



# Ureter Identification Using Near-Infrared Fluorescence Imaging

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## Indications

Although surgical procedures are performed to improve a medical condition, the risk for iatrogenic injury is always part of the operation. During colorectal, urologic, and gynecologic surgery, ureteric injury is a feared complication. In order to prevent such iatrogenic damage, the surgeon must be aware of the exact location of the ureter.

Ureteric injury can result in pain, intra-abdominal sepsis, systemic infection, abscesses, urinoma, ureteral stricture, ureteric fistula, renal failure, and even loss of the ipsilateral renal unit [1–3]. The majority of ureteral injuries are often found only after other, more profound injuries are addressed, which may lead to worse outcomes.

Previous pelvic operations, infection, and inflammatory bowel disease are among the

known risk factors for iatrogenic ureteral injury during laparoscopy, but most ureteral injuries occur in patients lacking these risk factors [4]. In earlier studies, an incidence of 0.1–7.6% of iatrogenic ureteral injury has been reported during colorectal and gynecologic surgery in which more than 80% of cases were unrecognized intraoperatively [5–7]. Failure to identify the relevant anatomy is the main factor leading to ureteral damage [8]. In an earlier study, Assimos et al. [7] compared the incidence of iatrogenic ureteral injuries between the pre-laparoscopic and laparoscopic era and found that the incidence of iatrogenic ureteral injuries was significantly greater in the latter. Thus, the early detection and prevention of ureteral injury have become even more important. Now laparoscopic surgery is the standard of care in the majority of pelvic surgical procedures. Therefore, a technique or method that improves visualization of the ureter by the surgeon would be beneficial in preventing ureteral damage.

A method to detect the ureters pre-operatively is by the use of retrograde pyelography or urologic computed tomography [9]. However, intraoperative visualization would be more desirable since the anatomy changes during surgery. A method used in open surgery to assist in manually identifying the ureters is by placement of ureteral stents. However, laparoscopic pelvic surgery is increasingly performed and tactile

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feedback is strongly reduced when using laparoscopic instruments as compared to open surgery. In laparoscopic surgery ureteral stent placement is therefore less helpful. Moreover, stent placement as a procedure itself can cause complications to the ureter [10–12].

Intraoperative fluorescence ureteral identification with preoperative optical dye administration is a new technique for easier and earlier intraoperative visualization of the ureter and could therefore improve the safety and efficiency of laparoscopic colorectal surgery [13, 14]. In fluorescence imaging, a near infrared (NIR) fluorescent dye is administered intravenously and is excited by a specific light source and detected by the use of a laparoscope with near-infrared (NIR) imaging properties.

However, at present no dye for ureteral identification is commercially available for use in the human setting. Methylene blue (MB) is a registered dye with fluorescent characteristics that is excreted by the kidneys and would therefore be applicable for ureter imaging. Unfortunately, the results of clinical experiments so far give conflicting results. These may be due to the characteristics of the dye itself with only a weak fluorescent signal or the currently available laparoscopic equipment. The latter refers to a disadvantage of MB: this dye is excited at 600 nm, which is a different wavelength than most other dyes like indocyanine green in other indications which is excited at around 800 nm. The use of MB therefore demands specifically developed equipment. The equipment used in the experiments with MB was experimental and is not available commercially for laparoscopic use so far.

Three experimental dyes are now available which are cleared by the kidneys and therefore excreted through urine with excitation around 800 nm and therefore applicable with the usual equipment. The first of these dyes was IRDye® 800CW (LI-COR Biotechnology, Lincoln, United States). Because of its high cost, two other dyes have been developed: IRDye® 800NOS and IRDye® 800BK. Their price is in the range of that of ICG which can be considered affordable for daily practice.

The present chapter describes and illustrates the use of these dyes in an experimental setting in pigs.

Surgeries particularly at risk are obviously surgeries concerning the area near the ureters: pelvic surgery including rectal cancer surgery, low anterior resection, but possibly also gynecological surgery.

Therefore, in these surgeries NIR ureter imaging is most required.

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## Technical Description of the Procedure(s)

In the experiments described in this chapter, the dye to illuminate the ureter is administered intravenously. Another application is the use through ureteral stents that are introduced with cystoscopy. In the following, only applications using intravenously (IV) administered dyes are described and the more invasive ureteral stenting technique is not described further. In current literature, several IV administered dyes have been tested for ureter visualization.

In 2007, the first use of NIR imaging for ureter visualization was described by Tanaka et al. in 2007. In pigs, IRDye® 800CW (earlier referred to as CW-800-CA) was injected in several concentrations. An NIR imaging system prototype was used to detect the fluorescent signal. Illumination of the ureters in both rats and pigs was obtained at 10 minutes post injection until 60 minutes post injection [14].

In 2013, Schols et al. published a second article on the use of this dye for ureter visualization in pigs. A low dose of 0.25 mg IRDye® 800CW was injected in the first pig and 3 mg in the second. In the first pig, no ureter fluorescence was observed, but in the second pig both ureters could be clearly visualized from 10 minutes onward after IV administration of the dye [15].

Korb et al. showed comparable results after systemic administration of 30, 60, or 120 µg/kg injection of IRDye® 800CW in pigs. An optimal signal was reached by 30 minutes and with a dose of 60 µg/kg [16].

MB as a dye for ureteral imaging was described in several studies. In 2010, Matsui et al. published a study on female Yorkshire pigs that were included for open surgery and laparoscopic surgery. The FLARE™ image guided surgery system was used for the open surgery setting, and for the laparoscopic setting a prototype was used. Animals were divided into three subgroups: slow infusion over 5 minutes with 0.01 mg/kg MB diluted in 30 mL saline, pretreatment with 10 mg intravenous furosemide 1 hour prior to MB injection, and injection with a higher dose of 0.5 mg/kg MB. In all included animals the ureter could be identified from 10 up to 75 minutes after MB administration, with a higher fluorescence to background ratio in the third group. No added value of the use of furosemide was found. In the laparoscopic setting, a dose of 0.1 mg/kg did not provide sufficient fluorescence intensity, but after intravenous injection of 1 mg/kg MB the ureters could be readily identified from 10 to 30 minutes post injection [13].

The first described in-human use of MB for ureter visualization was in 2013 by Verbeek et al. In their article, the intravenous administration of several concentrations of MB followed by NIR imaging using the mini-FLARE™ system was assessed. In all included 12 patients, the ureter could be visualized 10 minutes after infusion with MB, with a higher fluorescence intensity achieved when administering a higher dose of MB [17].

A second article on the in-human use of MB showed less favorable results. The injected dose of MB in this study ranged between 0.125 and 1 mg/kg. The ureter was successfully detected in 5 out of the 10 patients, in whom the highest doses were administered (0.75–1 mg/kg), but the fluorescent signal was only picked up after the ureter was already visible in the conventional white light mode. Therefore, the technique was considered to be of no added value in these patients [18].

Most recently, Barnes et al. used MB for ureter visualization in 40 patients in a dose ranging from 0.25 to 1 mg/kg. Of the 69 ureters analyzed, 64 were seen under fluorescence of which 14 were seen earlier with fluorescence than white light. Fluorescence was deemed useful in 13

cases, whereas in 10 cases, the fluorescence revealed the ureter to be in a different location than expected. The highest signal to background ratio was found when using 0.75 mg/kg [19].

Friedman-Levi and colleagues used ICG loaded into liposomes, enabling IV administration and renal excretion. In the liposomal-ICG treated animals, the ureters could be visualized, up to 90 minutes after administration. However, background fluorescence was quite bright using this technique diminishing the discrimination of the ureter [20].

Verbeek et al. assessed the use of ZW800–1 conjugated to the cyclic RDG-peptide to both visualize the ureter and colorectal cancer in mice. ZW800 has an absorption peak at 772 nm and emission peak at 788 nm. The Flare system was used to detect the fluorescent signal. With these dyes both the experimentally induced tumors and the ureters could be visualized using cRDG-ZW800–1 [21].

De Valk et al. recently published an article describing the first in-human use of ZW800–1. After a low dose injection of this dye, the ureters were clearly visible up to 3 hours after injection, without observable toxicity [22].

Dip et al. described the use of IV sodium fluorescein in rats for ureteral imaging after laparotomy. A 530 nm excitation light was used to visualize the fluorescent signal. Using this light, the peristaltic movement of the ureters was seen in all rats [23].

A new dye, UreterGlow, with emission at 800 nm and excitation at 830 nm was tested by Mahalingam et al. in mice using a commercially available NIR system. Ureter identification with white light was achieved in none of the pigs, but within 15 minutes after injection of the fluorescent dye, both ureters could be clearly visualized for up to 2 hours without background fluorescence [24].

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## Regarding the Presented Video

Video 22.1 is derived from a study that has been reported before in a peer-reviewed journal [25]. This study was approved by the local animal ethics

committee, and the experiments were conducted at the central animal facilities of Maastricht University (Maastricht, the Netherlands). Animals were used in compliance with the regulations of the Dutch legislation concerning animal research. A pig model was chosen because of the similarities between pig anatomy and human anatomy and because of earlier successful application of NIR imaging in pigs [15]. The experiments were done in three female Dutch landrace pigs (each weighing 40 kg). After surgery, the pigs were sacrificed by the veterinarian-anesthesiologist.

### Laparoscopic Fluorescence Imaging System

A commercially available laparoscopic fluorescence imaging system (Karl Storz SE & Co. KG, Tuttlingen, Germany) was used. This system with a xenon based light source enables both excitation and detection of all three dyes used in this experiment: IRDye® 800CW ( $\lambda_{EX}/EM = 775/796$  nm), IRDye® 800BK ( $\lambda_{EX}/EM = 774/790$  nm), and IRDye® 800NOS ( $\lambda_{EX}/EM = 767/786$  nm). A foot pedal allows the surgeon to switch easily between the white and NIR light imaging modalities. The same NIR imaging settings were used for all three dyes tested. All procedures were digitally recorded with the built-in recording equipment.

### Characteristics of the Dyes

The characteristics of the dyes used (IRDye® 800CW, IRDye® 800BK, IRDye® 800NOS) have been previously described [26].

### Preparation of the Dyes

The dyes were prepared and used following instructions of the manufacturer. They were diluted in sterile phosphate buffered saline (PBS) solution to a concentration of 1 mg/mL. In this dilution, 6 mg of each dye was prepared for intravenous injection in one pig.

### Surgical Technique and Assessment

The surgical procedures were performed under general anesthesia. Premedication consisted of intramuscular injection of azaperone 3 mg/kg, ketamine 10 mg/kg, and atropine 0.05 mg/kg. Anesthesia was induced with intravenous thiopental 10–15 mg/kg. After intubation, the pigs were maintained under anesthesia with isoflurane and oxygen.

Surgical residents performed a laparoscopic partial excision of the bicornuate uterus, mimicking a laparoscopic appendectomy. These procedures were strictly supervised by two expert endoscopic gastrointestinal surgeons. One dye was tested per animal, using a 6 mg dose (1 mg/mL) which was intravenously administered.

Fluorescence imaging was initiated immediately after the intravenous administration of the dye. Further imaging was performed intermittently in fluorescence mode and white light mode. Intraoperatively, it was systematically documented whether the ureters could be identified in fluorescence mode by filling in a registration form. The attending surgeon was consulted to reach agreement on the identification of the ureters. A structure was defined as “identified” if its localization was confirmed with absolute certainty by the experienced surgeon.

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### Interpretation

All three dyes enabled clear and satisfactory visualization of the anatomical course of both ureters, in which the highest fluorescence intensity compared to the background was found in the pigs in which IRDye® 800BK and IRDye® 800CW were used.

The first identification of the ureters occurred within minutes after dye administration in all three pigs because of vascular imaging of the wall of the organ. At this time, also other structures are illuminated such as the uterus, bowel wall, and lymph nodes. No peristaltic movement of fluorescent dye through the ureter is yet visualized. This occurs in IRDye® 800BK, IRDye® 800CW, and IRDye® 800NOS 35, 10, and

45 minutes after dye administration, respectively, when the ureters become visible through excretion of the dyes in the urine. The ureters remained fluorescent during pulsatile movement of the ureter for 3.5 hours after administration.

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## Pitfalls

Despite the promising results, the findings of this study have to be interpreted with caution. Since each dye was only tested *in vivo* at one specific dosage and each dye was only tested in one pig, further experiments are needed to determine optimal dosing and timing of the dyes which are dependent on the pharmacokinetic properties of the dyes. The ureter wall thickness of pigs is slightly less than the thickness of the ureter wall in humans; therefore, the use of NIR in humans might give different results [27].

Before visible pulsatile flow occurs, NIR highlights the ureter and other well vascularized structures because of intravascular presence of the dye. In this phase, the ureter might be confused with other structures. When the dye appears in the urine, the ureter is easy to distinguish from the surrounding vascularized structures. These structures have lost most fluorescence at that time because of washout, while the remaining pulsatile movement of urine through the ureter is quite characteristic. The relationship with peristalsis has one slight drawback, as the signal is not present permanently and the surgeon must wait for the ureter to make these peristaltic movements. In addition, adequate renal function is required for sufficient excretion of the fluorescent dye into the urine.

No adverse reactions as a result of the administration of the dyes were observed.

A transient decrease in measured SpO<sub>2</sub> oxygen saturation is displayed. This is due to the measurement method, in which the color of the blood is used, which changes after administration of the dyes.

Of course, one should be aware that the present results were obtained in an experimental setting, studying pigs. Two clinical studies are being performed evaluating the use in the human set-

ting. One of these studies focuses on the safety and efficacy of IRDye® 800BK in laparoscopic bowel resection and laparoscopic donor nephrectomy (NCT03387410). The second clinical trial currently performed is a dose-escalation study in gynecological surgery (NCT03106038). The information and video presented in this chapter should be regarded as indicative of what the use in the clinical setting could be like, but the results of these trials will determine whether these expectations were justified.

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