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# Perioperative characterization of anastomotic doughnuts with high-resolution probe-based confocal laser endomicroscopy in colorectal cancer surgery: a feasibility study

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**Abstract**
*Background* Confocal laser enables in vivo real-time histopathology of the mucosa layer of gastrointestinal tract. The aim of this study was to assess the feasibility and the role of probe-based confocal laser endomicroscopy in extemporary examination of staple rings of patients with colorectal cancer.
*Methods* This was a prospective, single-center pilot study. Patients who underwent end-to-end stapled surgical resection for colorectal cancer were included. Confocal imaging was analyzed with great attention to image quality evaluation of cellular morphology and cellular structures of the serosal, muscular, and mucosal layers of the doughnuts than comparing results with definitive histopathology.
*Results* Twenty-six doughnuts were analyzed. Real-time video sequences were obtained in all patients, with a total of 204 mosaic images. For each doughnut, most of the images were adequate for evaluation of cellular morphology and cellular structure of the serosal, muscular, and mucosal layers.
*Conclusions* Perioperative assessment of doughnut tissues in patients with colorectal cancer by confocal laser endomicroscopy is feasible and safe. Prospective studies are warranted for further evaluation of the clinical impact of this technology during surgery.

**Keywords** Colorectal cancer · Confocal laser endomicroscopy · Doughnuts · Stapled anastomosis · Surgery

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Gastrointestinal (GI) doughnuts are rings of bowel wall removed from the staple gun after mechanical GI anastomosis. The staple gun cuts two circular doughnuts of tissue from inside the anastomosis, one proximal and one distal [1, 2]. Inspection of doughnuts serves two purposes. First, when the staple ring is not complete, a higher risk of anastomotic leak might be expected, while a complete circumferential full-thickness ring is indicative of a satisfactory anastomosis. Second, doughnuts histology might be considered a useful tool to assess or confirm disease-free margins.

Although the Royal College of Surgeons of England and the Association of Coloproctology of Great Britain and Ireland recommended that doughnuts should be always sent for histology, some studies suggest that when the tumor is more than 3 cm away from the cut edge, routine histological examination doughnuts is not clinically justified, and the decision to examine doughnuts should be made case by case. Moreover, preparation and histological examination of two doughnuts is a costly procedure, and it requires up to 40 min of a pathologist's time [3–7].

Recent technological advances in miniaturization allowed the integration of confocal scanning microscope into a conventional flexible endoscope or into transendoscopic probes, which is now known as confocal laser endomicroscopy (CLE). This newly developed technology has enabled

endoscopists to collect real-time in vivo histological images, or virtual biopsies, of the GI mucosa during endoscopy. It has stimulated significant interest in the application of this technique in clinical endoscopy [8, 9].

Recently, several studies have addressed the clinical applications of CLE, particularly in the field of precancerous lesions of the upper and lower GI tract, thanks to its high agreement with histopathology [10–14].

The aim of this study was to assess the feasibility and the role of probe-based confocal laser endomicroscopy (p-CLE) in extemporary examination of staple rings of patients with colorectal cancer to establish whether this technique might help select specimens eventually requiring conventional histology or even to understand whether this technique might become a new tool to change the surgical strategy when pathological alterations are detected.

## Materials and methods

### Patients

This was a prospective single-center feasibility and pilot study. Patients who underwent end-to-end stapled surgical resection for colorectal cancer from November 2012 to May 2013 were included. Exclusion criteria were cancers pretreated with neoadjuvant therapy, obstructing cancers, and cancers arising from inflammatory bowel disease. Patients with known allergic disease and impaired renal function were also excluded from this study. Eligible patients were invited to participate in the study, and a specific informed consent was obtained. The study was approved by the internal clinical research ethics committee (protocol 134/11).

### Equipment

CLE was performed using the Cellvizio Endomicroscopy System (Mauna Kea Technologies, Paris, France) using a coloflex UHD-type probe (1 μm lateral resolution; 12 frames/second). This probe has a field of view of 240 μm × 200 μm, with a lateral resolution of 1 μm. p-CLE imaging data were collected at a scan rate of 12 frames/s with a scanning field of 30,000 pixels. Single video frames were reconstructed into a single larger static image (4 mm × 2 mm) by a special computer software ("mosaicing"; Mauna Kea Technologies).

### Contrast agent

Five milliliters of 10 % sodium fluorescein were used as an intravenous contrast enhancement agent before CLE image acquisition.

**Table 1** Demographic, clinical, and pathologic characteristics of 13 study patients

| Characteristic | Value |
|---|---|
| Sex, M/F | 10/3 |
| Age, years, average (range) | 66.3 (23–79) |
| Tumor location | |
| Ra | 5 |
| Rb | 2 |
| Sigmoid | 5 |
| Colon (FAP) | 1 |
| Intervention | |
| Anterior resection | 12 |
| Total colectomy + IRA | 1 |
| Histopathology | |
| pT1 | 2 |
| pT2 | 6 |
| pT3 | 5 |
| G1/G2/G3 | 3/8/2 |
| N+/N− | 5/8 |
| Doughnut involvement | |
| No | 12 |
| Yes (mucosal) | 1 |

*Ra* rectum above the peritoneal reflection, *Rb* rectum below the peritoneal reflection, *FAP* familial adenomatous polyposis, *IRA* ileorectal anastomosis

### Procedure

After adequate colon mobilization, 5 ml of 10 % sodium fluorescein were injected intravenously about 5 min before colon edge transsection. Soon after the end-to-end stapled anastomosis was performed, the O rings were removed from the stapler. Confocal imaging of the serosal, muscular, and mucosal layers of the doughnuts were then obtained. CLE image acquisition was performed by placing the tip of the probe in direct contact with the target tissue.

### Main outcome measurements

Confocal imaging was analyzed with great attention to image quality evaluation of cellular morphology and cellular structures of the serosal, muscular, and mucosal layers of the doughnuts, then comparing results with definitive histopathology.

CLE images were stored digitally in specific folders into a database and compared in a blinded fashion with the histopathological sections analyzed by an expert GI pathologist.

## Results

Thirteen patients met the inclusion criteria, and 26 doughnuts were analyzed.

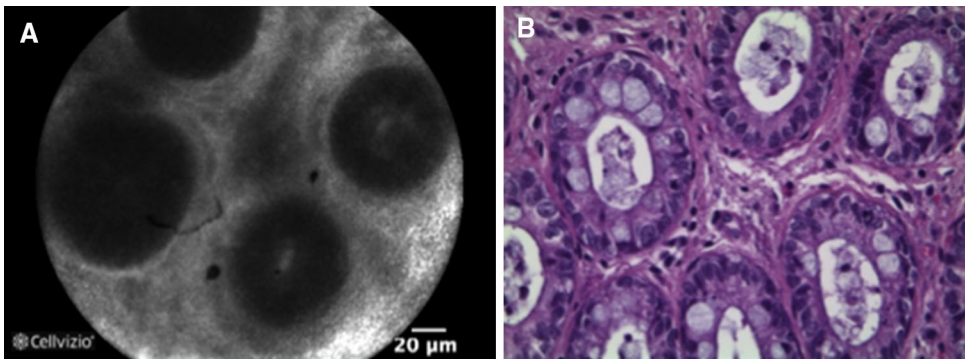


**Fig. 1** **A** p-CLE fluorescein sodium 10 % image of normal colon mucosa. **B** Corresponding normal tissue (hematoxylin and eosin staining)

Demographic, clinical, and pathologic characteristics of the study patients are shown in Table 1. Surgical procedures performed in the 13 study patients included anterior resection in 12 patients, and total colectomy followed by ileorectal anastomosis in 1 patient.

Real-time video sequences were obtained in all patients, for a total of 204 mosaic images. For each doughnut, most of the images were good and were adequate for evaluation of cellular morphology and cellular structure of the serosal, muscular, and mucosal layers.

There were no adverse reaction to sodium fluorescein, apart from a slight yellowish discoloration of the skin, which usually disappeared within 60 to 180 min.

### Confocal image data

Normal mucosa was characterized by a hexagonal honeycomb appearance with a regularly ordered network of capillaries demarcating the luminal crypt orifice. Surface crypt architecture was classically represented by ordered and regular crypt orifices covered by a homogeneous epithelial layer with visible black-hole goblet cells within the subcellular matrix (Fig. 1). Muscles were characterized by longitudinal structure of single myofibrils. These single muscle cells, which have a cylindrical shape, contain contractile fibers and peripherally located nuclei. Bright-stained capillaries were also visible (Fig. 2). Imaging of the serosal layer enabled clear visualization of adipocytes, other than capillaries and venules (Fig. 3). Neoplastic mucosa, which was evident in one patient, was represented by dark cells, with mucin depletion and goblet cell/crypt density attenuation; the architectural pattern was irregular, as was the epithelial thickness, with villiform structures and a dark epithelial border. Blood vessels were dilated and branched irregularly (Fig. 4). This pattern was confirmed by definitive histology, which diagnosed a high-grade dysplasia in the examined doughnut. No other pathological alterations were found in the remaining specimens in definitive sections. We found no pathologic images of muscular and serosal layers.

## Discussion

Confocal laser endomicroscopy is a newly introduced procedure that allows the capturing of images of virtual histology of the GI mucosa during endoscopy, thus offering the opportunity to obtain a real-time visualization of pathology aspects of mucosal epithelium with its cellular and subcellular structures. CLE evaluation and its relative high-quality images have shown high agreement with the real histology of the tissue, thus opening a wide spectrum of potential applications, which are mainly focused on the possibility of reducing and/or targeting the biopsies during endoscopy [15–18].

The current potential indications for CLE imaging are broad and include almost all the cases in which endoscopic biopsy is needed. More recent studies have demonstrated that transgastric or laparoscopic CLE for in vivo histology in the peritoneal cavity, including visualization of solid organs, was technically feasible. Visualized organs were readily identified and characterized by typical histologic aspects known from standard histopathology [19–22].

The next frontier for CLE might be represented by the development of confocal probes that fit within the shaft of a needle, providing real-time assessment of histology of extraluminal organs such as the pancreas, liver, and lymph nodes. A preliminary report suggests that cellular elements within pancreatic masses can be identified by CLE when the probe is inserted into the mass through an endoscopic ultrasonography–guided fine needle aspiration needle [23, 24].

In this study, we present, for the first time, the use of confocal imaging to examine cellular morphology and cellular structures of the serosal, muscular, and mucosal layers of resected colon wall. This pilot study has demonstrated that in patients undergoing colon resection for cancer, CLE imaging of doughnut tissues is feasible, has a good quality, and may at least select bowel rings for which traditional histology is recommended.

Cellular morphology and cellular structures of the serosal, muscular, and mucosal layers of the doughnuts

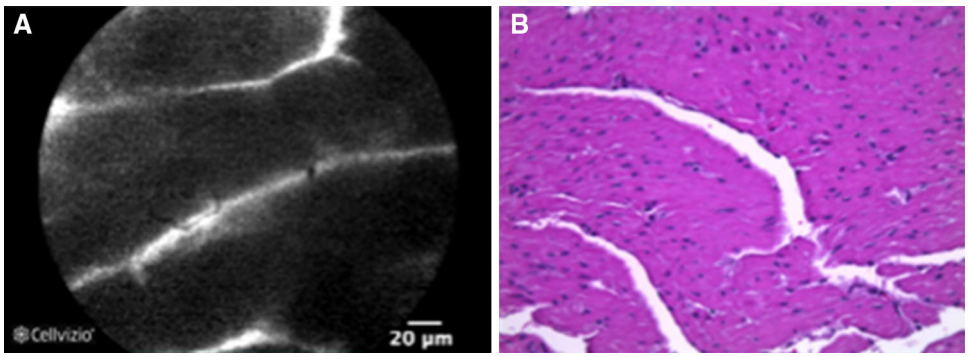


**Fig. 2** **A** p-CLE image of muscular layer characterized by longitudinal structure of single myofibrils. **B** Corresponding histopathologic image

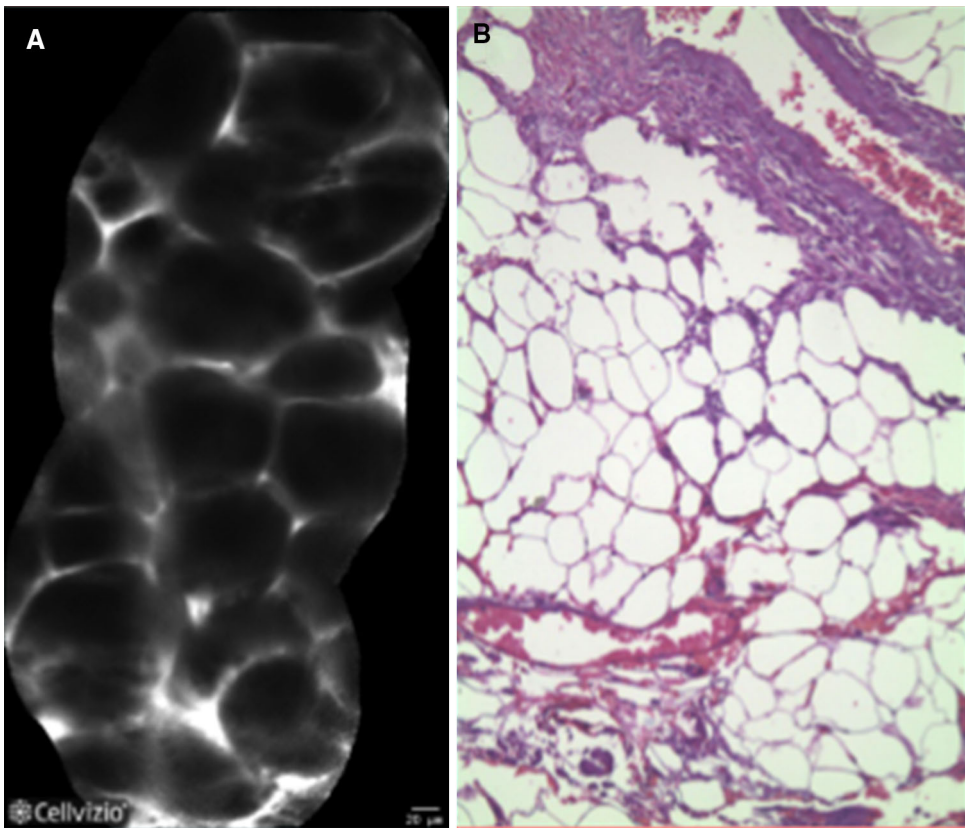


**Fig. 3** **A** p-CLE image of serosal layer enabled clear visualization of adipocytes and capillaries and venules. **B** Corresponding histopathologic image

were clearly visualized with a resolution quality comparable to conventional histology. Direct contact of the probe tip with the surface of the resected tissues generally provided adequate stability of the optics relative to the tissue for reliable image acquisition. The absence of breathing movements and/or peristaltic movements represented a key factor for the acquisition of high-quality images in a large proportion of specimens.

As previously emphasized, a clear consensus does not exist regarding the opportunity of routine histologic examination of staple rings after resection; nevertheless, some reports suggest that this would be recommendable. p-CLE might become a useful and safe technique to select those cases when doubts exist regarding microscopic invasion of resected margins and their need to be sent for definitive histology. In this sense, the cost of the equipment might be at least partially rewarded by the cost savings coming from avoiding several histological examinations in all the cases negative to p-CLE.

Moreover, in the era of neoadjuvant therapy and sphincter-saving surgery, several reports exist on the adequacy of 2 cm or even 1 cm free distal margins in rectal cancer [25, 26]. p-CLE could be particularly helpful in this setting: the presence of abnormalities or signs of not macroscopically evident invasion of the distal ring, after an ultralow anterior resection, might lead the surgeon to change the surgical strategy and convert into and abdominal perineal resection. In addition, in cases of even lower

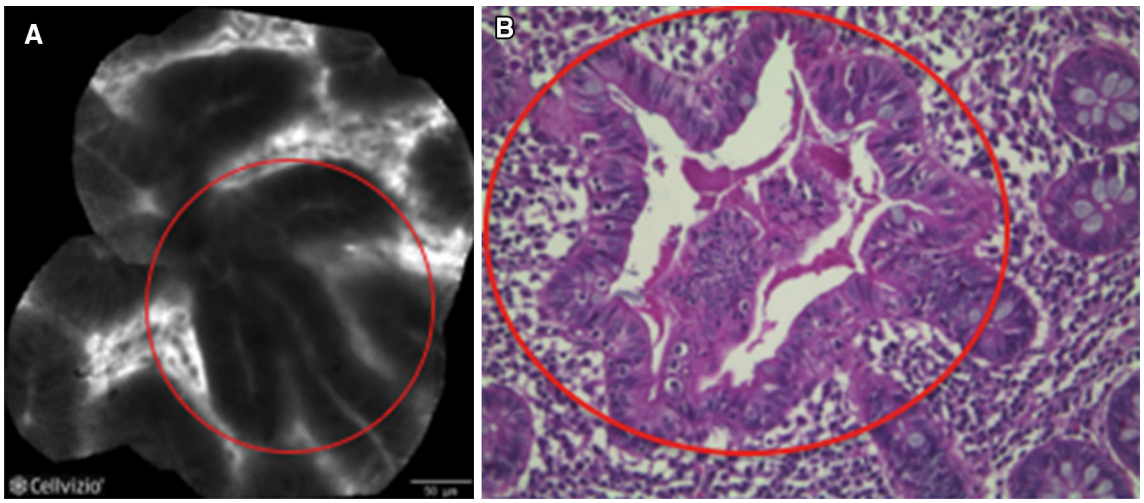


**Fig. 4** **A** p-CLE image showing adenomatous tissue (*red circle*) at level of mucosa of doughnut. **B** Histopathology of lesion (*red circle*) (hematoxylin and eosin staining) (Color figure online)

cancer, in which an intersphincteric resection has been planned, the transanal examination (with the aid of an anal dilator) of the rectal mucosa above the dentate line with p-CLE, performed before resection, may reveal microscopic invasion and then guide the surgeon to an abdominal perineal resection. This possibility seems to be of great clinical importance, particularly in the era of neoadjuvant radiotherapy, because some reports have demonstrated the possibility of tumor-cell scatter or distal spread up to 3 cm from the visible tumor edge [27]. From this point of view, one of the limitations of the present study is the lack of analysis of patients who received radiotherapy, who were excluded; the reason of this choice lay in the pilot and feasibility nature of the present report, the aim of which was to assess the possibility of doughnut analysis, together with the imaging quality evaluation, compared with conventional histology. This said, we decided to exclude all those situations (e.g., inflammatory bowel disease, radiotherapy) that might have introduced a bias into this preliminary image quality assessment. Future research should certainly be conducted that focuses on the role of p-CLE imaging on irradiated tissue.

Another limitation of the current study is that data concerning the muscular and serosal layers were assessed only on healthy tissues because we had no cases of neoplastic invasion of doughnuts in the current series. However, the only case with a residual neoplastic tissue involving the mucosa was easily shown to CLE, and this was confirmed by histology.

In conclusion, this report provides evidence that perioperative assessment of doughnut tissues in patients with colorectal cancer by CLE is feasible and safe; in fact, the only risk to the patient is related to a possible adverse reaction to the fluorescein infusion, which is not higher than for any other intravenous contrast agent.

Although more prospective studies are warranted to further elucidate the clinical value of this technology during surgery, the present results are encouraging. This method carries a high potential for intraoperative real-time assessment of resection margins, including, for rectal cancers, the circumferential (lateral or radial) resection plane, thus ensuring R0 resection while preserving the rectal sphincter.

**Disclosures** Drs. Giovanni D. De Palma, Gaetano Luglio, Stefania Staibano, Luigi Bucci, Dario Esposito, Francesco Maione, Massimo Mascolo, Gennaro Ilardi, and Pietro Forestieri have no conflicts of interest or financial ties to disclose.

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