



# Simultaneous multipurpose fluorescence imaging with IRDye® 800BK during laparoscopic surgery

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## Abstract

**Background** IRDye® 800BK is a fluorophore, currently undergoing clinical translation, which has both biliary and renal clearance. To date, there is no description of a fluorophore, which can be simultaneously used for non-invasive, near-infrared fluorescence-based (NIRF) visualization of different structures and perfusion evaluation. The purpose of this study was to evaluate IRDye® 800BK for the simultaneous assessment of bowel perfusion, lymphography, ureter and bile duct delineation.

**Methods** Six pigs received a 0.15 mg/kg dye as a single bolus intravenous injection (IV). With the FLER (fluorescence-based enhanced reality) software, fluorescence intensity (FI) of 5 regions of interest (ROI) in an ischemic bowel loop was measured along with the time to reach the FI peak, and capillary lactate was measured from the same ROI, followed by the assessment of the ureters and bile ducts for a maximal duration of 180 min after dye administration. In 3 animals, the procedure was initiated via gastroscopic injection of a 0.6 mg (1 mg/mL) dye in the gastric submucosa followed by lymphography in a NIRF setting.

**Results** Excellent visualization of the ureters and bowel perfusion was obtained under NIRF imaging. Additionally, the bile duct and gastric lymph ducts and nodes were visualized. A positive correlation was found between the time to peak FI in the ischemic bowel loop and the corresponding capillary lactate levels ( $\rho$  0.59,  $p < 0.001$ ).

**Conclusion** In this study, we successfully demonstrated the simultaneous multipurpose IRDye® 800BK applicability during laparoscopic surgery. This fluorophore has the potential to become a powerful and versatile image-guided surgery tool.

**Keywords** Ureteral visualization · Bowel perfusion · Lymphography · Bile duct imaging · Laparoscopic surgery · Near-infrared fluorescence

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Near-infrared fluorescence (NIRF) imaging is being rapidly adopted as a surgical navigation tool [1]. NIRF provides a real-time enhanced visualization of anatomical structures and/or metabolic information, thanks to the increased tissue penetration of NIR when compared to white light [2, 3]. NIRF imaging is being studied in multiple surgical applications, including the assessment of bowel perfusion [4–6] to decrease the risk of anastomotic leakage; bile duct [7, 8] and ureteral delineation [9, 10] to decrease the risk of iatrogenic injury; and lymph node identification [11, 12] to aid in oncological resections.

Indocyanine green (ICG) [7, 13–17] is the dye which is most extensively studied and used in clinical research and practice, as it has been approved by the FDA (Food and Drug Administration) for clinical use and it is well-known due to its established clinical applications in the assessment of hepatic blood flow, assessment of the choroidal blood flow,

and measurement of cardiac output [18]. This facilitated its introduction to new applications in NIRF imaging. Since ICG is exclusively cleared by the liver, it cannot be used for non-invasive ureteral delineation and it requires direct intra-ureteral injection or retrograde catheter insertion for this purpose [19]. The clinically available methylene blue (MB) is a dye, which is partially cleared by the kidney and it was studied for this purpose. However, although the feasibility of intraoperative ureteral delineation under NIRF imaging with MB has been proven [9, 20], it failed to visualize all the ureters studied in clinical studies, which may originate from the optical properties of MB that are suboptimal for high tissue penetration, since MB has a lower brightness and emits a fluorescence signal near the lower edge of the NIRF range [19].

Consequently, several new fluorophores have been designed for ureteral imaging [10, 19, 21] including the IRDye® 800BK dye (nerindocyanine sodium) (LI-COR Inc., USA). The IRDye® 800BK dye has a molecular weight of 1113 g/mol, an average mass of 1025.213 Da and consists of the chemical components C<sub>44</sub>H<sub>52</sub>N<sub>2</sub>O<sub>16</sub>S<sub>5</sub> which enable a partial renal and hepatic clearance. It has a maximum absorption at 774 nm and a maximum emission at 790 nm. Due to its hydrophilicity, it is primarily cleared by the kidneys, which allows non-invasive intraoperative ureteral imaging [22]. Due to its partial hepatic clearance, this dye was also successfully studied for bile duct visualization in an earlier pre-clinical study [23]. However, the possibility that the IRDye® 800BK dye could be useful in other applications including perfusion evaluation and fluorescence-based lymphography has not been evaluated yet.

During laparoscopic surgery, it is essential for the surgeon to simultaneously clearly recognize various structures to improve the safety and efficiency of the procedure. However, to date, the majority of the studies on NIRF have been focusing on the visualization of a single structure or organ during laparoscopy.

In our current study, we evaluated the potential of the IRDye® 800BK dye for the simultaneous, intraoperative, multipurpose visualization of organs and tissues in combination with a commercially available NIRF imaging system.

## Materials and methods

### Animals

Six mature, female pigs (*Sus scrofa domestica*, ssp. Large White; mean weight  $44.7 \pm 7.97$  kg) were included and were managed according to French laws for animal use and care and according to the directives of the European Community Council (2010/63/EU) and ARRIVE guidelines [24]. The present study is part of the Endoscopic Luminescent

Imaging for Oncology Surgery (ELIOS) project and was approved by the local ethical committee on animal experimentation (ICOMETH No. 38.2016.01.085), and by the French Ministry of Superior Education and Research (MESR) (APAFIS#8721-2017013010316298-v2). The pigs were housed in individual stable boxes and had access to food and water.

Animals received an intramuscular (IM) injection of Zolazepam + Tiletamine 10 mg/kg (Zoletil ND, Virbac, France) as premedication. Anesthesia was induced by means of IV injection of Propofol 3 mg/kg (Propofol Lipuro ND, B Braun, France) + Rocuronium 0.8 mg/kg (Esmeron ND, MSD, France) allowing intubation and mechanical ventilation. Pigs were sedated during the experiment via inhalation of isoflurane 2–3% (Isoflurin ND, Axience France) + Oxygen. Buprenorphine (Buprecare ND, Axience, France) 0.01 mg/kg IM was used as a painkiller. At the end of the experiments, pigs were sacrificed under deep anesthesia (Isofluran 5%) with a lethal IV injection of Pentobarbital 40 mg/kg (Exagon ND, Axience, France).

### Dye preparation

The IRDye® 800BK dye was prepared and used following the manufacturer's instructions. The powder was diluted in a sterile phosphate-buffered saline (PBS) solution to a concentration of 1 mg/mL. Based on earlier experiences [21, 23], which have shown a successful ureteral and bile duct delineation in a pig model, we chose an IV concentration of 0.15 mg/kg body weight. For the gastric submucosal dye injection, we chose 0.6 mg (1 mg/mL, 0.6 mL) as the empirical total amount of dye representing a preliminary dose.

### Surgical procedure

A pneumoperitoneum was created with a Veress needle and a 12 mmHg pressure of carbon dioxide gas. A 10 mm supraumbilical trocar was introduced into the abdominal cavity. The rest of the procedure was performed under laparoscopic vision with a commercially available NIR laparoscope (D-Light-P, KARL STORZ, Germany). Four additional 10 mm and 5 mm working trocars were inserted. A jejunal loop with a length of approximately 10 cm was identified and loosely suspended using 3 transperitoneal sutures with polypropylene (Prolene, Ethicon™, Johnson & Johnson Health Care Systems Inc., USA) 3/0 threads, followed by the ligation of 3 to 4 peripheral mesenteric arteries and veins of this loop with a bipolar vessel-sealing device (LigaSure™ Maryland, Covidien, USA), as can be seen in Fig. 1. This model is a simulation of an ischemic loop as described in an earlier study [25]. In this ischemic intestinal loop, 5 regions of interest (ROIs) were identified and marked with a surgical pen. The locations of these regions of interest

were as follows: (1) left lateral border, (2) 2.5 cm from the left lateral border, (3) middle of the loop, (4) 2.5 cm from the right lateral border, and (5) right lateral border (Fig. 1). After 30 min of ischemia, the laparoscope was shifted to a NIRF mode and 0.15 mg/kg of IRDye® 800BK was injected intravenously. Ventilation was suppressed for 60 s to stop the breathing motion during video capture. Directly after NIRF imaging, local capillary lactates were measured on blood samples obtained by puncturing the serosa at each of the 5 ROIs. Blood was aspirated with a 2 mL serological pipet (Falcon, Beckton Dickinson Labware, USA) attached to an electric aspirator (PipetBoy™, Integra biosciences, USA) and instantly measured using a handheld portable lactate analyzer (EDGE®, ApexBio, Taipei, Taiwan). Lactate is the end product of the glycolysis and its accumulation reflects a lowered mitochondrial activity in the presence of reduced O<sub>2</sub> tension. This method has been described earlier in a study on “metabolism-guided bowel resection” [26].

Since the fluorescent intensity depends on the distance between light source and target, a standard reference calibration aid, yielding a constant signal when illuminated by the near-infrared light [25], was used. This so-called reference card (Green balance ICG reference card, Diagnostic Green GmbH, Germany) was introduced through a 10 mm trocar and was constantly held in the laparoscopic view to help for the future analysis of the video recordings of the procedure.

After the NIRF analysis of bowel perfusion and blood sampling for lactate analysis, alternating NIRF imaging of the left ureter and bile duct was performed at fixed time points for a total duration of 120 to 180 min. For these structures, no dissection was performed, and the peritoneum was kept intact.

Additionally, in the last 3 included pigs, the procedure was initiated with the gastroscopic injection of a 0.6 mg (1 mg/mL) dye in the gastric submucosa under laparoscopic guidance, followed by a laparoscopic assessment of lymph nodes and lymph ducts in a NIRF setting for a total duration of 10 min.

### Perfusion analysis software

The amplitude of fluorescence intensity variation is proportional to the amount of fluorescent dye diffused in the tissue and is consequently a marker of tissue perfusion [27]. The majority of studies on NIRF evaluation of bowel perfusion were performed in a static manner, taking only the relative FI into account without considering the diffusion of the fluorescent dye over time. To overcome these issues, we evaluated the IRDye® 800BK dye with the dedicated image analyzer software (ER-PERFUSION, IRCAD, France) to obtain fluorescence-enhanced reality (FLER). This method has been described in detail by our group in a previous study [4]. The software computes the time to peak (TTP) for each pixel, the

time for the fluorescence signal to reach its maximum. To prevent the risk of bias, the software does not use the minimum and the maximum of the fluorescence signal but the first and last quartile. Using the first and last quartile ensures that fluorescence signals are analyzed during a period during which the increase in the fluorescence signal is constant.

### Statistical analysis

Statistics were performed using GraphPad 8.3 (GraphPad Software®, USA). A Spearman’s rho was calculated to correlate local lactates with TTP parameters measured with the FLER software. A  $p < 0.05$  was considered statistically significant. Normalized fluorescence (NF) is calculated by the FI of the target divided by the FI of the reference card in the same screenshot to minimize the bias of the differences in distance between the light source and the target organ. By using the reference card as a calibration tool, it was possible to compare interoperative and intraoperative findings in all pigs.

### Cell culture and microscopy details

To evaluate the cellular uptake of IRDye® 800BK and hence to determine the potential complete clearance of the dye from the body, fluorescence microscopy analysis was performed on a cell culture. HeLa cells (Human cervical cancer cell lines) (ATCC® CCL-2) were grown in Dulbecco’s modified Eagle medium (DMEM, Gibco-Invitrogen, USA), supplemented with a 10% fetal bovine serum (FBS, Lonza, Switzerland) and a 1% antibiotic solution (penicillin–streptomycin, Gibco-Invitrogen) at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cells were seeded onto a chambered coverglass (IBiDi®, Germany) at a density of  $1 \times 10^5$  cells/well 24 h before microscopy measurement. For imaging, the medium was removed, and the remaining attached cells were washed with 1X PBS and added with a reduced serum medium (Opti-MEM, Gibco-Invitrogen) containing the IRDye® 800BK dye (1 μM) and incubated for 2 h at 37 °C. Fluorescence images were captured before and after washing the cells with 1X PBS. Fluorescence imaging was performed in an epifluorescence mode using the Nikon Ti-E inverted microscope with a 60× objective. The sample was excited with a 730 nm LED source and the emission was collected in the NIR channel with an 810/90 band-pass filter. The *ImageJ* software was used to process fluorescence images.

## Results

In Table 1, the weight, total amount of dye, lactate values of the regions of interest in the ischemic bowel loop, and the length of the surgical observation are summarized

as well as the organs which were successfully visualized with NIRF imaging. The intraoperative visual results of this study are shown in the supplementary video (Supplemental Digital Content 1).

### Bowel perfusion model

In all included pigs, a clear macroscopic NIRF visualization of the perfusion was performed (Fig. 1), in which regions 1 and 5 had a faster development of brightness as compared to regions 2, 3, and 4. This is also objectively proven with the FLER analysis of the TTP in which ROI 3 had the highest TTP with a significant difference with ROI 1 and ROI 5 (Fig. 2a). ROI 3 also had a significant higher level of lactate than ROI 1, 4, and 5 (Fig. 2b). A significant positive correlation was found between the time to peak FI in the ischemic bowel loop and the corresponding capillary lactate levels (Spearman's rho 0.59,  $p < 0.001$ ) (Fig. 2c).

### Ureteral imaging

The first ureteral evaluation under NIRF imaging was performed between 10 and 20 min after dye administration. At this first evaluation, the ureter could be clearly distinguished from its surroundings under NIRF imaging (Figs. 3, 4a). All ureters remained clearly visible until the end of the procedure (max. 180 min) with no significant change in the fluorescence signal over time between the time points and between the analyzed porcine ureters (Kolmogorov–Smirnov  $p > 0.11$  for each measured time point). Maximum fluorescence intensities were seen during the peristaltic contractions of the ureter (Fig. 3). In one pig, the left ureter was transected on purpose at the end of the procedure. Under NIRF imaging, this ureteral damage and the leakage of urine could be clearly visualized as shown in the supplementary video.

### Bile duct imaging

The first bile duct evaluation under NIRF imaging was performed between 10 and 20 min after dye administrations.

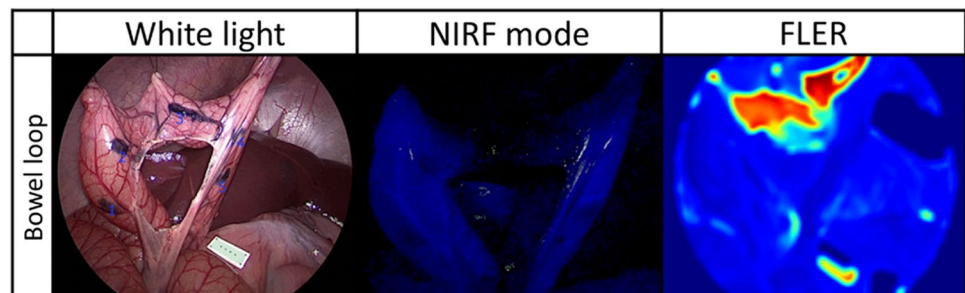
**Table 1** Summary of experimental results

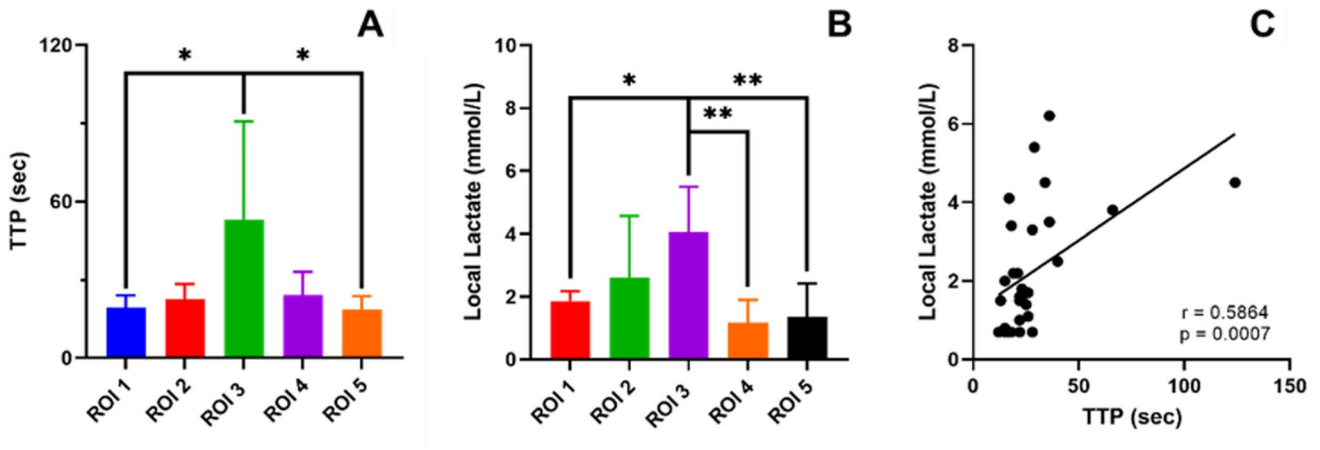
	Pig 1	Pig 2	Pig 3	Pig 4 <sup>b</sup>	Pig 5	Pig 6
Weight (kg)	44	33	58	45	43	45
Dye in mg	6.6	4.95	8.7	6.75	6.45	6.75
Systemic lactate <sup>a</sup>	0.7	1.5	0.8	4.4	0.7	1.3
Lactate region of interest 1	2.2	1.6	1.5	2.2	2	1.7
Lactate region of interest 2	0.7	3.3	4.1	5.4	0.8	1.4
Lactate region of interest 3	6.2	3.5	4.5	4.5	1.8	3.8
Lactate region of interest 4	1	1.5	0.7	2.5	0.7	0.7
Lactate region of interest 5	0.7	1.6	0.7	3.4	0.7	1.1
Structures visualized	Perfusion Bile duct Ureter	Perfusion Bile duct Ureter	Perfusion Bile duct Ureter	Perfusion Bile duct Ureter Lymph nodes	Perfusion Bile duct Ureter Lymph nodes	Perfusion Bile duct Ureter Lymph nodes
Length of observation (minutes)	120	180	180	180	180	120

<sup>a</sup>Lactate values are in mmol/L

<sup>b</sup>In this pig, a bronchospasm occurred before intubation leading to low saturation levels and tachycardia for several minutes. This may have influenced lactate values

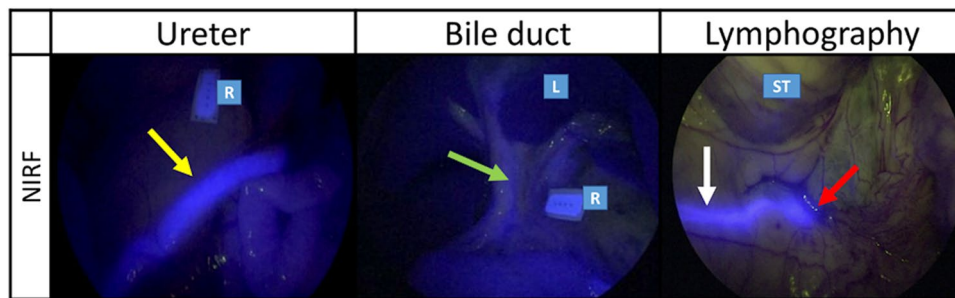
**Fig. 1** Near-infrared fluorescence visualization of the ischemic bowel loop. An ischemic bowel loop set up in white light, near-infrared fluorescence (NIRF), and fluorescence-enhanced reality (FLER) mode, respectively. 1–5: marked regions of interest (ROIs)





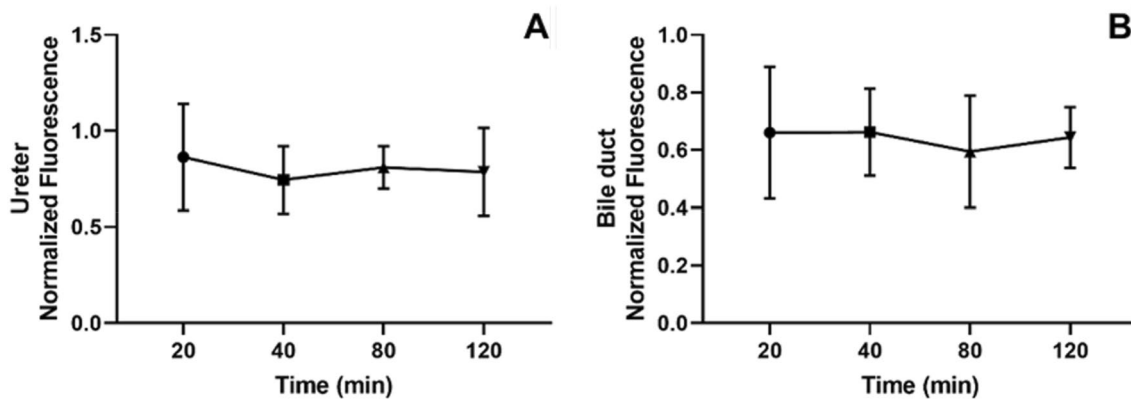
**Fig. 2** Results of bowel perfusion analysis. **A** Time to peak fluorescence in seconds (TTP) for each region of interest (ROI) studied, showing a significant difference between ROI 3 and ROI 1 and ROI 5. **B** The local lactate level was significantly different between ROI

3 and ROI 1, 4, and 5. **C** A significant positive correlation was found between the time to peak FI in the ischemic bowel loop and corresponding capillary lactate levels (Spearman's rho 0.59,  $p < 0.001$ )



**Fig. 3** Multipurpose fluorescence visualization in various applications. Left ureter after 20 min (yellow arrow), bile duct after 30 min (green arrow), gastric lymph duct (white arrow) and lymph node (red

arrow) after 5 min of dye injection, respectively, seen under NIRF imaging. *L* Liver, *R* ICG reference card, *ST* stomach (Color figure online)



**Fig. 4** Results of ureteral and bile duct NIRF imaging. **A** Normalized fluorescence of the ureter at different time points after dye administration. **B** Normalized fluorescence of the ureter at different time points

after dye administration. Normalized fluorescence is the product of the fluorescence intensity of the target divided by the fluorescence intensity of the reference card

At this first evaluation, the bile duct could be distinguished from its surroundings under NIRF imaging (Figs. 3, 4b). Subjectively, based on visual findings, the bile duct became less distinguishable from its surroundings over time. This finding is supported by a statistically reduced NF of the bile duct after 40 min of dye administration onwards. A Mann–Whitney test to assess whether the NF of the bile duct is greater than the NF of the background, showed a statistically significant difference for time points up to 40 min ( $p < 0.0473$ ).

### Gastric lymph node imaging

In the 3 randomly selected pigs in which a gastroscopy-assisted submucosal dye injection was performed, a clear delineation of one or more lymph ducts appeared, and within 5 min, the corresponding lymph node could be clearly identified under NIRF imaging as seen in Fig. 3.

### Cell culture

HeLa cells were incubated with the IRDye® 800BK dye (1  $\mu\text{M}$ ) in optiMEM, for 2 h at 37 °C after which fluorescence images were obtained. Fluorescence images showed no cellular uptake and only background fluorescence was observed. Analysis of fluorescence images confirmed that there was no cellular uptake of the dye (Fig. 5). The negatively charged sulfonate groups, present on IRDye® 800BK, prevent the dye molecule from crossing the cell plasma membrane, which results in poor cellular uptake.

### Complications

No intraoperative dye-related complications occurred. However, pig No. 4 developed a bronchospasm after induction

but before intubation, which resulted in reduced levels of blood oxygen saturation for several minutes after which a successful intubation was achieved. This may have influenced the higher levels of systemic lactate found in this pig.

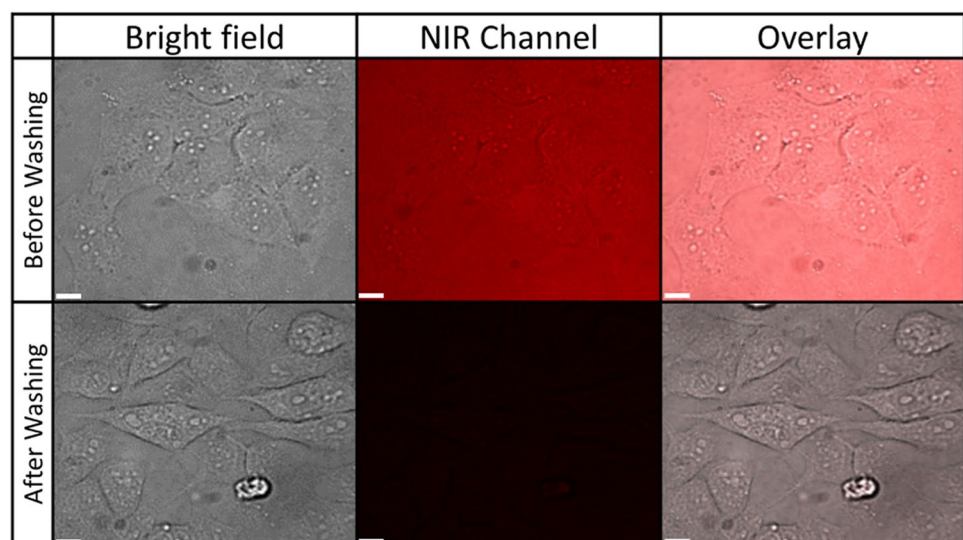
## Discussion

In this pre-clinical study, we have successfully demonstrated the simultaneous, multipurpose NIRF imaging of several intra-abdominal organs using the IRDye® 800BK dye and a commercially available fluorescence imaging system during laparoscopic surgery.

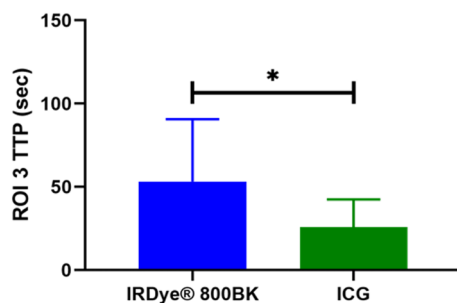
One of the advantages, as compared to ICG, is that this dye has both renal and hepatic clearance, which allows its use for ureteral and biliary imaging as demonstrated in this study and in earlier studies. The majority of ureteral injuries are identified postoperatively [28]. In this study, the ureters were clearly identified under NIRF imaging in all animals. Additionally, by deliberately transecting the ureter, it was possible to easily recognize and locate the ureteral injury under NIRF imaging intraoperatively. This finding potentially allows the surgeon to prevent any ureteral injury, and if an injury occurs, to repair the injury during the same procedure and overcome the sequelae [29] of missed ureteral lesions. In a study by Barnes et al., it was demonstrated that the IRDye® 800BK dye was also a promising alternative to ICG in visualizing the urethra using NIRF imaging. It was shown that this dye has a greater depth of penetration and may allow for an earlier detection of the urethra intraoperatively, thereby preventing wrong plane surgery during low rectal resections [30].

Importantly, as the clinically most relevant finding of this study, we have demonstrated that this dye allows to clearly visualize intestinal perfusion in combination with ureteral

**Fig. 5** Fluorescence microscopy images of HeLa cells stained with IRDye® 800BK. HeLa cells were incubated with IRDye® 800BK (1  $\mu\text{M}$ ) in optiMEM, incubated for 2 h at 37 °C. The rows represent the images before and after washing respectively. Samples were excited with a 730 nm LED source and the emission was collected in NIR channel with an 810/90 band-pass filter. No cellular uptake was observed even after 2 h of incubation with the dye. Scale bar 10  $\mu\text{m}$



delineation. This finding is particularly valuable for colorectal surgeons as it is pivotal for the surgeon to identify the course of the ureter to prevent any iatrogenic ureteral injuries and to assess bowel vascularization prior to anastomosis creation to minimize the risk of anastomotic leakage. With the approach presented, there is no need to insert a ureteral stent to locate the ureter, nor is there a need for 2 different fluorescence dyes to visualize bowel perfusion and the ureter simultaneously. Additionally, a significant positive correlation was found between the time to peak FI in the ischemic bowel loop and corresponding capillary lactate levels ( $\rho$  0.59,  $p < 0.001$ ). This highlights the importance of an intraoperative quantitative analysis of the NIRF imaging of bowel perfusion using dedicated analysis software such as FLER, which may help to reduce the incidence of anastomotic leaks [26]. We have compared the NIRF findings of this bowel loop to the findings of historical data in which a similar approach was used to create an ischemic bowel loop and visualize the perfusion with a similar NIRF imaging system, after intravenous ICG injection in pigs two hours after the creation of the ischemic bowel loop [4]. An interesting finding is that there was a significantly (Mann–Whitney,  $p < 0.039$ ) increased absolute value of the TTP for the IRDye® 800BK dye in the ischemic zone (ROI 3) as compared to ICG, despite a longer ischemia time (Fig. 6). This suggests that there is a decreased diffusion of the IRDye® 800BK dye in less perfused zones, possibly due to the higher molecular weight of this dye (1113 g/mol vs. 775 g/mol for ICG), which may improve intraoperative decision-making when assessing the viability of the bowel under NIRF imaging. Since several ICG agents usually contain Sodium Iodide, it is thought that this is the general cause of noted allergic reactions and not the molecular weight per se. IRDye® 800BK is not associated with Sodium Iodides and was reconstituted in sterile phosphate-buffered saline for this study. Our group aims to design a comparative study



**Fig. 6** Comparison of absolute time to peak values at the ischemic region of interest between IRDye® 800BK and ICG. Time to peak fluorescence in seconds (TTP) for ROI 3 (ischemic region of interest), showing a significant (Mann–Whitney,  $p < 0.039$ ) higher TTP for IRDye® 800BK compared to ICG

between the IRDye® 800BK dye and ICG for bowel perfusion in an acute pig model.

In this study, we have also shown the clear visualization of lymph ducts and corresponding lymph nodes in the draining delta within several minutes after local dye injection in the gastric submucosa. This can potentially be used to road-map lymphatic drainage, which can be a determinant for the oncological status of lymph nodes and may aid in decision-making regarding the extent of operative dissection and resection [31] in oncological surgery.

Finally, we successfully visualized the bile ducts in all animals. However, this was time-dependent as NF decreased over time and the bile duct became less distinguishable from its surroundings from 40 min onwards after dye injection. In this study, it was not possible to study the bile ducts within 10 to 20 min after dye injection. In future studies, it would be of interest to continuously evaluate the behavior of this dye in bile duct imaging directly after administration to find the optimal moment of dye administration in respect to peak fluorescence and to evaluate its potential use for hilar vascular anatomy assessment.

In a study by Ashitate et al. [32], the simultaneous visualization of the bile ducts and the hepatic artery were explored successfully. However, this was performed with a combination of MB, ICG, and ZW800-1 with a dual-channel NIRF system. In this study, the use of two independent wavelengths of invisible NIRF light were explored to provide simultaneous contrast of extrahepatic bile ducts and hepatic arteries. A similar approach was described by Wada et al. [33] where the combination of T700-F with ZW800-1 for NIRF imaging of vessels and kidney, ZW800-3C for lymph nodes, and ESNF31 for adrenal glands, were studied respectively.

The IRDye® 800BK dye is currently under evaluation in clinical trials for use in the human setting. Such studies will also provide data on the side effects and adverse reactions to the injection of this dye. One of these studies is a clinical trial performed as a dose escalation study in gynecologic surgery (NCT03106038). The second focuses on the safety and efficacy of this dye in laparoscopic bowel resection and laparoscopic donor nephrectomy (NCT03387410). It is anticipated that FDA approval for clinical use will be achieved (personal communication with the manufacturer).

It is rare in surgery to simultaneously need the visualization of the bile duct, ureter and perigastric lymph nodes at any time as was shown feasible in this study. However, this study was meant to explore the potential width of utility of this dye. In future studies, clinically relevant combinations such as ureteral + bowel perfusion and gastric perfusion + draining lymph nodes could be studied separately.

In this study, which served as a proof-of-concept, we evaluated a fixed dose (0.15 mg of dye) per kg body weight. In future research, a dose escalation trial should be performed

to find the ideal dose for this dye for a combination of organs studied simultaneously.

Due to the operative regimen in which we started with perfusion analysis and moved to ureteral and bile duct imaging afterwards, we cannot draw conclusions on the speed of NIRF visualization of the ureter and bile duct after dye administration. Based on our earlier findings, it is likely that the onset of ureteral and bile duct visualization occurs within a few minutes (around 1 for ureters and around 15 for bile ducts) after dye administration [22, 23].

The novelty of this study lies in the fact that, to the best of our knowledge, there is no prior study presenting the successful identification of the ureter, bile duct, lymph nodes, and quantitative bowel perfusion in a single operative procedure and with a single commercially available NIRF system. This provides surgeons with a potentially powerful tool to enhance the visibility of several organs intraoperatively with a single dye and imaging system. Additionally, the described method was safe, may be easily reproduced, and may subsequently be integrated in the surgical workflow. Based on microscopic analysis, no cellular dye uptake was present, which suggests the complete clearance of the dye from the body over time. In a recently published study, our group successfully demonstrated the feasibility of this dye for ureteral imaging in robotic surgery using the Firefly technology in a pig model. This underscores the potential future application of this dye for both laparoscopic and robotic surgery procedures [34].

Despite the promising results of the current study, the findings must be interpreted with caution. It is known that the porcine peritoneum contains less fat as compared to humans. Consequently, NIRF imaging with this dye during laparoscopy in humans could be negatively influenced by the greater layer of fat. Moreover, although the current pre-clinical work does suggest a broad application, it is critical that the understanding of the intended use for any potential indication would require a thorough review to include dosing, timing of visualization of any or all targets of interest. This should be the focus of future studies exploring the potential of this novel dye.

## Conclusion

In this pre-clinical study, we successfully demonstrated the simultaneous multipurpose IRDye® 800BK dye applicability during laparoscopic surgery. This dye has the potential to become a powerful and versatile image-guided surgery tool.

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## Compliance with ethical standards

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