Beyond Standard Image-enhanced Endoscopy Confocal Endomicroscopy

Daniel Teubner, MD[a], Ralf Kiesslich, MD[a,*], Takayuki Matsumoto, MD[b], Johannes W. Rey, MD[a], Arthur Hoffman, MD[a]

KEYWORDS

• IBD • Confocal endomicroscopy • Chromoendoscopy

KEY POINTS

- Endomicroscopy is a new imaging tool for gastrointestinal endoscopy.
- Panchromoendoscopy with targeted biopsies has become the method of choice for surveillance of patients with inflammatory bowel disease.
- Endomicroscopy can be added after chromoendoscopy to clarify whether standard biopsies are still needed.
- This smart biopsy concept can increase the diagnostic yield of intraepithelial neoplasia and substantially reduce the need for biopsies.
- Endomicroscopy is still mainly used for research but clinical acceptance is increasing because of a multitude of positive studies about the diagnostic value of endomicroscopy.

INTRODUCTION

Patients with long-standing extensive chronic inflammatory bowel disease (IBD) have an increased risk to develop intraepithelial neoplasia and colitis-associated cancer compared with the average population risk. Triggers to neoplasia are chronic inflammation and sporadic adenoma.[1] Thus, colonoscopic surveillance is recommended in patients with long-lasting ulcerative colitis (left side and pancolitis) as well as Crohn's colitis.[2] Guidelines recommend performing targeted (visible lesions) and random biopsies. Here, 2 to 4 random biopsies every 10 cm within the colon should be performed.[2] Dysplastic lesions are often multifocal, flat, and difficult to detect with white light endoscopy.[2]

[a] Department for Internal Medicine, Gastroenterology and Oncology, St Marienkrankenhaus, Richard-Wagner-Street, 14, Frankfurt 60318, Germany; [b] Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
* Corresponding author.
E-mail address: info@ralf-kiesslich.de

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In 2003, the first randomized controlled trial[3] was published evaluating lesions in the colon according to a modified pit pattern classification after panchromoendoscopy with methylene blue (0.1%) (pit pattern I–II, endoscopic prediction of nonneoplastic lesions; pit pattern III–V, endoscopic prediction of neoplastic lesions). Chromoendoscopy made it possible to identify dysplastic lesions and to clarify the borders between neoplastic and normal tissue. This development has led to the smart biopsy concept, in which more targeted biopsies become possible after enhanced endoscopy (chromoendoscopy) (**Figs. 1–3**). Panchromoendoscopy has become the method of choice for endoscopic surveillance of patients with IBD (European consensus guidelines).[2]

Confocal laser endomicroscopy (CLE) is a research and clinical tool that promises to improve diagnostics and therapeutic algorithms in patients with IBD. Endomicroscopy has been shown to be useful in dysplasia detection and differentiation of lesions to optimize their management (differentiation between colitis-associated neoplasia, sporadic neoplasia, and nonneoplastic lesions) and to reduce the number of unnecessary biopsies.[4] Confocal endomicroscopy has for the first time revealed in vivo tissue microscopy to gastroenterologists.[4] Using this technology, changes in vessel, connective tissue, and cellular-subcellular structures can be graduated during ongoing colonoscopy at subcellular resolution.[5,6]

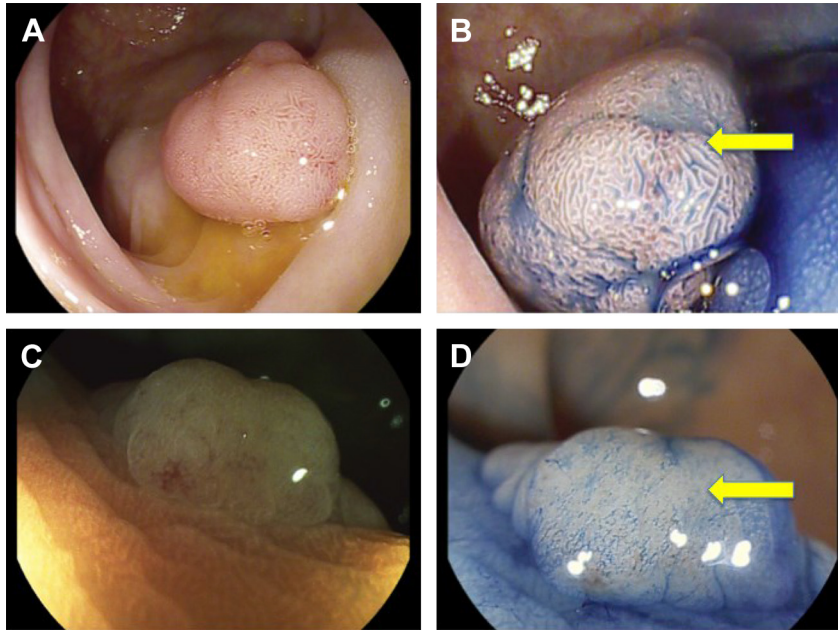

Fig. 1. Chromoendoscopy of colorectal lesions. (*A*) A polypoid lesion can be identified in the ascending colon of a 64-year-old patient who has had ulcerative colitis for 34 years. (*B*) Chromoendoscopy with methylene blue (0.1%) clarifies the mucosal pattern (pit pattern IIIL, *arrow*), which predicts tubular adenoma. Endoscopic resection was performed and final histology confirmed adenoma with low-grade intraepithelial neoplasia. (*C*) A sessile lesion can also be identified. A wide cryptal opening is seen (pit pattern II) using magnification and chromoendoscopy (*D*). Hyperplastic changes (nonneoplastic) could be confirmed histologically.

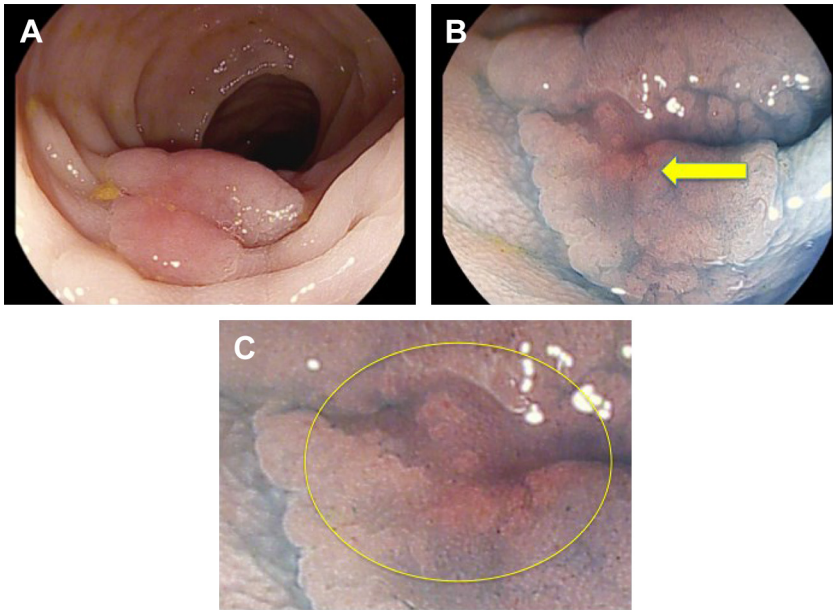

Fig. 2. Colitis-associated dysplasia. (*A*) A flat lesion is visible using white light high-definition colonoscopy. (*B*) Chromoendoscopy with methylene blue (0.1%) clarifies the borders and surface architecture. An irregular pattern with shallow depression (type IIc, pit pattern V) can be identified (*arrow* and magnified view [*C*]). Endoscopic resection revealed colitis-associated early cancer (shallow infiltration of the submucosal layer).

Confocal endomicroscopy has been shown to decrease the need for random biopsies because it has a high negative predictive value. Its use is often combined with chromoendoscopy. Intravital staining is used to identify lesions and targeted endomicroscopy is performed to clarify the need for standard biopsies. Thus, endomicroscopically normal-looking mucosa does not usually require further standard biopsies. Neoplastic changes and regenerative tissue can readily be identified using this method. However, detailed knowledge about the microarchitecture of the mucosa is necessary to achieve high diagnostic yields.[6,7]

TECHNICAL PRINCIPLES OF CONFOCAL ENDOMICROSCOPY

The CLE technique introduced in 2004 has been developed for cellular and subcellular imaging of the mucosal layer.[5] In confocal microscopy, a low-power laser is focused to a single point in a microscopic field of view and the same lens is used as both condenser and objective folding the optical path, so the point of illumination coincides with the point of detection within the specimen.[6] Light emanating from that point is focused through a pinhole to a detector and light emanating from outside the illuminated spot is not detected.

Because the illumination and detection systems are at the same focal plane, they are termed confocal.[6] All detected signals from the illuminated spot are captured and the created image is an optical section representing 1 focal plane within the examined specimen. The image of a scanned region can be constructed and digitized by measuring the light returning to the detector from successive points, and every point is typically scanned in a raster pattern.[6]

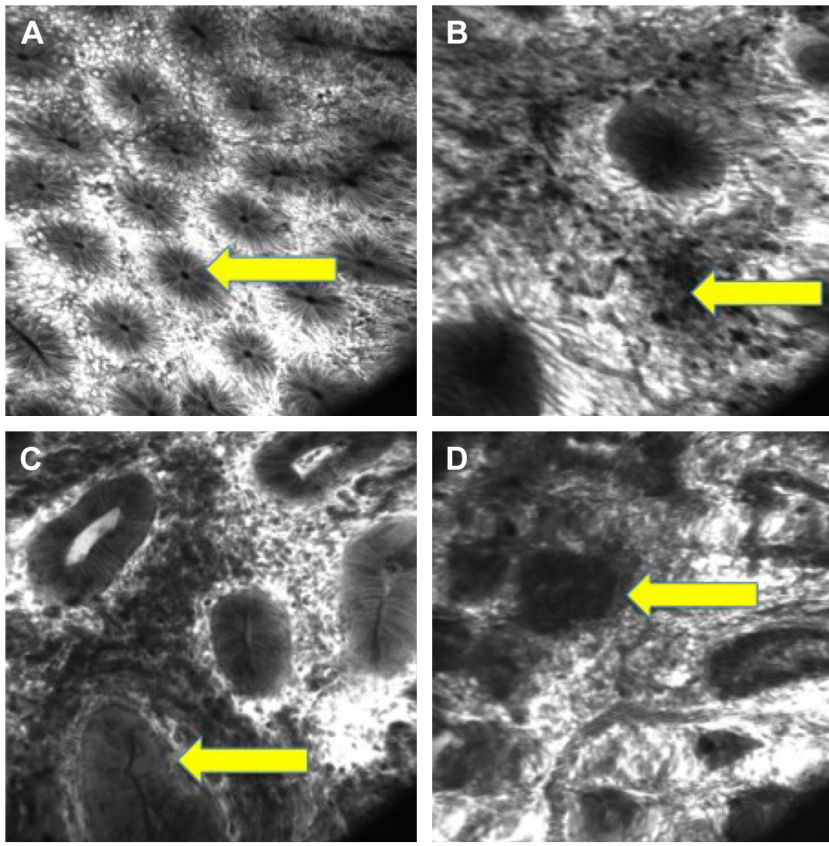

Fig. 3. Endomicroscopy in IBD. (*A*) Normal colonic mucosa with regular crypt (*arrow*) architecture can be seen. (*B*) Inflammatory cells can be identified within the lamina propria and are a sign of chronic inflammatory changes. (*C*) Inflammatory changes and dysplastic crypts (*arrow*) can be seen. The basement membrane is intact. Targeted biopsies confirmed the presence of low-grade intraepithelial neoplasia (colitis-associated dysplasia). (*D*) Colitis-associated cancer is present. Distorted glands with infiltration of malignant cells into the lamina propria (*arrow*) can be identified.

At present, 2 CLE-based systems are used in clinical routine and research (**Table 1**)[6,7]:

1. In CLE, a miniaturized confocal scanner is integrated into the distal tip of a flexible endoscope (Pentax Endomicroscopy System, Japan). A blue laser light source delivers an excitation wavelength of 488 nm, and light emission is detected at greater than 505 nm.[8] Successive points within the tissue are scanned in a raster pattern to construct serial en face optical section of 475 × 475 μm at a user-controlled variable imaging depth. Lateral resolution is 0.7 μm, and optical slice thickness is 7 μm (axial resolution). Images on the screen approximate a 1000-fold magnification of the tissue in vivo.[8]
2. The probe-based system (pCLE; Cellvizio Endomicroscopy System, Mauna Kea Technologies, Paris, France) consists of a 1.5-mm flexible miniprobe with lateral resolution between 3.5 μm and 1 μm, depending on the miniprobe, and axial resolution 5.0 μm. It is compatible with the working channel of any standard

Table 1
Technical aspects of endomicroscopic systems

	Endoscope Based	Probe Based
Outer diameter (mm)	12.8	1.0; 2.7; 2.6[a]
Length (cm)	120; 180	400; 300[a]
Field of view (μm)	475 × 475	320; 240; 600 μm^{2}[a]
Resolution	0.7	3.5; 1.0[a]
Magnification	×1000	×1000
Imaging plane depth (μm)	0–250 (dynamic)	40–70; 55–65; 70–130 (fixed)[a]

[a] Dependent on various probes.

endoscope.[7,8] These probes can be fitted through the working channel of most endoscopes for clinical use. Image acquisition is faster with this probe (12 frames/s) at the expense of resolution being limited by the number of fibers (30,000 single fibers = pixels).

Compared with probe-based CLE, endoscopic CLE has slightly higher lateral resolution (approximately 0.7 vs 1.0 μm), a larger field of view (approximately 475 vs 240 μm), and variable imaging plane depth (approximately 0–250 vs 0–65 μm). However, the miniprobe is currently the only commercially available system and it can be used in conjunction with any standard endoscope. It is simply passed over the working channel and endomicroscopic images at video-frame rates are obtained, which allows a dynamic examination of the vessels and microarchitecture (12 vs 0.8–1.6 frames per second)/14).

Endomicroscopy requires contrast agents. The most commonly used dyes are fluorescein (intravenous application), acriflavine (local application), and cresyl violet (local application).[8–11]

The potential of endomicroscopy is not only in vivo histology. Endomicroscopy is also able to display and observe physiologic and pathophysiologic changes during ongoing endoscopy. Molecular imaging also becomes possible.[12] In inflammatory bowel diseases, CLE was able to spot intramucosal bacteria within the lamina propria.[13] These intramucosal bacteria are more common in patients with IBD compared with normal controls. These new visible details might refine understanding of IBD, because increased cell shedding is linked to increased amounts of intramucosal bacteria as well as a higher risk to develop a flare within 12 months.[14] Most recently endomicroscopy was used for molecular imaging; labeled antibodies (adalimumab) were applied topically onto the affected (inflamed) mucosa in patients with Crohn's disease. The number of membranous TNF-alpha receptors within the mucosa could be quantified and the response to biologic therapy could be predicted with high accuracy based on the fluorescence pattern of the receptors.[15]

CLINICAL TRIALS

An increasing body of literature has provided evidence that supports the concept of taking smart biopsies instead of untargeted, random specimens. Image-enhanced endoscopy using a dye-based technique (chromoendoscopy) and endomicroscopy are performed in combination. Chromoendoscopy provides the means for detection[16] with endomicroscopy for characterization.[17] The combination allows more neoplastic lesions to be detected and they can be differentiated from nonneoplastic lesions based on surface pattern architecture. Note that endomicroscopy of the whole

gastrointestinal tract is not feasible because CLE has only a limited field of view (a maximum of 475–475 μm). The enhanced ability of chromoendoscopy and endomicroscopy to discriminate between nonneoplastic lesions, sporadic adenoma (adenomalike mass), and colitis-associated neoplasia (dysplasia-associated lesion masses) can potentially help to reduce the risk of colorectal cancer, lengthen surveillance intervals, and reduce the number of unnecessary biopsies (see **Fig. 3**).[2,3,15]

Panchromoendoscopy with either methylene blue or indigo carmine became a valid diagnostic tool for improving the diagnostic yield of intraepithelial neoplasia using the SURFACE guidelines in patients with IBD.[17] In the first randomized trial of endomicroscopy in ulcerative colitis, 153 patients with long-term ulcerative colitis who were in clinical remission were randomly assigned at a ratio of 1:1 to undergo either conventional colonoscopy or panchromoendoscopy using 0.1% methylene blue in conjunction with endomicroscopy to detect intraepithelial neoplasia or colorectal cancer.[4] Chromoendoscopy was used in this study to identify lesions for CLE and compared with standard white light endoscopy with random biopsies.

In vivo endomicroscopic prediction of the nature of lesions (neoplastic vs nonneoplastic) was accurate in 97.8% of lesions. In the conventional colonoscopy group, 42.2 biopsies were necessary. In the chromoendoscopy/CLE group, 3.9 biopsies per patient were sufficient, if only circumscribed lesions (by chromoendoscopy) with suspicious microarchitecture (by CLE) were biopsied.[4] The negative predictive value (NPV) for mucosa with a normal appearance on CLE to not harbor intraepithelial neoplasia was 99.1%, which reinforces the concept of taking smart biopsies instead of untargeted, random specimens.[4]

Sanduleanu and colleagues[18] showed that Acriflavine-guided endomicroscopy enables clinicians to differentiate between low-grade and high-grade intraepithelial neoplasia. Adenoma dysplasia score reliably discriminated high-grade dysplasia from low-grade dysplasia (accuracy, 96.7%). Interobserver agreement was high (K coefficients: pathologist, 0.92; endomicroscopist, 0.88). In vivo histology predicted ex vivo data with a sensitivity of 97.3%, specificity of 92.8%, and accuracy of 95.7%.

A meta-analysis of 91 studies, of which 11 on CLE by Wanders and colleagues[19] compared the pooled sensitivity, specificity, and real-time NPV of virtual chromoendoscopy (NBI, i-scan, FICE), CLE, and autofluorescence imaging for differentiation between neoplastic and nonneoplastic colonic lesions. This meta-analysis showed that virtual chromoendoscopy and CLE had an overall similar sensitivity and specificity, in that CLE produced the best results (sensitivity of 93% and specificity of 89%) and only CLE had a real-time NPV of more than 90%. A further meta-analysis of 15 studies of CLE, of which 4 on IBD by Su and colleagues[20] showed the effectiveness of CLE in discriminating between neoplastic and nonneoplastic lesions, showed similar results in pooled sensitivity and specificity, whereby specificity was even higher (sensitivity of 94% and specificity of 95%).

THE USE OF CRESYL VIOLET AND CONFOCAL ENDOMICROSCOPY

For tissue illumination with endomicroscopic low-power laser (488-nm blue laser light) application of fluorescence agents are necessary. Most studies in humans have been performed with intravenous fluorescein sodium (5 mL, 10%). Fluorescein quickly distributes within all compartments of the tissue, and CLE is possible within seconds after injection. It contrasts cellular and subcellular details, connective tissue, and vessel architecture at high resolution, but does not stain nuclei.[12]

Intravenous fluorescein is a nontoxic agent that is safe and mostly well tolerated, and only transient discoloration of the skin has been described.[12] CLE with intravenous

fluorescein sodium allows analysis of cellular structure, connective tissue, and blood cells of the colonic mucosa in vivo. However, the nuclei of the intestinal epithelium are not readily visible because of the pharmacokinetic properties of fluorescein. Acriflavine and cresyl violet are alternative dyes that are applied topically and highlight nuclei, cell membranes, cytoplasm, and to a lesser extent vessels. Acriflavine accumulates in nuclei and therefore carries a potential mutagenic risk. Cresyl violet, which enriches in the cytoplasm and visualizes nuclear morphology negatively, is an alternative.

A 2-step study approach made in 2007 by Goetz and colleagues[21] evaluated the staining characteristics and optimal concentration of a single topical contrast agent, cresyl violet (Merck, Darmstadt, Germany) for simultaneous chromoendoscopy and CLE for straightforward and reliable recognition of lesions and their immediate characterization in vivo. After establishing the optimal cresyl violet dye concentration of 0.13% with a pH of 3.8 in an animal preclinical study, 67 sites in 36 patients in a prospective clinical study were topically stained and subsurface serial images were generated at different depths using CLE. The results showed a good resolution with chromoendoscopy for pit pattern classification and good fluorescent contrast for endomicroscopy. Imaging at variable penetration depths permitted high-resolution visualization of tissue architecture and subcellular details, such as mucin in goblet cells, and, more importantly, cell nuclei so that in vivo distinction of low-grade versus high-grade intraepithelial neoplasia was possible for the first time. Endomicroscopic targeting of biopsies to a region of altered nucleus/cytoplasm ratio on intravital staining with cresyl violet has resulted in the diagnosis of 1 additional case of high-grade intraepithelial neoplasia, and the overall prediction rate of neoplastic changes by CLE was excellent, although the small number of sites investigated may limit the significance of this finding.[21]

SUMMARY

Endomicroscopy is a new imaging tool for gastrointestinal endoscopy. In vivo histology becomes possible at subcellular resolution during ongoing colonoscopy.

Panchromoendoscopy with targeted biopsies has become the method of choice for surveillance of patients with IBD with IBD. Endomicroscopy can be added after chromoendoscopy to clarify whether standard biopsies are still needed. This smart biopsy concept can increase the diagnostic yield of intraepithelial neoplasia and substantially reduce the need for biopsies.

Endomicroscopy is still mainly used for research but clinical acceptance is increasing because of a multitude of positive studies about the diagnostic value of endomicroscopy. Different contrast agents are available to identify cellular and subcellular structures. Fluorescent agents can also be combined with proteins or antibodies to enable molecular imaging. Smart biopsies, functional imaging (eg, defining local barrier dysfunction), and molecular imaging (predicting the response to biologic therapy) may represent the future for endomicroscopy.

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