoma	
Type G <sub>2</sub>	Normal pits disappearing, with the appearance of atypical glands	Differentiated tubular adenocarcinoma	

Figure 4. [7] Gastric pit patterns-G1 and G2 correspond to pathological patterns for gastric cancer.

3.3. Colon polyps

Pathological analysis is the only method to accurately distinguish benign and malignant polyps. This is performed postpolypectomy. Confocal allows us to perform a histological analysis prior to polypectomy with an in vivo diagnosis. Polyps with low malignant potential can be left behind while those with malignant potential can be removed. Confocal endomicroscopy differentiates hyperplastic and neoplastic polyps based on the appearance of dark thickened epithelium and dysplasia. Figure 5 [8] shows a comparison between the confocal laser microscopy image and hematoxylin and eosin staining of a hyperplastic polyps. A classification system, the Mainz classification, shown in Table 1 [9] was developed based on the appearance of the vessel and crypt architecture. Molecular imaging during confocal endoscopy is also being used for diagnosis of colonic malignancies. A labeled peptide is applied topically which binds to a neoplastic area. These could provide a means for surveillance for gastrointestinal neoplasms.

Grade	Vessel architecture	Crypt architecture
Normal	Hexagonal, honeycomb appearance	Regular luminal openings, homogenous layer of epithelial cells
Regeneration	Hexagonal, honeycomb appearance with no or mild increase in the number of capillaries	Star-shaped luminal crypt openings or focal aggregation of regular-shaped crypts with a regular or reduced amount of goblet cells
Neoplasia	Dilated and distorted vessels; irregular architecture with little or no orientation to adjunct tissue	

Table 1. Mainz classification

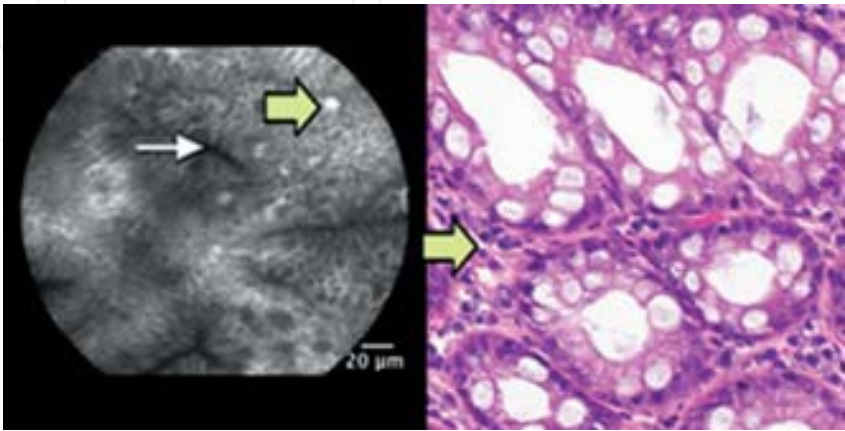


Figure 5. [8] Confocal image of hyperplastic polyp and hematoxylin and eosin image. Thin arrow shows dark goblet cells and thick arrows in both images show the small vessels.

### 3.4. Inflammatory colitis

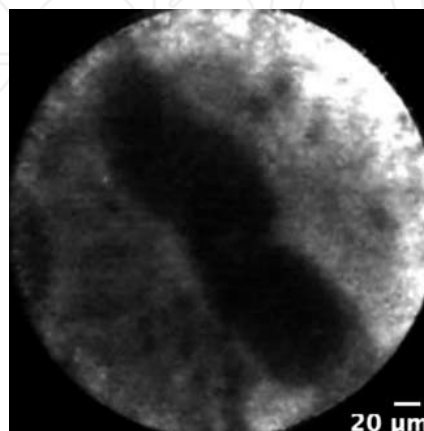
Confocal endomicroscopy may be used for diagnosis and cancer surveillance in patients with inflammatory bowel disease. Endomicroscopy in combination with panchromoendoscopy was compared to white light endoscopy in a prospective trial for surveillance. The accuracy of endomicroscopy in predicting intraepithelial neoplasia was found to be 97.8% [4]. Confocal endomicroscopy can also diagnose inflammatory changes in the mucosa.

Confocal endomicroscopy has also been used in microscopic colitis with success. Microscopic colitis is frequently difficult to diagnose due to the patchy distribution. CLE enables targeted biopsies of abnormal mucosa. Similarly confocal endomicroscopy has also found a use in graft-vs-host disease post bone marrow biopsy in patients with diarrhea [10].

### 3.5. Pancreaticobiliary strictures

Pancreaticobiliary strictures have varying etiologies and maybe benign or malignant. Several methods are used to diagnose the nature of the stricture, i.e., benign or malignant. These include biopsy, brushing and needle aspiration. The diagnostic yield for malignant biliary strictures is limited as most cholangiocarcinomas arise in the bile duct wall. Confocal endomicroscopy provides real-time images. The pCLE is used for evaluation of the biliary system. Due to its small size, it is optimal for biliary imaging. The confocal miniprobe is passed through the channel of a side-viewing endoscope.

A classification for biliary and pancreatic findings on pCLE were devised in 2012-MIAMI Classification [11]. Characteristics most suggestive of malignancy were thick white or dark bands, or dark clumps. Figure 6 demonstrates thick bands as seen via confocal microscopy [12]. Meining et al. tested the validity of the Miami classification by conducting a blinded consensus review of pCLE videos from 47 patients. The sensitivity for the criteria was found to be 97%, specificity was 33%, and positive and negative predictive values of 80% each [12]. Current data on pCLE for diagnosis of biliary strictures is limited. Further studies are needed to determine the accuracy of the confocal miniprobe in diagnosing malignant biliary strictures and differentiating these from inflammatory strictures.



**Figure 6.** [12] Image from probe based confocal laser endomicroscopy of thick dark band



## 4. Limitations

Although confocal endomicroscopy is a novel and emerging technology, it has its technical and practical limitations. There is a learning curve for the endoscopists as evidenced by Wallace et al. [13] who evaluated the accuracy of confocal endomicroscopy of 9 international endoscopists in patients with Barrett's esophagus associated dysplasia. They found an overall accuracy for diagnosis of high grade dysplasia in Barrett's esophagus to be 90.5%, sensitivity of 88% and specificity of 94%.

Buchner et al. [14] studied the learning curve for pCLE in the diagnosis of colorectal neoplasia and found that most GI endoscopists required 2 hours of training and review of approximately 50-70 cases with high quality pCLE images to become proficient.

## 5. Overview of comparable modalities

Several modalities with similar applications as confocal endomicroscopy are being developed and used for detection of gastrointestinal pathologies. These include Optical coherence tomography, Virtual chromoendoscopy and magnification endoscopy.

Optical coherence tomography uses a wavelength of light to provide real-time sectional imaging of the mucosa, pit patterns and glandular architecture. It is however limited due to inability to assess large surface areas.

Chromoendoscopy uses dye application as a method to enhance the architecture of the mucosa for visualization. The dyes used include acetic acid, methylene blue and indigo carmine. Chromoendoscopy is not routinely used as it is time consuming due to the preparation and the performance of the procedure. Its use is limited to tertiary care centers due to the experience required for its use.

Magnification endoscopy uses a movable lens on the endoscope which allows the endoscopist to alter the degree of magnification to provide higher resolution. Magnification endoscopes have a pixel density of 850,000 in comparison to conventional endoscopes which have a density of 100,000 to 200,000. Magnification endoscopy allows for histologic identification by increasing the resolution of the image.

## 6. Summary

Confocal endomicroscopy is a developing method for diagnosis of various gastrointestinal disorders such as Barrett's esophagus, gastric cancer, inflammatory bowel disorder, colon polyps and pancreaticobiliary strictures. It provides real-time images to aid in the diagnosis and management for these conditions. Confocal microscopy also achieves a more targeted biopsy of the abnormal tissue to expedite the therapeutic planning and decisions regarding

endoscopic intervention possibly eliminating the need for repeated procedures. Future trends in confocal endomicroscopy include the wide spread use of molecular imaging with labeled peptides to aid in a more accurate diagnosis of malignancies and for therapeutic planning.

## Author details

Anjali Basil and Wahid Wassef

UMass Memorial Medical Center, Worcester, MA, USA

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