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Usefulness of Preoperative Endoscopic Fluorescent Clip Marking in Laparoscopic Gastrointestinal Surgery

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Abstract. *Background/Aim: Precise tumor localization during gastrointestinal surgery improves curability and function preservation. We investigated the efficacy of preoperative endoscopic fluorescent clip marking using a Zeoclip FS with built-in near-infrared fluorescent resins in delineating gastrointestinal cancer for surgery. Patients and Methods: We evaluated the intraoperative visibility of the Zeoclip FS using a VISERA ELITE 2 and the short-term outcomes of 37 cancer patients (colorectal, n=23; gastric, n=14) who underwent preoperative fluorescent clip marking. Results: The study included 23 male and 14 female subjects with a mean age of 73 years (range=39-87 years). Thirty-three patients (89.1%) exhibited clear fluorescent clip marking and easily determined transection lines. Fluorescence was not observed in 1 sigmoid colon cancer patient (2.7%), who required a colonic stent for preoperative obstruction. Three patients (8.1%) required additional procedures for fluorescence visualization. Conclusion: Endoscopic fluorescent clip marking can delineate tumors well for determining the extent of resection.*

Accurately locating cancer lesions that are not clearly visible from the serosal side, and determining the extent of resection during surgery are very important for curability and functional preservation.

Endoscopic tattooing with India ink is popular for preoperative marking of cancer lesions in colorectal cancer surgery (1-3). However, adverse effects of India ink have been reported, including peritonitis and ileus (4-7). Additionally, intraperitoneal ink spraying or misinjection into the mesentery discolors the dissection layer black, which makes it difficult to recognize it and may decrease the safety and curability of the surgery. Furthermore, India ink is not approved as a pharmaceutical product. Thus, the use of India ink is currently the responsibility of doctors.

In gastric cancer surgery, determining the gastric transection line on the oral side is especially important. Intraoperative upper gastrointestinal endoscopy or intraoperative X-rays are more popular than marking with pigments or ink in colorectal surgery (8-12). However, the complexity of the procedure, radiation exposure and the need for an endoscopist are major limitations.

Therefore, we tested a new technique, *i.e.*, preoperative endoscopic fluorescent clip marking (FCM), to identify cancer lesions accurately and determine the intestinal transection line during laparoscopic colorectal and gastric cancer surgery. Narihiro *et al.* (13, 14) have described FCM with the Zeoclip FS (Zeon Medical, Tokyo, Japan) for colorectal and gastric cancer. This clip produced by Prof. Masashi Yoshida has built-in near-infrared fluorescent resin (14, 15) and is intended for disposable use (Figure 1). The peak excitation and fluorescence wavelengths of this clip are 760 and 790 nm.

In the study by Narihiro *et al.*, the VisionSense (Medtronic, Co., Minneapolis, MN, USA) was used for fluorescence laparoscopy. However, the usefulness of the Zeoclip FS relative to that of other fluorescence laparoscopic systems has not been reported. In this study, the efficacy and safety of preoperative FCM with the Zeoclip FS (Zeon Medical) and the VISERA ELITE 2 system (Olympus, Tokyo, Japan) were investigated.

Patients and Methods

Participants in this study comprised 37 patients who underwent laparoscopic colorectal and gastric cancer surgery between August 2019 and April 2020. They underwent preoperative endoscopic FCM with the Zeoclip FS one day before surgery.

In colorectal cancer, clipping was performed on the anal side of the tumor, at 3-5 sites central to the abdominal site. In gastric cancer, clipping was performed on the oral side at 3-5 places on the planned resection line.

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Key Words: Fluorescence, preoperative marking, colorectal cancer, gastric cancer.

Table I. *Patient characteristics (n=37).*

Patient characteristics	
Age (years)	73 [39-87]
Gender	
Male	23 (62%)
Female	14 (38%)
Body mass index (kg/m^2)	21.2 [14.3-29.0]
Location of cancer	
Upper part of stomach	3 (8%)
Middle part of stomach	9 (24%)
Lower part of stomach	2 (5%)
Ascending colon	4 (11%)
Transverse colon	3 (8%)
Sigmoid colon	6 (16%)
Rectosigmoid colon	4 (11%)
Upper rectum	4 (11%)
Lower rectum	2 (5%)
Comorbidity	
Angina pectoris	2 (5%)
Valvular heart disease	1 (3%)
Hypertension	6 (16%)
Diabetes	3 (8%)
Respiratory disease	3 (8%)
Neurological disease	2 (5%)
Neurogenic bladder	1 (3%)
Arteriosclerosis obliterans	1 (3%)

Values are presented as the median [range] or n (%).

Table II. *Operative procedure.*

Operative procedure	n (%)
Ileocecal resection	1 (3)
Right hemicolectomy	6 (16)
Sigmoidectomy	4 (11)
High anterior resection	4 (11)
Low anterior resection	4 (11)
Super-low anterior resection	2 (5)
Hartmann operation	2 (5)
Distal gastrectomy	14 (38)
Additional cholecystectomy	2 (5)
Additional partial hepatectomy	1 (3)
Additional partial resection of small intestine	1 (3)
Covering ileostomy	3 (8)

Outcome measurements were patient characteristics and surgical outcomes, including the surgical margin, intraoperative visibility of the Zeoclip FS and the number of remaining clips after specimen removal. Laparoscopic surgery was performed as usual, and the visibility of the Zeoclip FS was examined using the VISERA ELITE 2 system. The intestinal transection line was determined with real-time fluorescence observation. The results are expressed as the mean values. The Zeoclip FS was approved for clinical use (Registration No. 13B1X001111000020). Furthermore, this study was approved by the Research Ethics Committee of the Kawaguchi Municipal Medical Center (Saitama, Japan) (approval number: 2019-33 and 2020-3).

Results

A total of 37 patients (colorectal cancer, n=23; gastric cancer, n=14) who underwent laparoscopic colorectal and gastric cancer surgery with preoperative endoscopic FCM with the Zeoclip FS were analyzed in this study. The cohort had a mean age of 73 years (range=39-87), and consisted of 23 men and 14 women (Table I).

The ratio of upper, middle, and lower gastric cancer tumors was 3:9:2. The ratio of colorectal cancer tumors in the ascending colon, transverse colon, sigmoid colon, rectosigmoid colon, upper rectum, and lower rectum was 4:3:6:4:4:2.

Table II shows the operative procedures. Surgery was performed for each case of cancer. In 4 cases additional surgeries were simultaneously performed (2 cholecystectomies, 1 partial hepatectomy, and 1 partial resection of the small intestine). In 3 rectal cancer cases, ileostomy was added (Table II).

In 33 (89.1%) cases, FCM was clearly visible, and the transection lines were determined (Figures 2 and 3). Among all cases, the total number of remaining clips was 130/134 (97.0%) (Figure 4). There were no cases in which two clips deviated (Table III). Fluorescence was not be observed in 1 case (2.7%), which involved sigmoid colon cancer with a colon stent for preoperative obstruction. In 3 cases (8.1%), fluorescence could not be observed at first, but the clips were visible with additional procedures. Table IV shows the TNM classification (16), pathological data and short-term outcomes (Table IV). The mean surgical duration was 286 min (range=162-586 min), the mean blood loss was 5 ml (range=1-300 ml), and the mean postoperative hospital stay was 10 days (range=7-30 days). The transection margins at the oral and anal sites in colorectal cancer were 100 (range=30-300 mm) and 60 mm (range=25-210 mm), respectively, and those in gastric cancer were 27.5 (10-105) and 90 (11-150) mm, respectively. Postoperative complications [Clavien-Dindo grade III (17) or over] occurred in 2 cases (5.4%), and included a ureter injury and abdominal abscess formation. There were no complications related to FCM.

Discussion

Fluorescence is a phenomenon in which a fluorescent dye molecule absorbs high-energy light and emits light with a longer wavelength than the light absorbed by this molecule (18). Indocyanine green (ICG) bound to plasma protein (β-lipoprotein) *in vivo* is excited by irradiation with near-infrared light at 760 to 780 nm and emits near-infrared fluorescence at 800 to 850 nm (18). The ICG fluorescence method is a technique of visualizing ICG fluorescence *in vivo* using a special camera that can visualize near-infrared

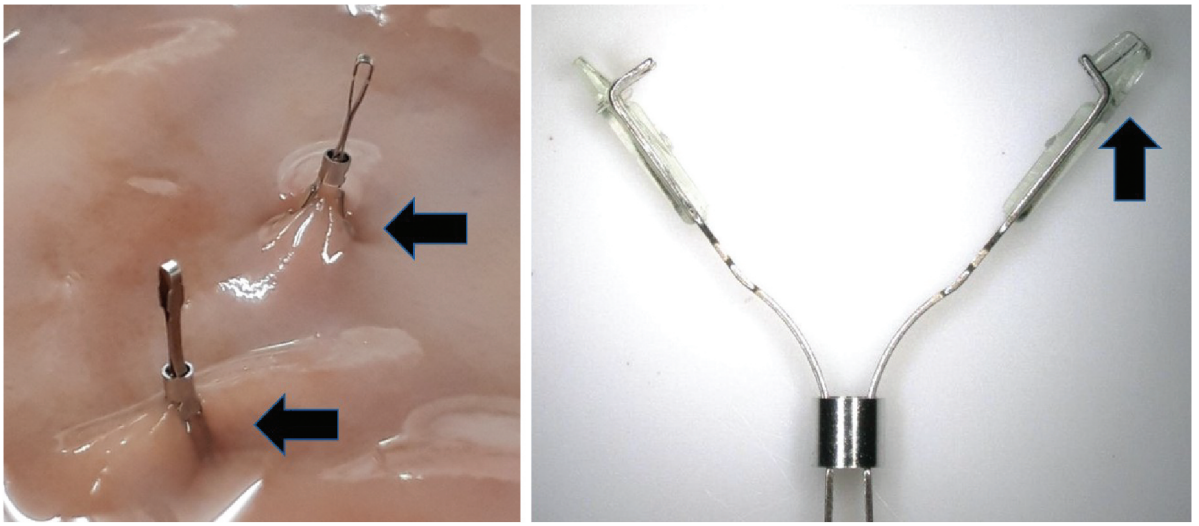

Figure 1. *The Zeoclip FS is an endoscopic clip containing resin (black arrow) that shows near-infrared fluorescence.*

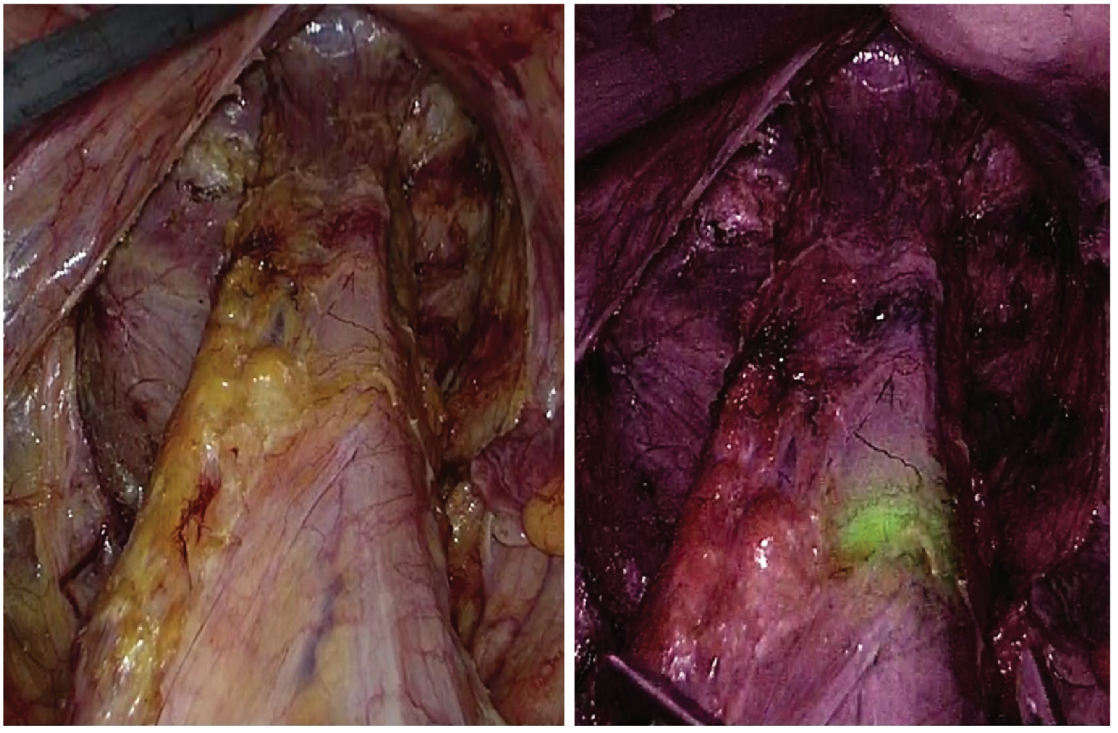

Figure 2. *This case involved early lower rectal cancer. FCM is depicted clearly. Left: normal observation. Right: fluorescent observation.*

rays in this range. Near-infrared fluorescent resin emits near-infrared fluorescence with near-infrared light similarly to ICG. A near-infrared fluorescence camera can visualize the fluorescence emitted from the resin.

India ink marking. India ink marking is a method that causes black discoloration of the serosal surface by local injection of ink into the mucous membrane of the intestine (1-3). This is a useful technique to visually identify colorectal lesions

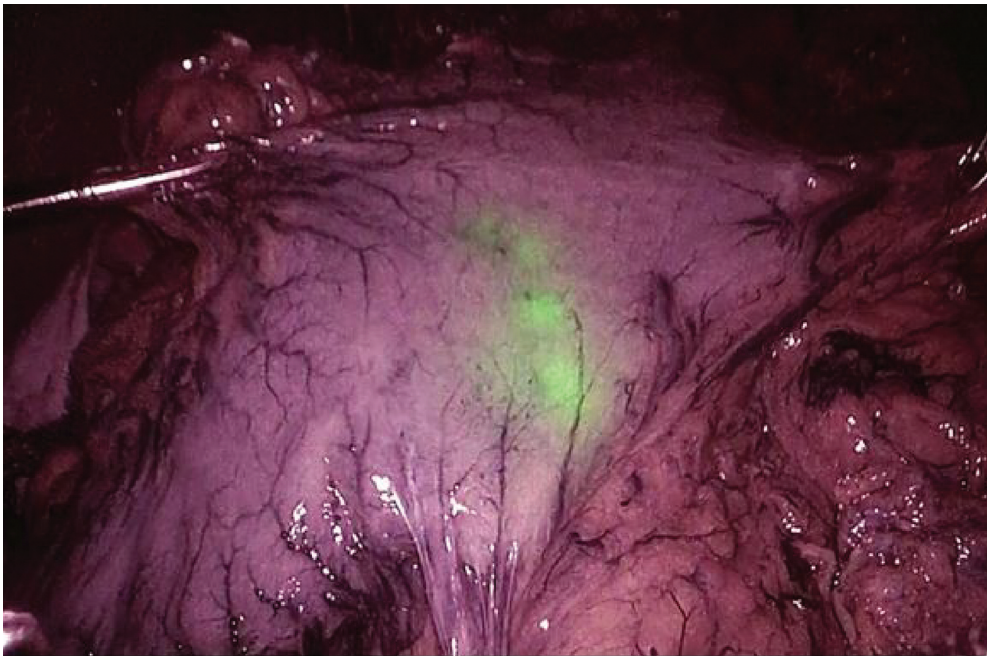

Figure 3. *This case involved gastric cancer. FCM is depicted clearly on the oral side.*

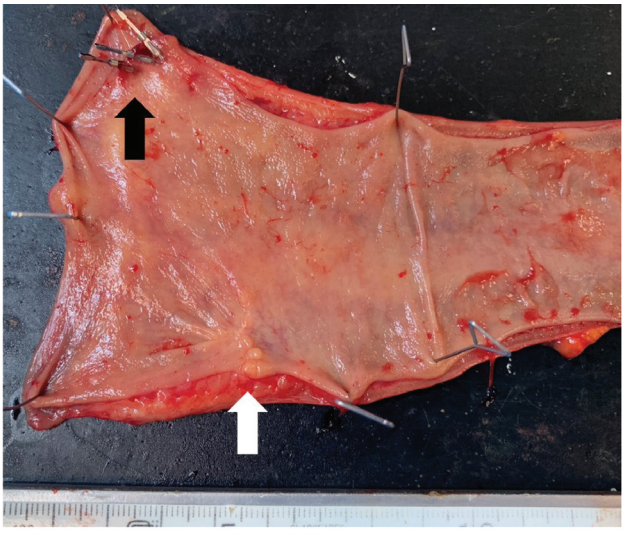

Figure 4. *Specimen of lower rectal cancer. The white arrow indicates the scar of the previous endoscopic mucosal resection of a tumor. The black arrow indicates three Zeoclip FS devices.*

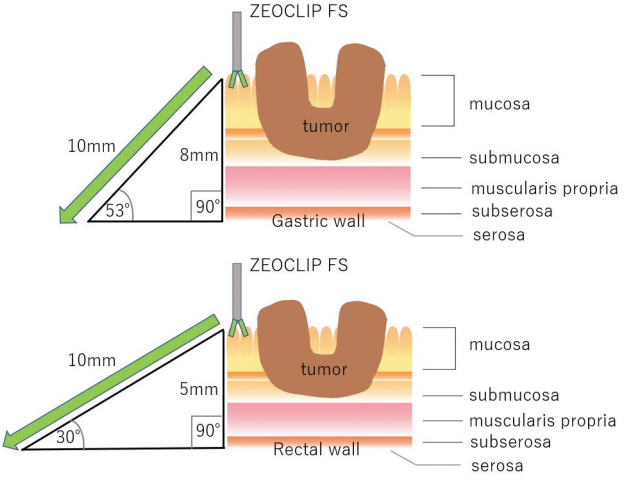

Figure 5. *Schematic diagram showing the relationship between the angle between the laparoscope and gastric or rectal wall and transmission distance of the near-infrared light. If the gastric wall is 8 mm thick, an angle of 53 degrees would result in a near-infrared penetration depth of 10 mm; if the rectal wall is 5 mm, an angle of 30 degrees would result in a near-infrared penetration depth of 10 mm.*

that are invisible from the serosal side. However, adverse effects of India ink have been reported. In some cases, the ink cannot be identified despite the marking, or the discoloration is too wide to capture the details of the marked position. When the ink is injected deep outside the intestine, the ink spreads into the abdominal cavity, or into the mesentery. Intraperitoneal ink spraying or misinjection into the mesentery discolors the dissection layer black, which

Table III. *Fluorescence clip marking*.

Results of fluorescence clip marking	n (%)
Clear FCM	33 (89)
Unclear FCM	3 (8)
Invisible FCM	1 (3)
Total number of deviated clips	4/134 (3)
Cases of one deviated clip	4 (11)

Table IV. *Postoperative data*.

Postoperative data	
Operative time (min)	286 [162-586]
Blood loss (ml)	5 [1-300]
Postoperative hospital stay (days)	10 [7-30]
Pathological type of cancer	
Tub1	19 (51%)
Tub2	12 (32%)
Por1	1 (3%)
Sig	4 (11%)
Scc	1 (3%)
Margin of gastric cancer	
Proximal margin (mm)	27.5 [10-105]
Distal margin (mm)	90 [11-150]
Margin of colorectal cancer	
Proximal margin (mm)	100 [30-300]
Distal margin (mm)	60 [25-210]
TNM classification	
T1	12 (32%)
T2	5 (14%)
T3	16 (43%)
T4	4 (11%)
N0	28 (76%)
N1	8 (22%)
N2	1 (3%)
M0	35 (95%)
M1	22 (5%)

Values are presented as the median [range] or n (%).

makes it difficult to recognize the surgical dissection layer and may decrease the safety and curability of the surgery. Moreover, peritonitis and ileus have been reported to occur when India ink is spread into the abdominal cavity (4-7). India ink marking may also spread within the intestinal tract and obscure the exact position, making it difficult to perform surgery that requires precise determination of the extent of resection, such as surgery for rectal cancer. If ink remains in the rectum, intestinal tissue may undergo inflammatory changes that interfere with anastomosis.

Endoscopic local injection of ICG. Marking by endoscopic local injection of ICG, as well as FCM, enables tumor identification by near-infrared light observation during surgery (19-21). However, the side effects of ICG need to be considered. In Japan, the incidence of ICG side effects has been reported to be 0.17% (22). More than 100 cases of side effects have been reported; shock symptoms occurred in 20 or fewer cases and there were 5 fatalities (22). Furthermore, ICG and India ink are not approved for local injection as a pharmaceutical product. Thus, the use of India ink and ICG is currently the responsibility of doctors.

Gastrointestinal endoscopy and X-rays for gastric cancer. For gastric cancer surgery, in most cases, determining the gastric transection line on the oral side is important for curability and functional preservation. Intraoperative upper gastrointestinal endoscopy and X-rays are popular techniques in addition to pigments and ink in colorectal surgery (8-12). Furthermore, with X-ray techniques, radiation exposure is a heavy burden (8, 9), and staff must wear protective clothing. Moreover, endoscopic techniques require an endoscopist (10-12).

Laparoscopic and gastroendoscopic observations are performed at the same time. The light from the laparoscope is very bright and increases the difficulty of gastroendoscopic observation. This is not an easy procedure, and the time required for endoscopic observation generally extends the operation time.

Because there is no consensus on the best techniques, new techniques, such as endoscopic autologous blood tattooing and intraoperative laparoscopic ultrasonography, are even being attempted (23, 24).

Fluorescent clip marking. Since FCM is performed by clipping the intestinal mucosa, the procedure is more useful, easier than local injection, and without chemical side effects. Marking with a fluorescent clip delineates the marked site and makes it visible with fluorescence. FCM has no complications due to leakage of the ink, and may contribute to determination of the resection margin because the marking site is accurately delineated by fluorescence.

The resection margin on the anal side in rectal cancer according to the Japanese Treatment Guidelines is over 3 cm for rectal sigmoid colon cancer and upper rectal cancer and over 2 cm for lower rectal cancer (25).

FCM does not require an X-ray device, and it is possible to omit the intraoperative endoscopy, thus shortening the operation time. Also, near-infrared penetration in living bodies is said to be approximately 5-10 mm (26), and it could seem difficult to identify this fluorescence when the wall of the digestive tract is thick. Fluorescence visibility is associated with the thickness of the colon or stomach wall and the object-camera angle. If the gastric wall is 8 mm thick, an angle of 53 degrees would result in a near-infrared penetration depth of 10 mm (Figure 5). If the rectal wall was 5 mm, an angle of 30 degrees would result in a near-infrared penetration depth of 10 mm (Figure 5). To improve visibility,

observing at an angle as close to a right angle as possible and stretching the intestine to make it thinner are important. Since the stomach is relatively thick, it is important that visibility is further improved by stretching the stomach and placing the scope at a right angle. Stretching of the stomach by the operator and assistant using forceps made the wall thinner and improved visibility in our cases.

To reduce the necessary near-infrared penetration depth in the intestine, the fluorescence resin is placed at the mucosal tip of the Zeoclip, which is designed to deeply bite into the mucosa. In addition, clips should not be placed on the greater or lesser curvature side where fat is not dissected, because FCMs are not visible at these sites. Therefore, endoscopic clips should be placed on the anterior or posterior wall. In rectal cancer, because the dorsal side is the mesorectum, the clips should be placed on the anterior wall. In colon cancer, in almost all cases, the ventral side is without mesocolon. However, attachment of the omentum may make viewing the fluorescence difficult. In cases of excess fat or transverse colon cancer, clips should be placed over the entire circumference of the intestinal tract. In this study, fluorescence was not observed in 1 case that involved sigmoid colon cancer with a colon stent for preoperative obstruction. The reason may be that the clip was hidden under the colon stent side by the thick fatty layer of the mesocolon. Therefore, in patients with colon stents, clips should not be placed near the stent. In 3 other cases, fluorescence was not observed at first, but additional procedures made the clips visible. In the first case of lower rectal cancer, digital rectal palpation made the clip clearly visible. In the second case of transverse colon cancer, observation via a small laparotomy after the laparoscopic procedure made the clip visible. In both cases, a change in the camera angle seemed to be effective. In the third case of gastric cancer, only one clip was observed, and endoscopy was performed. Extension of the stomach wall by insufflation thins the gastric wall and makes the clip visible. While endoscopy was required in this case, FCM facilitated the decision of the resection line more than intraoperative endoscopy would because the marking clips were clearly observed from the serosal side.

In 97% of cases, the fluorescent clips were visible, including a few cases requiring additional technology. Among all cases, the total number of remaining clips was 130/134 (97.0%). One clip deviated in 4 cases (11%). For FCM, at least two clips should be used. FCM accurately indicates the location of the lesion. It may contribute to the curability and functional preservation of cancer surgery and shorten the operation time. Endoscopic FCM can delineate tumors well, and the procedure is useful for determining the extent of resection. The limitation of this study is its retrospective design, small number of patients from a single institution and possible selection bias.

Conflicts of Interest

None of the Authors have any conflicts of interest or financial ties to disclose in relation to this study.

Authors' Contributions

Ryu S: Project development, Data collection, data analysis, manuscript writing. Okamoto A, Nakashima K, Hara K, Ishida K, Ito R, Nakabayashi Y: Data collection. Eto K, Ikegami T: Manuscript editing.

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Clinical Trials

Examination of fluorescence navigation for laparoscopic colorectal cancer surgery. The Research Ethics Committee of the Kawaguchi Municipal Medical Center approved the study (Saitama, Japan) (approval number: 2019-33 and 2020-3). https://kawaguchi-mmc.org/wp-content/uploads/clinicalresearch-r02.pdf

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