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Real-time navigation by fluorescence-based enhanced reality for precise estimation of future anastomotic site in digestive surgery

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Abstract
Background Fluorescence-based enhanced reality (FLER) is a technique to evaluate intestinal perfusion based on the elaboration of the Indocyanine Green fluorescence signal. The aim of the study was to assess FLER's performances in evaluating perfusion in an animal model of long-lasting intestinal ischemia.
Materials and methods An ischemic segment was created in 18 small bowel loops in 6 pigs. After 2 h ($n = 6$), 4 h ($n = 6$), and 6 h ($n = 6$), loops were evaluated clinically and by FLER to delineate five regions of interest (ROIs): ischemic zone (ROI 1), presumed viable margins (ROI 2a–2b), and vascularized areas (3a–3b). Capillary lactates were measured to compare clinical vs. FLER assessment. Basal (V_0) and maximal (V_{max}) mitochondrial respiration rates were determined according to FLER.
Results Lactates (mmol/L) at clinically identified resection lines were significantly higher when compared to those identified by FLER (2.43 ± 0.95 vs. 1.55 ± 0.33 $p = 0.02$) after 4 h of ischemia. Lactates at 2 h at ROI 1 were 5.45 ± 2.44 vs. 1.9 ± 0.6 (2a–2b; $p < 0.0001$) vs. 1.2 ± 0.3 (3a–3b; $p < 0.0001$). At 4 h, lactates were 4.36 ± 1.32 (ROI 1) vs. 1.83 ± 0.81 (2a–2b; $p < 0.0001$) vs. 1.35 ± 0.67 (3a–3b; $p < 0.0001$). At 6 h, lactates were 4.16 ± 2.55 vs. 1.8 ± 1.2 vs. 1.45 ± 0.83 at ROI 1 vs. 2a–-2b ($p = 0.013$) vs. 3a–3b ($p = 0.0035$). Mean V_0 and V_{max} (pmolO2/second/mg of tissue) were significantly impaired after 4 and 6 h at ROI 1 ($V_0^{4h} = 34.83 \pm 10.39$; $V_{max}^{4h} = 76.6 \pm 29.09$; $V_0^{6h} = 44.1 \pm 12.37$ and $V_{max}^{6h} = 116.1 \pm 40.1$) when compared to 2a–-2b ($V_0^{4h} = 67.1 \pm 17.47$ $p = 0.00039$; $V_{max}^{4h} = 146.8 \pm 55.47$ $p = 0.0054$; $V_0^{6h} = 63.9 \pm 28.99$ $p = 0.03$; $V_{max}^{6h} = 167.2 \pm 56.96$ $p = 0.01$). V_0 and V_{max} were significantly higher at 3a–3b.
Conclusions FLER may identify the future anastomotic site even after repetitive assessments and long-standing bowel ischemia.

Keywords Fluorescence-guided surgery · Indocyanine green · Near-infrared fluorescence videography · Augmented reality · Enhanced reality · Bowel perfusion

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Intraoperative navigation using near-infrared fluorescent light is a relatively novel technology which allows to visualize "the invisible," and may inform on morphology but also on organ function to some extent. Generally, fluorescence videography captures the fluorescence signal emitted by an injected dye (mostly Indocyanine Green or fluorescein) when illuminated by a laser. Fluorescence videography falls within the concept of image-guided surgery, with some advantages over futuristic intraoperative navigation modalities based on CT-scan, Magnetic Resonance Imaging, PET-scan, and fusion 4D ultrasonography, which may require complex set-up. Fluorescence videography has the potential to provide real-time information. It is independent of preoperative imaging, and does not disrupt surgical flow. It is fast, safe, easy to perform, and does not require the presence of an additional operator to function [1–4].

A variety of fluorescence-based optical systems have been developed and the range of clinical applications of this technology has constantly increased over recent years (e.g., sentinel lymph node navigation [5], biliary tree anatomy detection [6], tissue perfusion in complex abdominal wall repair [7], real-time assessment of anastomotic perfusion in colorectal resections [8, 9], or before gastric pull-up after esophageal resections [10]).

Fluorescence-based enhanced reality (FLER) is a novel technique used to evaluate tissue perfusion based on the use of a near-infrared laparoscopic endoscope (D-Light P; Karl Storz Endoskope, Tuttlingen, Germany) to detect the indocyanine green (ICG) fluorescence signal combined with a specifically designed imaging analyzer software (ER-PERFUSION, IRCAD). This software provides a digital perfusion cartography, based on the time-to-peak of the fluorescent signal, which is superimposed to the intraoperative laparoscopic image [11].

In the previous experimental essay about the evaluation of early bowel ischemia, FLER was able to detect a subtle drop in bowel perfusion and demonstrated a significant concordance with changes in lactate levels, mitochondria respiratory rate, and metabonomics fingerprint of ischemia [11].

The aim of this study was to compare the ability of FLER and clinical judgment to identify perfused margins in a model of bowel resection, after longer periods of ischemia, when boundaries become more apparent at clinical evaluation.

Materials and methods

Animals

The present experimental study is part of a larger experimental protocol on intestinal ischemia (No. 38.2012.01.039), approved by the local Ethical Committee on Animal Experimentation. All animals used in the experimental laboratory were managed according to French laws for animal use and care and according to the directives of the European Community Council (2010/63/EU). A total of 6 (4 males) swine (Sus scrofa domesticus, ssp. Large White; mean weight 27.01 ± 6.01 kg) were used in this non-survival study. Pigs were fasted for 24 h before surgery with free access to water. Premedication by intramuscular injection of ketamine (20 mg/kg) and azaperone (2 mg/kg) (Stressnil; Janssen-Cilag, Belgium) was administered 1 h before surgery. Induction was achieved by intravenous propofol (3 mg/kg) combined with pancuronium (0.2 mg/kg). Anesthesia was maintained with 2 % isoflurane. Pigs were equipped with an esophageal probe capable of measuring endoesophageal temperature before starting the procedure. Care was taken to prevent hypothermia: warming blankets were placed below the animals and, during procedure interval times, a thermal emergency blanket sheet was used to cover the animals. At the end of the procedure, animals were humanely sacrificed with an intravenous injection of a lethal dose of potassium chloride.

Procedures and equipment

An ischemic segment (approximately 5 cm in length) was created laparoscopically in 18 small bowel loops (3 loops/pig) by sealing 3 to 4 mesenteric vessels using the LigaSure™ vessel-sealing device (Covidien, Boulder, Colorado). After 2 h ($n = 6$), 4 h ($n = 6$), and 6 h ($n = 6$), ischemic segments were evaluated by clinical assessment and by FLER, to determine presumed viable margins. Five regions of interest (ROIs) were identified: ischemic (ROI 1), presumed viable margins (ROI 2a and 2b, respectively, left and right), and vascular areas (3a and 3b, respectively, left and right).

Blinded clinical evaluation of the ischemic area

2, 4, and 6 h after sealing of mesenteric vessels, the small bowel was examined to determine "clinical" viable margins by a second surgeon, who was observing the white light laparoscopic images on a laptop computer. Clinical criteria used to identify the resection lines were the relative duskiness of serosa of the ischemic area. Blinding was obtained by superimposing a white spot to cover the extent of the mesenteric window and sealed vessels. Covering the mesenteric window was deemed necessary since the porcine mesentery is thin and transparent, which is not the case in humans, and allows to easily follow vascular tree distribution to the bowel. Presumed resection lines ($2a_{CLINIC}$ and $2b_{CLINIC}$) were first drawn on the laptop screen by the blinded surgeon using a pen tool, overlapped onto laparoscopic images, and finally marked laparoscopically with a surgical clip on the serosal anti-mesenteric site.

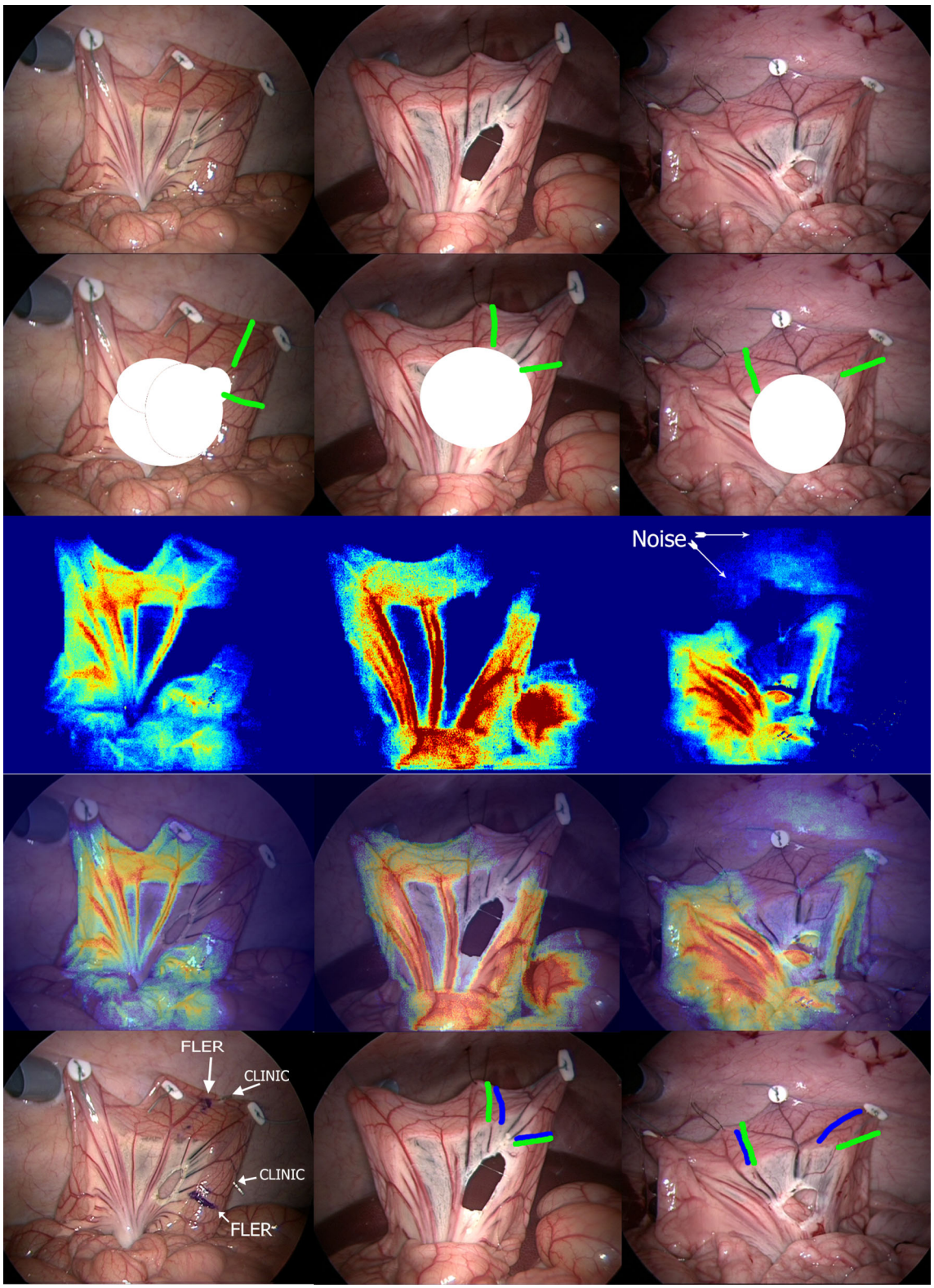

Fig. 1 Fluorescence-based Enhanced Reality after 2, 4, and 6 h of ischemia .*First row* 3 bowel loops were exposed and an ischemic segment was created, sealing some mesenteric vessels by means of the LigaSure™ vessel-sealing device. *Second row* blinded clinical evaluation after 2, 4, and 6 h of ischemia. *Third row* a virtual perfusion cartography was generated by the ER PERFUSION software based on time-to-peak fluorescence signals (in seconds) recorded with the D-Light-P (Karl Storz, Tuttlingen, Germany). *Fourth row* FLER was obtained by superimposing the virtual perfusion cartography onto the screen. *Fifth row* resection lines identified by FLER were marked with a surgical pen (over the *blue lines*) while those identified by the clinic were marked with a surgical clip (over the *green lines*)

FLER

Immediately after clinical evaluation, 0.5 mg/kg of Indocyanine Green (Infracyanine®, Serb, Paris) was injected intravenously into the animal, and the D-Light-P was shifted to a near-infrared mode to capture the fluorescence signal. The automatic shutter was turned off and set to a fixed ratio of 1/50. The fluorescence signal was analyzed using *ad hoc* software to construct virtual perfusion cartography of the bowel based on time-to-peak (in seconds). Time-to-peak is the mean time for the fluorescence signal to reach the maximum intensity in a given area. This is averaged on the recorded video for 20–40 s at the speed of 5 frames per second. The software algorithm was set to observe a time-to-peak delay of at least 50 % when compared to a control area (3a–3b). The steepness of the curve of time-to-peak depends on perfusion. Virtual perfusion cartography generated by the software was overlapped onto laparoscopic images, hence creating Enhanced Reality (Fig. 1). FLER and clinical evaluation for future resection lines 2a and 2b ($n = 36$ in total; $n = 12$ per time point) were considered concordant when the distance between the two landmarks, i.e., clip for clinical (CLINIC) and surgical marker for FLER on the bowel serosa, was $\leq$1 cm. The distance between the clip (Clinic) and the pen marker (FLER) was measured on the exposed bowel after laparotomy using a scale.

Systemic and local lactates

Systemic arterial lactatemia was measured in blood samples obtained from the femoral artery using a standard blood gas analysis unit. Capillary systemic lactatemia was measured in blood samples obtained by puncturing the pig's groin. Capillary blood samples were placed on test strips connected to the EDGE™ Blood Lactate Analyzer (Apex Biotechnology Corp. Hsinchu, Taiwan, ROC). A laparotomy was performed and local capillary lactates were measured in blood samples obtained by puncturing the bowel serosa at ROIs as identified by FLER. In case of discordance (>1 cm distance) between FLER and CLINIC at future resection lines, additional capillary lactates were measured at the sites identified by CLINIC (Fig. 2).

Mitochondrial respiratory chain assessment

Full-thickness bowel biopsies were taken at the previously marked ROI following the same pre-determined randomized order. Mitochondrial activity was determined as previously reported [11, 12]. Intestinal biopsies were quickly cut into 5 pieces each (1–2 mm^3) allowing mitochondria challenge within their cellular environment. Samples were placed in a 2 mL water-jacketed oxygraphic cell at 37 °C

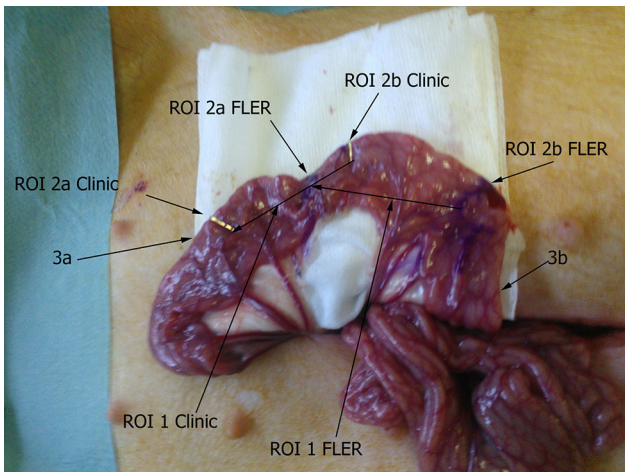

Fig. 2 Laparotomy and analysis of the regions of interest (ROI). Every 2 h, the small bowel loop was exteriorized and the ROIs identified by FLER and CLINIC were analyzed. Distance between resection lines was measured. Capillary lactates were withdrawn at the center of ROI1 (ischemic area), resection lines 2a–2b according to FLER, and CLINIC and control areas (3a–3b, 1 cm lateral to 2a–2b). Surgical biopsies for mitochondria respiratory rate were taken at the center of ROI1, at 2a–2b (according to FLER), and alternately at 3a or 3b. Surgical biopsies for pathology were taken at the center of ROI1 and alternately at 3a or 3b

under continuous stirring (Oxygraph-2 k©, Oroboros instruments®, Innsbruck, Austria) equipped with a Clark electrode. With the presence of glutamate (5 mmol/L), malate (2 mmol/L), and succinate (25 mmol/L), basal oxygen consumption (V_0) was determined. Maximal tissue respiration rates were measured in the presence of a saturating amount of ADP as phosphate acceptor (V_{max}). Once V_{max} has been recorded, electron flow goes through complexes I, II, III, and IV. After the experiments, tissues were harvested and dried (15 min at 150 °C) and respiration rates were expressed as pmol of Oxygen/second/mg of dry weight.

Pathology

Full-thickness surgical biopsies were taken at the ischemic area and at a control site (vascular areas 3a and 3b alternatively) of the sigmoid colon. Specimens were fixed in 4 % buffered formalin for at least 24 h. Sections (4-µm thick) were cut from paraffin-embedded tissues and stained with hematoxylin and eosin. Six sections per biopsy were analyzed. A standardized semi-quantitative histology score to evaluate ischemia was applied by a blinded pathologist to normal and ischemic areas. The score was composed as follows: 0 = normal mucosa; 1 = partial epithelial edema and necrosis; 2 = diffuse swelling and necrosis of epithelium; 3 = necrosis with submucosal neutrophil infiltration; 4 = widespread necrosis and massive neutrophil infiltration and hemorrhage.

Statistical analysis

A statistical analysis was performed using the GraphPad Prism Software. ANOVA followed by a Dunnett's multiple comparison test was performed to compare the ischemic zone to future resection lines and vascularized areas. A Student's t test was used to calculate p values for continuous variables. A p value <0.05 was considered statistically significant.

Results

Temperature

Endoesophageal temperature was measured at induction and at 2, 4, and 6 h, and remained stable over time and was 36.97 ± 0.44, 36.5 ± 1.3, 36.44 ± 1.75, and 36.16 ± 1.94 °C, respectively.

FLER

Clinical assessment and FLER were concordant regarding the position of resection lines (2a and 2b) in 50 % (6/12) of cases after 2 and 4 h of ischemia, and in 75 % (8/12) at 6 h of ischemia. In discordant cases, clinical resection lines were traced closer to the ischemic area in 50 % (3/6), 83 % (5/6), and 75 % (3/4) of cases after 2, 4, and 6 h, respectively. Mean distance between clinical assessment and FLER was 2 ± 0.63 cm, 1.91 ± 0.2, and 1.87 ± 0.25 after 2, 4, and 6 h, respectively.

Local bowel capillary lactates

Mean capillary lactate levels at 2, 4, and 6 h were significantly higher in the ischemic zone (ROI1) than in the others regions 2 h: 5.45 ± 2.44 versus 1.9 ± 0.6 versus 1.2 ± 0.3 mmol/L at ROI 1 versus 2a–2b ($p < 0.0001$) versus 3a–3b ($p < 0.0001$), respectively; 4 h: 4.36 ± 1.32 versus 1.83 ± 0.81 versus 1.35 ± 0.67 mmol/L at ROI 1 versus 2a–2b ($p < 0.0001$) versus 3a–3b ($p < 0.0001$); 6 h: 4.16 ± 2.55 versus 1.8 ± 1.2 versus 1.45 ± 0.83 at ROI 1 versus 2a–2b ($p = 0.013$) versus 3a–3b ($p = 0.0035$). In discordant cases (distance between CLINIC and FLER >1 cm), lactate levels at presumed viable margins assessed by clinical evaluation ($2a_{CLINIC} + 2b_{CLINIC}$) were statistically significantly higher when compared to those at $2a_{FLER} + 2b_{FLER}$ (2.43 ± 0.95 mmol/L vs. 1.55 ± 0.33 $p = 0.02$) after 4 h of ischemia. There was no statistically significant difference at 2 and 6 h (Fig. 3).

Mitochondria respiratory rate (pmol O_2/second/mg of dry tissue)

Mean basal and maximal mitochondrial respiratory rates expressed in pmol O2/second/mg of dry tissue (V_0 and V_{max}) were significantly impaired after 4 and 6 h of ischemia at ROI1 ($V_0^{4h} = 34.83 \pm 10.39$; $V_{max}^{4h} = 76.6 \pm 29.09$; $V_0^{6h} = 44.1 \pm 12.37$, and $V_{max}^{6h} = 116.1 \pm 40.1$) when compared to 2a–2b ($V_0^{4h} = 67.1 \pm 17.47$ $p = 0.00039$; $V_{max}^{4h} = 146.8 \pm 55.47$ $p = 0.0054$; $V_0^{6h} = 63.9 \pm 28.99$ $p = 0.03$; $V_{max}^{6h} = 167.2 \pm 56.96$ $p = 0.01$). V_0 and V_{max} were statistically significantly higher at vascular areas at all time points (except V_{max} at 4 h). No differences between ROIs 2a–2b and 3a-3b were found in lactate levels and mitochondrial respiratory rate (Fig. 4).

Pathology

Mean ischemia score at ROI1 was 2 ± 0.63, 1.83 ± 0.98, and 2.33 ± 0.81 after 2, 4, and 6 h of ischemia, respectively. Widespread necrosis (score 4) was not observed (Fig. 5). Ischemia score at control areas was invariably 0 (normal mucosa) at all time points.

Discussion

Fluorescence videography is a surgical navigation modality which is flexible, rapid, and easy to use and to integrate in the routine surgical work flow [1–4].

However, correct evaluation of the fluorescent signal is the key factor when assessing dynamic elements, such as intestinal perfusion. In addition, repeated injection of fluorescents may perturb the evaluation of the signal.

Recently, Carus et al. [9] proposed the use of laparoscopic ICG fluorescence angiography to intraoperatively assess anastomosis perfusion in a series of 45 patients undergoing colorectal resections and four patients undergoing sleeve gastrectomy. To confirm perfusion adequacy, Carus et al. used the degree of "relative blueness," as provided by computer analysis.

Ris et al. [13] reported a successful evaluation in 30 patients undergoing colorectal resections using the Pinpoint system (Novadaq, Mississauga, ON, Canada), which integrates a software providing a superimposition of white light and near-infrared modalities providing a real-time evaluation of perfusion. Evaluation of anastomosis perfusion was based on the duration of visible ICG fluorescence, which was 35 s on average.

In the previous study, we describe a method to merge the concept of fluorescence videography with augmented reality (AR) to guide intestinal resection and assess vascular supply at the future anastomotic site. In our method, FLER, virtual images of bowel perfusion (virtual vascular cartography) are generated from intraoperative live images based on fluorescence time-to-peak of Indocyanine Green as detected by a near-infrared endoscope and interpreted by an image analyzer software [11].

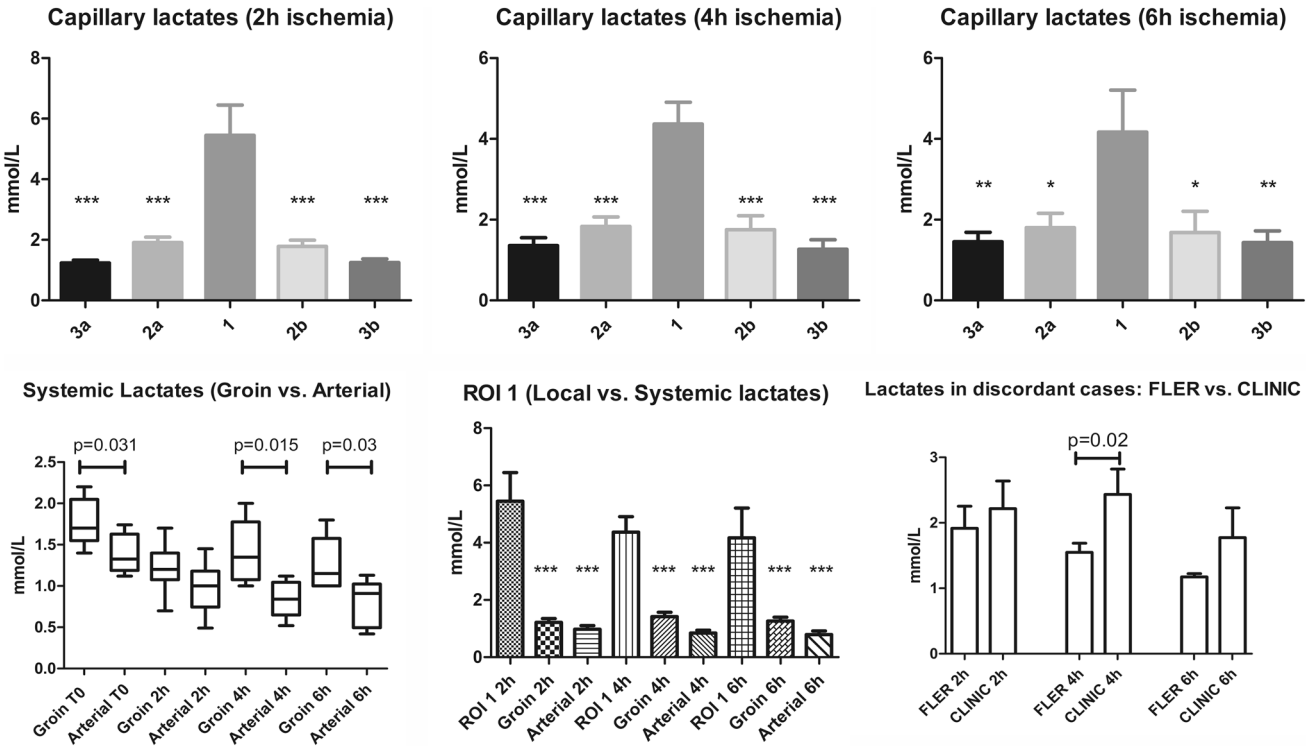

Fig. 3 Systemic and local lactates .The *graph* shows the evolution of capillary lactates over time (*first row*) at the ischemic ROI1, resection lines 2a–2b, and control areas 3a–3b. Local lactates were significantly higher ($p < 0.0001$) at the ischemic area when compared to both systemic lactates (groin and arterial) per all time points. *Second row* groin capillary lactates were statistically significantly higher when compared to arterial lactates at induction, after 4 and 6 h of ischemia. Systemic lactates (groin and arterial) were comparable to local capillary lactates at resection lines (2a–2b) and to vascularized areas (3a–3b) per all time points. Local lactates at presumed viable margins in discordant cases (distance between CLINIC and FLER >1 cm) were significantly higher at ($2a_{CLINIC} + 2b_{CLINIC}$) when compared to those at $2a_{FLER} + 2b_{FLER}$ ($p = 0.02$) after 4 h of ischemia (*$p < 0.01$; **p<0.001; ***p<0.0001)

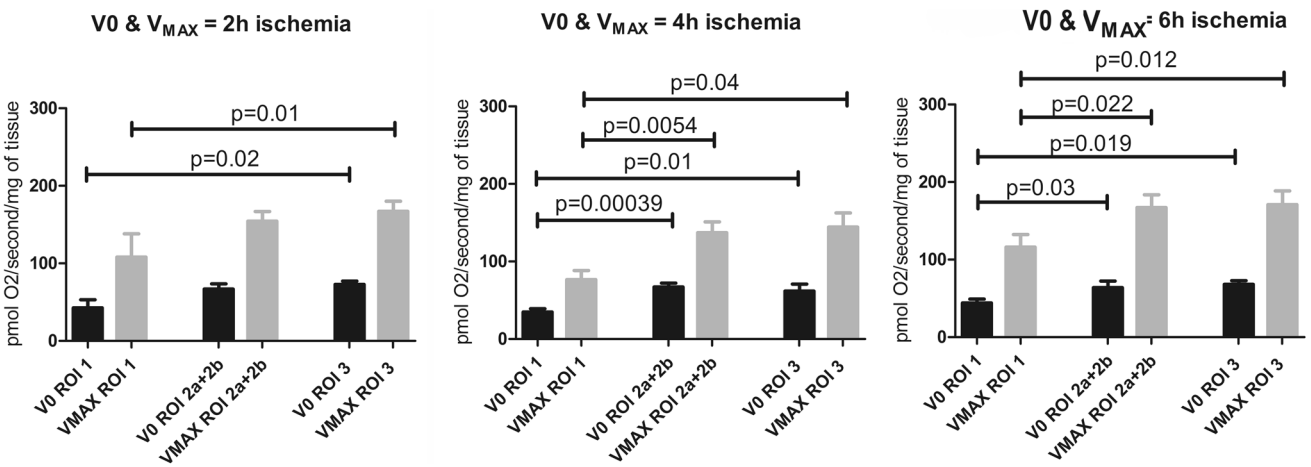

Fig. 4 Evolution of mitochondria respiratory rate (pmol O_2/second/mg of dry tissue) in small bowel regions identified by FLER. Basal and maximal mitochondrial respiratory rates (V_0 and V_{max}) were significantly impaired after 4 and 6 h of ischemia at ROI1 when compared to 2a–2b. V_0 and V_{max} were statistically significantly higher at vascular areas at all time points (except V_{max} at 4 h). No differences between ROIs 2a–2b and 3a–3b were found for lactate levels and mitochondrial respiratory rate

The use of the fluorescence time-to-peak slope has two advantages when compared to the use of the absolute value of "fluorescence intensity".

First, time-to-peak is independent of the distance between the light source and the imaged area. It is not the case with fluorescence intensity, which is highly dependent

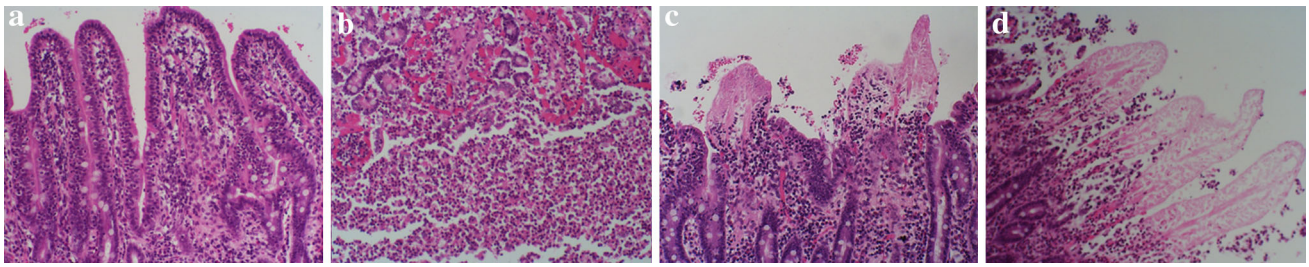

Fig. 5 Pathology ischemic score .The figure shows some examples of small bowel biopsies taken at the center of the ischemic area (ROI1). Hematoxylin and Eosin staining× 20. **a** Normal mucosa. **b** Score 1: partial epithelial edema and necrosis. **c** Score 2: diffuse swelling and necrosis of the epithelium. **d** Epithelial necrosis with submucosal neutrophil infiltration

on distance. A low perfused area may look more intensively fluorescent when observed closely and vice versa; a highly perfused area may look poorly fluorescent when observed from afar. Using time-to-peak allows to eliminate the need for the calibration tool and allows for objective inter-patient comparisons.

To verify this hypothesis, we measured the inverse relationship between distance (light source-target) and fluorescence intensity, using a customary electromagnetic tracking device (METRIS 3D) [14]. To track the position of the D-Light P laparoscope, the METRIS 3D system was attached to the shaft of the laparoscope, and a 2 cm plastic tool was also fixed to establish a set distance. The laparoscope was then used to target the fluorescence calibrating tool in a dark environment, and was moved in and out several times (Fig. 6).

Secondly, time-to-peak theoretically allows for multiple and repetitive assessments, since the "noise" produced by the accumulation of fluorescent dye should not affect the steepness of the slope. In a preliminary test on a living animal, before starting the research protocol, a series of injections of ICG (0.125 mg/kg = 25 % of the dose) were performed while constantly focusing on the same small bowel loop (without ischemia). ICG was injected every 15 min and time-to-peak was calculated on two separate points of the loop and on the calibration tool delivering a constant signal. This test shows saturation occurring even with small doses if interval time is too short to wash out ICG. Global fluorescence intensity raised progressively, even if there were no changes in perfusion, while the steepness of the slope for time-to-peak was constant at each assessment (Fig. 7). With time-to-peak, the background can be zeroed to allow for an additional dye injection. Only the additional signal is interpreted to generate virtual perfusion cartography.

The aim of the present study was to control the possibility to perform repeated injections and to evaluate FLER performances over time when compared to clinical assessment, in a longer ischemia model. Performances of fluorescence evaluation to detect perfused areas can decrease because of ICG accumulation from previous injections that might alter signal interpretation by increasing the noise given by a saturated background. Simultaneously, clinical evaluation could be superior because of a clearer delineation during ischemia progression.

The distance between resection lines as identified by FLER and clinical assessment and levels of local capillary lactates were used to compare performances.

The minimum discriminating distance between the two evaluation methods was fixed to 1 cm because the width of the laparoscopic linear staplers commercially available is 1 cm, and thus a lower distance would have minimal clinical impact. A second reason was that full-thickness biopsies of approximately 0.5 cm were taken at the ROIs to measure mitochondria activity and for histology.

The distance between resection lines (2a and 2b) at clinical assessment and FLER in discordant cases (distance >1 cm; $n = 18$) decreased with duration of ischemia. In such cases, a blinded clinical evaluation identified resection lines closer to the ischemic area or within the ischemic area in 3 out of 6 cases at 2 h, in 5 out of 6 cases at 4 h, and in 3 out of 4 at 6 h. The better performance of FLER in terms of distance at 4 h of ischemia was confirmed by the analysis of local capillary lactates.

Lactate is the end product of glycolysis which accumulates in the bloodstream and muscles as a result of oxygen debt. Lactate is also a precursor of glucose synthesis, and the lactate pool acts as an important dynamic substrate for oxidative energy production when oxygen debt is recovered. Systemic lactatemia is an unspecific marker of tissue hypoperfusion and slowly increases in response to mesenteric ischemia with significant elevation only after advanced damage [15]. In an experimental model of acute limb ischemia, Noll et al. [16] showed that local capillary lactates directly measured in blood obtained by limb needle puncture demonstrated both a rapid increase during ischemia and a rapid decrease during reperfusion, as they represent better markers than systemic lactates. In the previous study, to establish proof of the FLER concept in a 1-hour model of mesenteric ischemia, capillary lactates

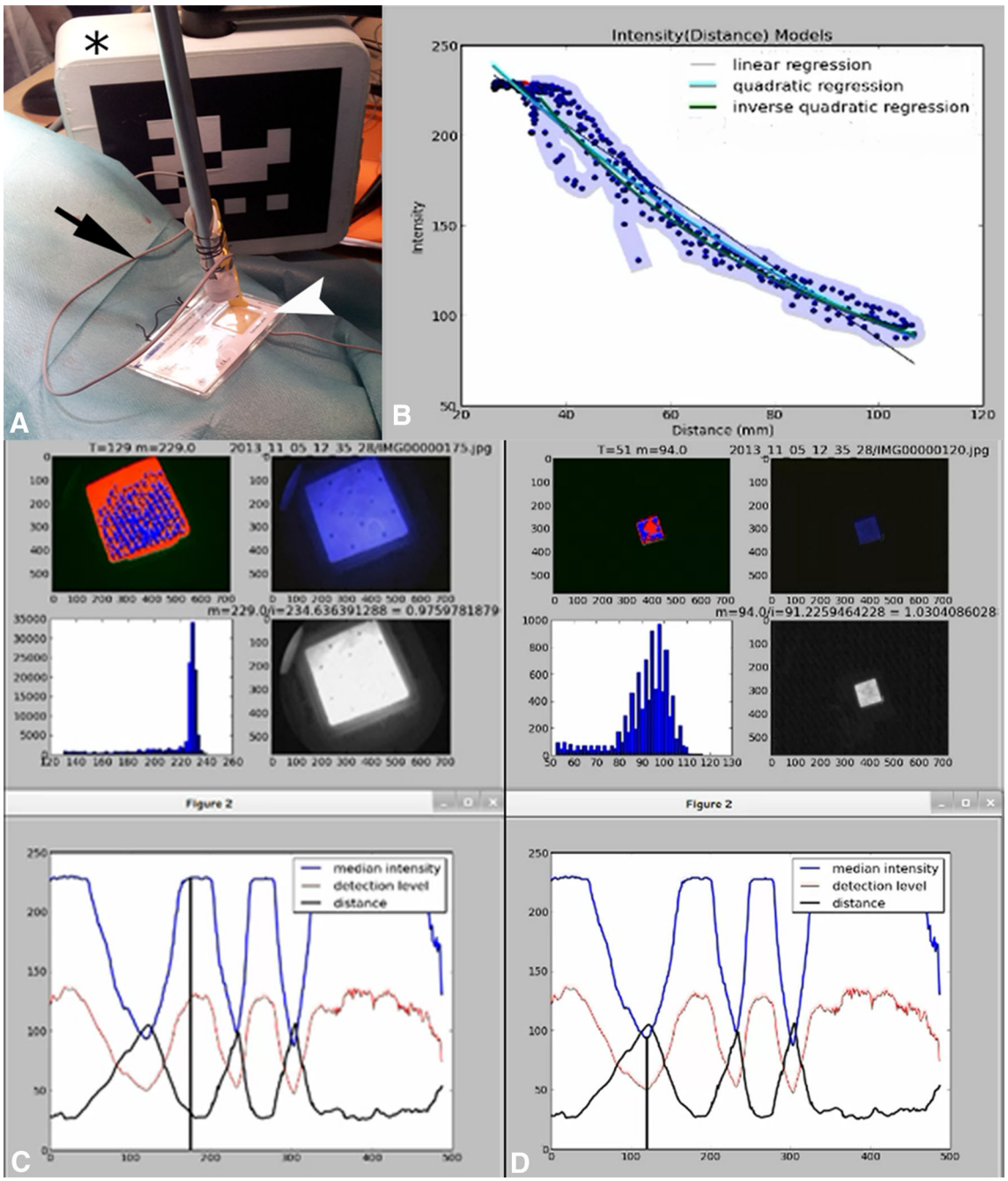

Fig. 6 Metris 3D system calibration. A customary electromagnetic tracking device (METRIS 3D) was used to verify the relationship between distance and fluorescence intensity. **a** The METRIS 3D system is made of a tube (1.2 m long and 2.2 mm in diameter, *black arrow*) that can be inserted into the operating channel of a flexible endoscope and contains 7 miniature electromagnetic coils distributed on its length. Electromagnetic coils are tracked by a magnetic tracking system (*asterisk*). To track the position of the D-Light P laparoscope, the METRIS 3D system was attached to the shaft of the laparoscope, and a 2 cm plastic tool was also fixed to establish a set distance. The laparoscope was then used to target the fluorescence calibrating tool (*white arrow*) in a dark environment, and was moved in and out several times. **b** The *graph* shows the inverse relationship between fluorescence intensity and distance. C and D) examples of the relationship: the higher part shows the appearance of the calibrating tool spot from close (2 cm) and afar (20 cm)

measured with the hand-held EDGE^{TM} lactate analyzer showed a good statistical correlation with fluorescence time-to-peak [11]. In a porcine model of free small bowel flaps, Birke-Sorensen et al. [17] used a microdialysis catheter to monitor local lactate and glucose. They could identify a cut-off of the lactate/glucose ratio yielding excellent accuracy to discriminate between ischemic and non-ischemic segments. Similarly, using magnetic

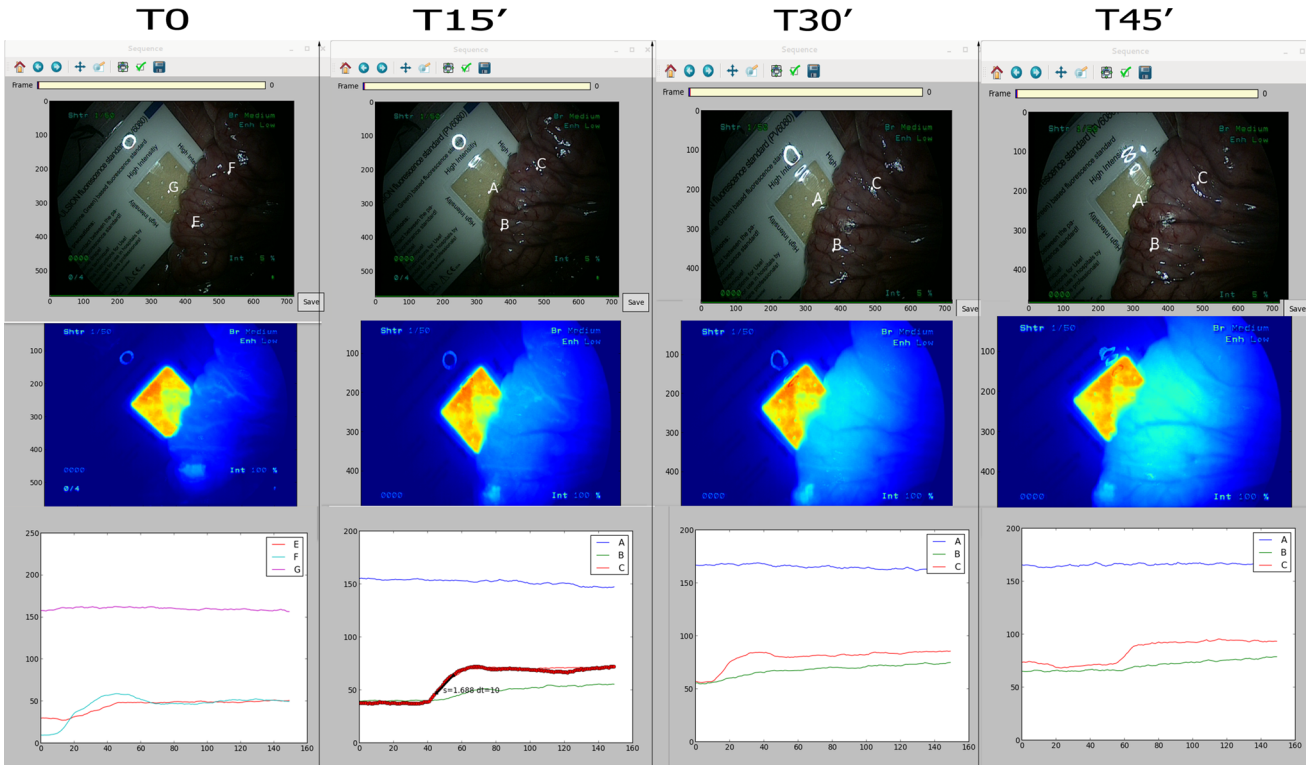

Fig. 7 Effect of repetitive injections of ICG within a short interval. In a preliminary test, a series of injections of ICG at a 15-second interval were performed while constantly focusing on the same small bowel loop (without ischemia). Time-to-peak was calculated at 2 separate points of the loop and on the calibration tool delivering a constant signal. This test showed the saturation occurring with a progressive rise in global fluorescence intensity in the absence of changes in perfusion. Conversely, the steepness of the slope for time-to-peak (*red line*) was constant at each assessment

resonance spectroscopy to assess the metabolic profile of small bowel ischemia, we found decreased glucose levels and increased tissue lactates and amino acids, showing increased anaerobic glycolysis [11].

Systemic capillary lactates, measured in a drop of blood obtained by needle puncture of the pigs' groins, were compared to systemic arterial lactates, measured in blood samples of the femoral artery. Groin (systemic) capillary lactates were statistically significantly higher when compared to systemic arterial lactates at induction ($p = 0.031$), after 4 h of ischemia ($p = 0.015$) and after 6 h of ischemia ($p = 0.03$). Local (bowel) lactates were significantly higher ($p < 0.0001$) at the ischemic area when compared to both systemic lactates (groin and arterial) per all time points. Additionally, systemic lactates (groin and arterial) were comparable to local capillary lactates at resection lines (2a–2b) and to vascularized areas (3a–3b) per all time points, reflecting that systemic lactatemia is not reliable to identify mesenteric ischemia, at least in a small portion of the bowel.

Local lactates at presumed viable margins in discordant cases (distance between Clinic and FLER >1 cm) were statistically significantly higher at ($2a_{CLINIC} + 2b_{CLINIC}$) when compared to those at $2a_{FLER} + 2b_{FLER}$ ($p = 0.02$) after 4 h of ischemia.

Pathology evaluation in our study did not show a clearly time-dependent progression of mucosal damage. On the contrary, the ischemia score was regularly worse after shorter ischemic periods. This could partly be explained by the limited size of the ischemic segment and the presence of some overlapping vascular networks on the bowel serosa which might have protected from further ischemic damage.

Oxygraphic assessment of the mitochondria respiratory rate allows to study the functional status of muscular mitochondria in a dynamic fashion. The activity of enzymatic complexes involved in oxidative phosphorylation can be studied individually with this method. Oxidative phosphorylation starts with a series of redox reactions (electron transport chain) in which electrons are transferred to the oxygen which is the most electronegative acceptor. These reactions generate the energy necessary to actively pump H + protons in the inter-membrane space and create an electrochemical transmembrane gradient which is used to produce adenosine triphosphate (ATP). There are 3 enzymatic proton pumps: complexes I, III, and IV. Ischemia, by reducing the presence of oxygen as terminal

electron acceptor, impairs the activity of those complexes, with progressive reduction of the gradient, energy depletion, and ultimately necrosis. In the same sample, to test the activity of those complexes, after determination of basal oxygen consumption (V_0), maximal respiratory rate is assessed in the presence of a saturating amount of substrate (Adenosine Diphosphate, ADP).

After 1 h of mesenteric ischemia, we found an already significant respiratory chain impairment in the ischemic zone as compared to the non-ischemic segments as expressed by V_{max} [11]. FLER could effectively differentiate the small bowel zones presenting these very early signs of energetic suffering. In the present study, oxygraphic measurement of the mitochondria respiratory rate in small bowel biopsies obtained at the ischemic area and at boundaries as identified by FLER confirmed the efficacy of ischemic versus non-ischemic bowel discrimination at all time points, with an increased accuracy after 4 and 6 h of ischemia.

Future works

The ER-PERFUSION software was recently upgraded to (1) improve the interface, and (2) to improve efficacy by reducing the time to calculate virtual perfusion cartography as well as registration of cartography with real images. In the latest version, only resection lines are projected onto the bowel with an enhanced reality effect. The accuracy of the software will be tested in a series of experimental bowel resections and anastomoses in a survival animal model (currently underway). Resection lines and subsequent anastomoses will be performed according to various degrees of perfusion, which will be determined by the ER-PERFUSION software. The latest version of the software also integrates a tracking function which has been developed to allow for constant registration of virtual perfusion cartography obtained by fluorescence time-to-peak onto real images, even during bowel manipulation. To track and constantly register FLER, virtual cartography is deformed by using an affine deformation created by pixel displacement estimated by a feature matching algorithm (BRIEF: binary robust independent elementary features) [18], in order to follow the motion of the regions of interest.

Conclusions

FLER is a promising technology to intraoperatively assess bowel perfusion and determine the future anastomotic site, as it is effective, rapid, and easy to use. FLER may effectively identify the future anastomotic site even after repetitive assessments. These would offer a better performance than a surgeon's subjective assessment. Developments are currently underway to enhance the software's capabilities and ensure optimal transfer of this technology to the clinical setting.

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