Optimization of indocyanine green angiography for colon perfusion during laparoscopic colorectal surgery

Hong-min Ahn1,2 | Gyung Mo Son1,2,3 | In Young Lee3 | Sang-Ho Park4 | Nam Su Kim4 | Kwang-Ryul Baek4

Aim: This study aims to evaluate the extrinsic effects of conditional factors affecting quantitative parameters and to establish the optimization of indocyanine green (ICG) angiography using in vitro experiments and a prospective observational study.

Method: In vitro experiments were performed to evaluate the correlation between conditional factors such as camera distance, surrounding lighting, fluorescence emission sources and ICG doses. The fluorescence intensity was measured from the ICG-containing test tube in each condition. In the clinical study, ICG angiography was applied to patients with colorectal cancer (n = 164). The quantitative perfusion parameters were the maximal fluorescence intensity ($F_{\text{MAX}}$), slope, $T_{1/2\text{MAX}}$ and perfusion time ratio (TR). Camera position, distance to colon, fluorescence emission source, surrounding lighting, site of angiography and ICG specific mode were considered as conditional factors and compared with the quantitative parameters to identify the optimal condition of ICG angiography.

Results: The fluorescence intensity had an inverse correlation with distance, and the transitional zone was shown at a distance of 4–5 cm by slope differential. $F_{\text{MAX}}$, $T_{1/2\text{MAX}}$ and slope were affected significantly by camera distance, site of angiography, fluorescence emission source and ICG mode as conditional factors. On multivariate analysis, $F_{\text{MAX}}$ was independently associated with spectral ICG mode with red inversion, laser mode and camera distance. Conversely, TR was not related to any conditional factors.

Conclusion: Since quantitative parameters of ICG angiography are influenced by various conditions, a standardized protocol is required. The application of ICG specific modes with a constant distance of 4–5 cm can provide optimized fluorescence images.

Keywords: colorectal surgery, fluorescein angiography, indocyanine green, laparoscopy, perfusion imaging
INTRODUCTION

Anastomotic leakage is a major complication that is reported as much as 10%–20% after colorectal surgery [1,2]. Insufficient mesenteric arterial flow can induce ischaemic changes and anastomotic complications [3]. Colonic perfusion can be determined by the bowel colour change, persistent peristalsis, or intentional bleeding of the colonic marginal artery, but these methods are too subjective to estimate colon perfusion [4].

Intra-operative angiography using a near-infrared (NIR) camera system was introduced as an objective perfusion assessment [5-7]. Since indocyanine green (ICG) yields fluorescence in the NIR spectrum, intravenous ICG injection can enable real-time colon perfusion status to be visualized [8]. Using ICG fluorescence angiography, surgeons have been able to detect the hypoperfusion segments and change the planned transection line, which can decrease the risk of anastomotic complications [9-11].

However, it is still challenging to establish a standardized protocol for ICG angiography and quantitative analysis to identify adequate perfusion status [6,12]. Quantitative parameters were calculated using fluorescence intensity and fluorescence enhancing time [13]. Fluorescence intensity can be affected by various conditional factors, such as surrounding light, fluorescence emission sources, or distance between the colon and camera [8,10]. The protocols for ICG angiography have also been varied between centres and surgeons in previous studies [14]. So, a standardized protocol is required to establish the quantitative analysis of ICG angiography as an objective method for evaluating perfusion status.

Thus, this study aims to evaluate the extrinsic effects of conditional factors affecting quantitative parameters of ICG angiography using in vitro experiments and a prospective observational study. Furthermore, we would like to establish an optimized protocol for ICG angiography in laparoscopic colorectal surgery.

METHOD

Patients

We prospectively enrolled 164 patients who underwent laparoscopic colorectal surgery between July 2015 and May 2019 at Pusan National University Yangsan Hospital. The inclusion criteria were patients aged 19–80 years who had sigmoid colon and rectal cancer and underwent laparoscopic surgery using an NIR camera system. Either anterior resection or low anterior resection was performed according to the cancer location.

The exclusion criteria were haemodynamic instability, emergency surgery or pregnancy. Patients who underwent surgery without colonic anastomosis were also excluded. All patients enrolled in the study had no history of allergies or adverse effects to either the contrast agent used for CT or the iodine-containing drugs. We used clinical data from the prospectively collected database and the recorded surgical videos. We defined the anastomotic complications as leakage, colonic necrosis, pelvic abscess and stricture requiring endoscopic or surgical treatment.

If postoperative anastomotic complications were suspected, pelvic CT and sigmoidoscopy were performed. This study was conducted after receiving the approval of the Institutional Review Board (IRB no. 05-2020-103) of the Pusan National University Yangsan Hospital. Written informed consent was obtained from all patients.

Indocyanine green angiography and quantitative analysis

All patients underwent intra-operative ICG angiography before colon transection. ICG (Diagnostin Inj, 25 mg, Daichi Sankyo) was diluted in 10 ml distilled water and injected by the anaesthesiologist attending the surgery. Using a dose of 0.25 mg/kg, the ICG was injected slowly for 10 s. Colon perfusion was monitored for 2 min after ICG injection, and for a further 3 min if the angiogram was not visible after 2 min. The change in fluorescence intensity was measured sequentially to produce time–fluorescence intensity graphs using video analysis and a modelling tool (MATLAB R2019a, MathWorks) [15]. From the graphs, we analysed quantitative parameters such as maximal fluorescence intensity ($F_{\text{MAX}}$), time to half of $F_{\text{MAX}}$ ($T_{1/2\text{MAX}}$), fluorescence rising slope (slope = $F_{\text{MAX}}/T_{\text{MAX}}$) and perfusion time ratio (TR = $T_{1/2\text{MAX}}/T_{\text{MAX}}$) (Figure 1A).

Conditional factors

The distance between the colon and camera was calculated using the formula $l = kW$, where $l$ is the length between the lens and the subject, $k$ is a coefficient and $W$ is distance on the ruler lying underneath the colon. When fluorescence angiography was performed outside the abdominal cavity, the distance was measured directly with a surgical ruler. In cases of extra-abdominal ICG angiography, the colon was extracted from the abdominal cavity through a transumbilical mini-laparotomy site. After dividing the colonic mesentery, ICG angiography was performed, leaving the operation room lights turned either on (light) or off (dark). Intra-abdominal ICG angiography was performed with the dark condition. We used two different camera systems using a xenon lamp (IMAGE1 S™, Karl Storz) or laser (1588 AIM camera system, Stryker). For ICG image modes of the laparoscopic NIR camera system, we used the conventional ICG mode (blue...
colour) and Spectra A mode with red inversion (light cyan colour) for the xenon lamp camera system and the endoscopic NIR visualization (ENV) mode (green colour) for the laser camera system (Figure 2).

**In vitro study**

We designed a series of in vitro experiments that explored the extrinsic effects of various conditional factors including distances, dilution concentrations of ICG, fluorescence emission sources, and lighting of surrounding conditions. First, we made a linear actuator device that could move the subject 0–15 cm away from the camera with constant velocity. The device consisted of a step motor, two limiter switches, a camera holder and a plate (Figure 3A). The plate moved at a speed of 0.33 cm/s, and the frame rate of the camera was 30 fps, so the subject was photographed using the NIR camera system while moving it by 0.011 cm per frame. We used two different fluorescence emission sources: a xenon lamp (300 W xenon light source, Karl Storz) and a laser (L10 LED light source, Stryker). With the laser, we used the ENV modes 3 and 5. The device was covered with a black box to demonstrate the ‘dark’ surrounding condition that could represent either intra-abdominal ICG angiography or extra-abdominal ICG angiography without room lights. Without the box, the ‘light’ surrounding condition was demonstrated, which represented extra-abdominal ICG angiography with the room lights on (Figure 3B). Diluted ICG solutions were prepared with dilution concentrations of 0.01 and 0.05 mg/ml in the test tubes (Figure 3C). From the recorded video, we analysed the fluorescence intensity from different distances with different conditional settings.

**Statistics**

The mean values of each parameter were compared using either an independent t test or Mann–Whitney U test according to the results of the Kolmogorov–Smirnov test. The correlation between the distance and each parameter was analysed using either Pearson correlation analysis or Spearman’s rank correlation analysis according to the distribution of data. Quantitative parameters on ICG image modes were compared using one-way ANOVA with the Bonferroni test for post hoc analysis. Multivariate analysis was performed with a binary logistic regression model using a forward condition analysis. The covariance input criterion was less than 0.1 and the elimination criterion was less than 0.05. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0, for Windows (SPSS, IBM). The results of continuous variables were expressed as mean ± standard deviation. All results with a P value less than 0.05 were considered significant.

**RESULTS**

**Clinical data analysis**

The clinical characteristics are shown in Table 1. Ten out of 164 (6.1%) patients had major anastomotic complications. The anastomotic complications were colonic necrosis (n = 1), anastomotic leak (n = 6), anastomotic stricture (n = 1), delayed pelvic abscess (n = 1) and delayed anastomotic dehiscence during intensive care for respiratory failure (n = 1). The transection line was changed in 11
patients (6.7%) based on ICG angiography, and anastomotic leakage occurred in one patient among them.

Conditional factors of ICG angiography are shown in Table 2. The correlation between distance and each quantitative parameter was estimated (Figure 4A–D). The distance from the colon to the laparoscopic camera was statistically related to $F_{\text{MAX}}$, $T_{1/2\text{MAX}}$ and slope but did not influence TR. In particular, $F_{\text{MAX}}$ tended to decrease when the distance increased, and it was statistically significant ($P < 0.001$). Intra-abdominal or extra-abdominal ICG angiography also showed different quantitative parameter levels (Figure 4E–H). $F_{\text{MAX}}$ and slope were significantly higher in intra-abdominal ICG angiography, but $T_{1/2\text{MAX}}$ and TR were statistically similar. However, the mean distance of intra-abdominal ICG angiography was 6.24 cm, while extra-abdominal ICG angiography was 8.56 cm, which was statistically significant ($P < 0.001$). Thus, in the intra-abdominal condition, the closer distance affected elevated $F_{\text{MAX}}$ and slope. The fluorescence emission source also affected $F_{\text{MAX}}$, $T_{1/2\text{MAX}}$ and slope, but not TR (Figure 4I–L).

A subgroup with extra-abdominal ICG angiography was analysed to compare each parameter with the room lights on and off. $F_{\text{MAX}}$ tended to display a higher value when the room lights were on (62.25 ± 25.41 AU) compared to when the room lights were off (58.67 ± 25.43 AU). However, all parameters, including $F_{\text{MAX}}$, were not statistically significant according to room lights ($P = 0.618$).

In the xenon camera system, the $F_{\text{MAX}}$ of conventional ICG mode (blue colour) and Spectra A mode with red inversion (light cyan colour) showed significant differences (48.29 ± 16.29 AU vs. 75.62 ± 29.14 AU, respectively, $P < 0.001$). However, there was no statistical difference between Spectra A mode and laser (ENV) mode (82.06 ± 29.39 AU, $P = 0.521$).

On multivariate analysis, $F_{\text{MAX}}$ (>70 AU) was independently associated with Spectra A mode, laser (ENV) mode and close distance (<8 cm) (Table 3). Conversely, TR (<0.6) was not affected by any conditional factors.
In vitro experiments

The fluorescence intensity graphs had an inverse correlation with distance (Figure 5A). When the fluorescence emission source was farther away, the estimated fluorescence intensity was lower. The decreasing slopes were steep (high slope region) in a distance within 4–5 cm, after which the slopes were almost flat (low-intensity region). This transitional section was obtained around 4–5 cm (Figure 5B). In various conditions, the graphs appeared as similar patterns of the transitional section where the optimal distance of fluorescence intensity was. The fluorescence intensity graphs were compared by altering the surrounding lighting and fluorescence emission sources (Figure 5C). The fluorescence slope of the laser camera was steeper and affected more easily by distance than the xenon camera. When the surrounding lights were turned on, the fluorescence intensity tended to be higher than in dark conditions.

FIGURE 3  In vitro study for conditional factors influencing the parameters of quantitative analysis. (A) A device named ‘Linear actuator’ in which a subject moves away with constant velocity (0.33 cm/s) was fabricated so that the fluorescence intensity could be recorded constantly from 0 to 15 cm. By changing camera systems, different fluorescence emission sources were implemented. We covered this device with the black-coloured box to demonstrate the dark surrounding conditions. (B) Combining the device with the NIR system, in vitro experiments were demonstrated. Without covering the black box, the light surrounding condition is made. By covering the device with the black box, dark surroundings are made. (C) We used a test tube to reduce the reflected light, which may interfere with the detection of fluorescence intensity.
DISCUSSION

ICG angiography protocols have been introduced for the objective assessment of colon perfusion [3,7,17,18,19,20,21] and are summarized in Table 4. All studies have described the intravenous dose of ICG and the NIR system. However, conditional factors such as distance and surrounding lighting varied from one to another. Recent meta-analysis studies on anastomotic leakage after ICG angiography have reviewed the papers but they considered different camera systems and ICG dose as their limitation, which would influence the diverse results [22]. They also commented that many previous studies were not standardized with the protocol for ICG angiography, including the dosage of ICG injected during surgery [23]. In this prospective observational cohort, the quantitative parameters of colon perfusion were significantly affected by conditional factors, including distance, surrounding light and fluorescence emission source. To standardize and optimize the ICG angiography protocol for quantitative analysis, we performed a series of in vitro experiments to explore how conditional factors affect the fluorescence intensity during ICG angiography.

First, the distance from the camera to the subject affects the fluorescence intensity inversely. In the quantitative analysis, $F_{\text{MAX}}$ tends to decrease when the distance increases between the laparoscopic camera and ICG test tube. A previous similar experiment for influencing environmental factors was conducted with ICG cholangiography [24]. Their experiment was based on different concentrations of ICG and distance from the laparoscopic camera to ICG dyed bile duct. The results were similar to ours: as the distance increased, the fluorescence intensity decreased. They also commented that maintaining the same distance might be necessary to obtain the optimal fluorescence signal. To standardize the various conditions that may affect the fluorescence intensity of ICG, we have designed a much more sophisticated in vitro experiment for fluorescence intensity under various conditions including wide distance range, dilute ICG solutions, surrounding light and fluorescence emission source. Interestingly, the fluorescence intensity graph patterns commonly show the inflection point around 4–5 cm, where the steep decreasing slope turns almost flat in a horizontal manner in most conditions. Thus, we considered that this transitional section could be one of the candidates for an optimal distance zone for ICG angiography.

In the high slope region, the camera becomes too close to visualize all the fields of view of the subject at a distance of 0–4 cm. In other words, the field of view is too small to properly analyze ICG angiography when the camera is too close. Also, a steep slope indicates that fluorescence intensity is very sensitive to slight changes in distance. Thus, a very small tremor of the handheld camera can make noise that interferes with quantitative analysis in clinical practice.

### TABLE 1 Clinical characteristics of patients ($n = 164$)

| Patient characteristics | n (%) |
|-------------------------|-------|
| Age ≥65 years           | 80 (48.8) |
| Sex                     |       |
| Male                    | 115 (70.1) |
| Female                  | 49 (29.9) |
| BMI ≥ 25 kg/m$^2$       | 53 (32.3) |
| Hypertension            | 69 (42.1) |
| Diabetes mellitus       | 37 (22.6) |
| Smoking                 | 38 (23.2) |
| Type of surgery         |       |
| Anterior resection      | 61 (37.2) |
| Low anterior resection  | 103 (62.8) |
| Cancer stage            |       |
| 0–2                     | 108 (65.9) |
| 3–4                     | 56 (34.1) |
| ASA score               |       |
| 1–2                     | 152 (92.7) |
| 3–4                     | 12 (7.3) |
| Cancer obstruction      | 38 (23.2) |
| Preoperative radiation therapy | 9 (5.5) |
| IMA ligation            |       |
| High ligation           | 120 (73.2) |
| Low ligation            | 44 (26.8) |
| Anastomosis level       |       |
| ≥5 cm                   | 106 (64.6) |
| <5 cm                   | 58 (35.4) |
| Splenic flexure mobilization | 130 (79.3) |
| Diverting ileostomy     | 55 (33.5) |
| Transection line change | 11 (6.7) |
| Anastomotic complications | 10 (6.1) |

Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index; IMA, inferior mesenteric artery.

### TABLE 2 Conditional factors of ICG angiography for laparoscopic colorectal surgery ($n = 164$)

| Conditional factors                                      | Values |
|---------------------------------------------------------|--------|
| Camera distance (cm), mean ± SD                         | 7.22 ± 1.95 |
| Site of angiography, n (%)                              |        |
| Intra-abdominal ICG angiography                         | 103 (62.8) |
| Extra-abdominal ICG angiography                         | 61 (37.2) |
| Surrounding lighting, n (%)                             |        |
| Light off (dark)                                        | 145 (88.4) |
| Light on (light)                                        | 19 (11.6) |
| Fluorescence emission source, n (%)                     |        |
| Xenon (Storz)                                           | 102 (62.2) |
| Laser (Stryker)                                         | 62 (37.8) |
| ICG mode, n (%)                                         |        |
| Conventional ICG (Storz)                               | 22 (13.4) |
| Spectra A mode with red inversion (Storz)               | 80 (48.8) |
| ENV mode (Stryker)                                      | 62 (37.8) |

Abbreviations: ENV, endoscopic near-infrared visualization; ICG, indocyanine green.
Moreover, in the low-intensity region above 5–6 cm from the subject, it is too dark to photograph which indicates less effective sections for obtaining a proper level of fluorescence intensity. From the technological point of view, the energy decreases in inverse proportion to the square of the distance in the radiation area. Additionally, if the fluorescence intensity is too dark, low-light noise is relatively emphasized and the quality of the image deteriorates. In our experiments, the best shooting distance is considered as 4–5 cm, wherein the slope is placed between high slope and low-intensity regions. Therefore, we would suggest that 4–5 cm is the optimal distance of ICG angiography for quantitative analysis.

We have assumed that \( F_{\text{MAX}} \) might be higher in dark conditions because fluorescence looks brighter in a dark field. The results of in vitro study have shown that the fluorescence intensity is higher in a surrounding light-on condition. This can be explained by room lights containing a similar wavelength of the NIR spectrum which can interfere with the fluorescence intensity of ICG. Not only our results but also a previous basic study recommended turning off shadowless lights during ICG fluorescence because of the existence of an additional NIR spectrum among the room lights [8]. Thus, we would also like to suggest that ICG angiography should be performed with all surrounding lights off during extra-abdominal ICG angiography.

In our clinical data, \( F_{\text{MAX}} \) in intra-abdominal ICG angiography was much higher than that of the extra-abdominal procedure. This clinical result is the opposite of the in vitro study; this can be explained by the fact that the mean distance is statistically closer in...
the intra-abdominal ICG angiography ($P < 0.001$). Thus, we speculate that distance has a greater influence on fluorescence intensity than surrounding lighting.

In this study, the fluorescence emission source affected $F_{\text{MAX}}$, $T_{1/2\text{MAX}}$, and slope with statistical significance. In particular, $F_{\text{MAX}}$ was higher with a laser source than a xenon lamp. In the xenon camera system, the original blue fluorescence image can be translated to light cyan colour by red inversion mode, and this ICG dedicated mode can express brighter fluorescence like a laser camera. On multivariate analysis, ICG specific mode and laser (ENV) mode were analysed as an independent factor for improving fluorescence intensity.

ICG angiography helps surgeons visualize the colon perfusion that is related to anastomotic leakage during laparoscopic colorectal surgery [21,25,26]. According to previous studies on ICG angiography from our institute, quantitative parameters including $T_{1/2\text{MAX}}$, slope and TR are associated with the prediction of anastomotic leakage [12,13]. In this study, we have found that TR was analysed as a stable quantitative parameter independent of conditional factors such as distance and fluorescence emission sources. Because TR is composed of the element of time rather than the level of fluorescence intensity, it can overcome the interference of extrinsic environmental factors. However, the slope and $F_{\text{MAX}}$ are based on the amount of fluorescence intensity change and are affected by conditional factors, so these parameters show a significant difference according to the external environmental conditions. This phenomenon can act as a hidden obstacle to quantitative analysis of perfusion status. Therefore, an optimized and standardized protocol of ICG angiography can serve as a basis for establishing the reliability of the quantitative perfusion analysis.

This study has several limitations. First, this was a small sample size study of a single cohort. Although the clinical data were collected for a prospective observational study, there is unequal size sampling within each different conditional group. This is due to consecutive trials for establishing an optimal protocol to obtain an improved quality of fluorescence intensity. Second, we have considered five different conditional factors; nevertheless, there may be more categories of conditional factors such as different ICG dosage and advanced fluorescence modes. We are looking for optimal ICG

### TABLE 3 Univariate and multivariate analysis for clinical and conditional factors associated with $F_{\text{MAX}}$ parameter ($n = 164$)

| Factors                        | Total | Univariate | Multivariate |
|-------------------------------|-------|------------|--------------|
|                               | n     | n (%)      | $P$ value    | HR            | 95% CI       | $P$ value |
| Age (years)                   | 164   |            |              |               |              |           |
| <65                           | 84    | 51 (60.7)  | 0.090        |               |              |           |
| ≥65                           | 80    | 38 (47.5)  |              |               |              |           |
| Preoperative radiation        |       |            |              |               |              |           |
| No                            | 155   | 81 (52.3)  | 0.032        |               |              |           |
| Yes                           | 9     | 8 (88.9)   |              |               |              |           |
| IMA ligation level            |       |            |              |               |              |           |
| High                          | 120   | 64 (53.3)  | 0.691        |               |              |           |
| Low                           | 44    | 25 (56.8)  |              |               |              |           |
| Anastomotic complications     |       |            |              |               |              |           |
| No                            | 154   | 85 (55.2)  | 0.350        |               |              |           |
| Yes                           | 10    | 4 (40.0)   |              |               |              |           |
| Fluorescence emission source  |       |            |              |               |              |           |
| Xenon                         | 102   | 48 (47.1)  | 0.017        |               |              |           |
| Laser                         | 62    | 41 (66.1)  |              |               |              |           |
| ICG image mode                |       |            |              |               |              |           |
| Conventional                  | 22    | 3 (13.6)   | <0.001       | 1             |              |           |
| Red inversion                 | 80    | 45 (56.3)  | 6.552        | 1.749–24.538  | 0.005        |           |
| Laser (ENV)                   | 62    | 41 (66.1)  | 7.556        | 1.862–30.663  | 0.005        |           |
| Surrounding light             |       |            |              |               |              |           |
| Off                           | 145   | 81 (55.9)  | 0.258        |               |              |           |
| On                            | 19    | 8 (42.1)   |              |               |              |           |
| Distance (cm)                 |       |            |              |               |              |           |
| ≥8                            | 40    | 12 (30.0)  | <0.001       | 1             |              |           |
| <8                            | 124   | 77 (62.1)  | 2.488        | 1.033–5.989   | 0.042        |           |

Abbreviations: ENV, endoscopic near-infrared visualization; $F_{\text{MAX}}$, maximal fluorescence intensity; HR, hazard ratio; ICG, indocyanine green; IMA, inferior mesenteric artery.
dosage for angiography; however, it is necessary to establish standardization of conditional factors ahead of the ICG dosage optimization study. As far as we know, there is no standard use of ICG dose for fluorescence angiography for colon perfusion [18]. So, we expect that this work can be a scaffold for future studies for the standardization of ICG angiography. Finally, this study was conducted based on the laparoscopic NIR system currently available in the operating room of our institution. In perspective, new camera systems will be introduced with the complementarity of the conditional factors. In particular, many instruments using not only ICG but also hyperspectral images and laser speckle contrast images are being developed to minimize the external influence of distance and room light. Along with developing the new image system, additional studies should be conducted to update the standardization protocol of angiography with advanced technology.

CONCLUSION

In conclusion, conditional factors, including distance, surrounding light and fluorescence emission source, significantly affect the quantitative parameters, especially $F_{\text{MAX}}$, $T_{1/2\text{MAX}}$ and slope. The standardization protocol of ICG angiography can improve the quality and consistency of quantitative analysis, and we would like to suggest a protocol with an optimal distance of 4–5 cm using ICG specific modes.
| Reference                        | Patients | Types of surgery                  | Intravenous dose of ICG | Distance (cm) | Extra-/intra-abdominal angiography | Surrounding light | Fluorescence emission source | NIR system                      |
|---------------------------------|----------|-----------------------------------|-------------------------|---------------|-----------------------------------|------------------|-------------------------------|---------------------------------|
| Aiba et al. 2021 [16]           | 110      | Colorectal resection              | 0.1 mg/kg               | 5             | Extra-peritoneal                   | Off              | Xenon                         | OPAL1 (Karl Storz)               |
| Benčurik et al. 2020 [17]       | 100      | Laparoscopic or robotic LAR        | 0.2 mg/kg               | -             | Extra-peritoneal                   | -                | Xenon laser                    | SPIES (Karl Storz) Firefly (Intuitive) |
| Watanabe et al. 2020 [18]       | 236      | Laparoscopic LAR                   | 0.25 mg/kg              | -             | Extra-peritoneal                   | -                | Xenon laser                    | D-light P (Karl Storz) 1588 AIM (Stryker) |
| De Nardi et al. 2020 [19]       | 240      | Laparoscopic left-sided colorectal resection | 0.3 mg/kg | - | - | - | Xenon | Image1 (Karl Storz) |
| Son et al. 2019 [13]            | 86       | Laparoscopic AR or LAR             | 0.25 mg/kg              | -             | Extra- or intra-peritoneal         | Off              | Xenon                         | Image1 S (Karl Storz) Photodynamic Eye System (Hamamatsu) |
| Ogino et al. 2019 [3]           | 74       | Colorectal surgery                | Bolus 5 mg              | 15            | Extra-peritoneal                   | -                | LED                           | Image1 S (Karl Storz) 1588 AIM (Stryker) |
| Morales-Conde et al. 2020 [20]  | 192      | Colorectal surgery                | Bolus 15 mg             | 5             | Extra-peritoneal                   | -                | Xenon laser                    | Image1 S (Karl Storz) 1588 AIM (Stryker) |
| Chang et al. 2019 [7]           | 110      | -                                 | Bolus 5 mg              | -             | Extra-peritoneal                   | Off              | Laser                         | SPY Elite System (Stryker) |
| Boni et al. 2017 [21]           | 42       | Laparoscopic LAR                   | 0.2 mg/kg               | -             | -                                 | -                | Xenon                         | Image1 (Karl Storz) |

Abbreviations: AR, anterior resection; ICG, indocyanine green; LAR, low anterior resection; LED, light emitting diode; NIR, near infrared.
INFORMED CONSENT
Informed consent was obtained from all individual participants included in the study.

ACKNOWLEDGEMENTS
The authors appreciate Myeong Sook Kwon, Kyung Hee Kim, Hyun Seok Jung and Mi Jeong Kim for technical assistance of fluorescence image guided surgery in the surgical field.

CONFLICT OF INTERESTS
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Conceptualization: SGM. Formal analysis: SGM, AHM. Investigation: SGM, AHM, PSH, KNS. Methodology: SGM, PSH, KNS, LIY. Project administration: LIY. Writing—original draft: SGM, AHM, PSH. Writing—review and editing: SGM, AHM.

ETHICAL STATEMENT
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

DATA AVAILABILITY STATEMENT
Research data are not shared.

ORCID
Gyung Mo Son https://orcid.org/0000-0002-8861-6293

REFERENCES
1. McDermott F, Heeney A, Kelly M, Steele R, Carlson G, Winter D. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. Br J Surg. 2015;102(5):462–79.
2. Shogan BD, Carlisle EM, Alverdy JC, Umanskiy K. Do we really know why colorectal anastomoses leak? J Gastrointest Surg. 2013;17(9):1698–707.
3. Ogino T, Hata T, Kawada J, Okano M, Kim Y, Okuyama M, et al. The risk factor of anastomotic hypoperfusion in colorectal surgery. J Surg Res. 2019;244:265–71.
4. Nachiappan S, Askari A, Currie A, Kennedy RH, Faiz O. Intraoperative assessment of colorectal anastomotic integrity: a systematic review. Surg Endosc. 2014;28(9):2513–30.
5. Kudszus S, Roesel C, Schachtrupp A, Höer JJ. Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. Langenbecks Arch Surg. 2010;395(8):1025–30.
6. Gröne J, Koch D, Kreis M. Impact of intraoperative microperfusion assessment with pinpoint perfusion imaging on surgical management of laparoscopic low rectal and anorectal anastomoses. Colorectal Dis. 2015;17:22–8.
7. Chang YK, Foo CC, Yip J, Wei R, Ng KK, Lo O, et al. The impact of indocyanine-green fluorescence angiogram on colorectal resection. Surgeon. 2019;17(5):270–6.
8. Miwa M. The principle of ICG fluorescence method. Open Surg Oncol J. 2010;2(1).
9. Boni L, David G, Dionigi G, Rausei S, Cassinotti E, Fingerhut A. Indocyanine green-enhanced fluorescence to assess bowel perfusion during laparoscopic colorectal resection. Surg Endosc. 2016;30(7):2736–42.
10. Kawada K, Hasegawa S, Wada T, Takahashi R, Hisamori S, Hida K, et al. Evaluation of intestinal perfusion by ICG fluorescence imaging in laparoscopic colorectal surgery with DST anastomosis. Surg Endosc. 2017;31(3):1061–9.
11. Jafari MD, Wexner SD, Martz JE, McMerec E, Margolin DA, Sherwinter DA, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. J Am Coll Surg. 2015;220(1):82–92 e1.
12. Wada T, Kawada K, Takahashi R, Yoshitomi M, Hida K, Hasegawa S, et al. ICG fluorescence imaging for quantitative evaluation of colonic perfusion in laparoscopic colorectal surgery. Surg Endosc. 2017;31(10):4184–93.
13. Son GM, Kwon MS, Kim Y, Kim J, Kim SH, Lee JW. Quantitative analysis of colon perfusion pattern using indocyanine green (ICG) angiography in laparoscopic colorectal surgery. Surg Endosc. 2019;33(5):1640–9.
14. Lutken CD, Achiam MP, Svendsen MB, Boni L, Nerup N. Optimizing quantitative fluorescence angiography for visceral perfusion assessment. Surg Endosc. 2020;34(12):5223–33.
15. Park S-H, Park H-M, Baek K-R, Ahn H-M, Lee IY, Son GM. Artificial intelligence based real-time microcirculation analysis system for laparoscopic colorectal surgery. World J Gastroenterol. 2020;26(44):6945–62.
16. Aiba T, Uehara K, Ogura A, Tanaka A, Yonekawa Y, Hattori N, et al. The significance of the time to arterial perfusion in intraoperative ICG angiography during colorectal surgery. Surg Endosc. 2021. https://doi.org/10.1007/s00464-020-08185-0
17. Benčurík V, Škrovina M, Martinek L, Bartoš J, Macháčková M, Dosoudil M, et al. Intraoperative fluorescence angiography and risk factors of anastomotic leakage in minimally invasive low rectal resections. Surg Endosc. 2020. https://doi.org/10.1007/s00464-020-07982-x
18. Watanabe J, Ishitake A, Suwa Y, Suwa H, Ota M, Kunisaki C, et al. Indocyanine green fluorescence imaging to reduce the risk of anastomotic leakage in laparoscopic low anterior resection for rectal cancer: a propensity score-matched cohort study. Surg Endosc. 2020;34(1):202–8.
19. De Nardi P, Elmore U, Maggi G, Maggiore R, Boni L, Cassinotti E, et al. Intraoperative angiography with indocyanine green to assess anastomosis perfusion in patients undergoing laparoscopic colorectal resection: results of a multicenter randomized controlled trial. Surg Endosc. 2020;34(1):53–60.
20. Morales-Conde S, Alarcon I, Yang T, Licardie E, Camacho V, Aguilar Del Castillo F, et al. Fluorescence angiography with indocyanine green (ICG) to evaluate anastomosis in colorectal surgery: where does it have more value? Surg Endosc. 2020;34(9):3897–907.
21. Boni L, Fingerhut A, Marzorati A, Rausei S, Dionigi G, Cassinotti E. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. Surg Endosc. 2017;31(4):1836–40.
22. Lin J, Zheng B, Lin S, Chen Z, Chen S. The efficacy of intraoperative ICG fluorescence angiography on anastomotic leak after resection for colorectal cancer: a meta-analysis. Int J Colorectal Dis. 2021;36(1):27–39.
23. Zhang W, Che X. Effect of indocyanine green fluorescence angiography on preventing anastomotic leakage after colorectal surgery: a meta-analysis. Surgery Today. 2021. https://doi.org/10.1007/s00595-020-02195-0
24. van den Bos J, Wieringa FP, Bouvy ND, Stassen LPS. Optimizing the image of fluorescence cholangiography using ICG: a systematic review and ex vivo experiments. Surg Endosc. 2018;32(12):4820–32.
25. Ris F, Hompes R, Cunningham C, Lindsey I, Guy R, Jones O, et al. Near-infrared (NIR) perfusion angiography in minimally invasive colorectal surgery. Surg Endosc. 2014;28(7):2221–6.

26. Arezzo A, Bonino MA, Ris F, Boni L, Cassinotti E, Foo DCC, et al. Intraoperative use of fluorescence with indocyanine green reduces anastomotic leak rates in rectal cancer surgery: an individual participant data analysis. Surg Endosc. 2020;34(10):4281–90.