UNIVERSITA' DEGLI STUDI DELL'INSUBRIA

DOTTORATO DI RICERCA IN BIOTECNOLOGIE,

BIOSCIENZE E TECNOLOGIE CHIRURGICHE

Curriculum Biotecnologie e Tecnologie Chirurgiche

XXX CICLO

Clinical Application of Fluorescence Guided Surgery

Sviluppo ed applicazioni dell'imaging a fluorescenza in chirurgia laparoscopica

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Dip. Biotecnologie e Scienze della Vita - Università degli Studi dell'Insubria

Anno Accademico 2016-2017

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The term fluorescence guided surgery (FGS) describes a medical technology based on real-time imaging intended to help and guide the surgeon during his operating practice.

In last years many innovations in surgical technique and minimally invasive technologies with laparoscopic, endoscopic and robotic techniques, has greatly improved surgical practice. Nevertheless, despite these constant advances, surgery still relies primarily on the surgeon's vision and on white-light reflectance.

The emerging field of fluorescent surgical imaging promises to be a powerful enhancement to improve surgical guidance.

Among all cromophores and fluorophores that could work as probes in medical imaging techniques, Near-infrared (NIR) fluorescence imaging with Indocyanine green (ICG) is emerging as major contributions to intraoperative surgical decisions and many different applications have already been described in literature.

ICG is a dye used in medicine since the mid-1950s for a variety of diagnostic applications in cardiology, ophthalmology and to test the hepatic clearance; however, its fluorescent properties have only recently been applied to new minimally invasive surgical instrumentations. ICG has some peculiar features that promote its widespread use: it is virtually harmless, due to lowest rate of adverse effects reported and to very high toxic dose for human body, and it is not expensive.

In addition FGS appears to have a great potential to become a standard in everyday clinical practice due to the multiple different possible applications and the ease of employ.

Our group started working on this technology since few years ago, cooperating with one of surgical imaging leading company on the market in developping new applications and tehcnical improvements.

As ICG, once injected, is excreted through the bile, it simply allows to outline the biliary tree anatomy by visualization under NIR light. This application find its ultimate use during laparoscopic cholecystectomy (LC).

LC is one of the most common procedure in general surgery worldwide (750.000 cases are performed every year in the United States); the most temible surgical complication of LC is biliary duct injuries (BDI), that could lead to severe consequences in terms of morbidity and mortality of patients.

Several studies have already reported that the the primary cause of BDI is misinterpretation of biliary anatomy (71%-97% of all cases), in some cases influenced by lack of tactile feedback and anatomic variations. FGS with ICG can be used effectively in LC to visualize the biliary system and avoid injuries; to date in literature several authors published case reports or small data series on this methodic, nevertheless larger randomized controlled studies are needed to confirm and validate the potential role of this technology as gold standard for LC.

Another extremely interesting field of employment of ICG fluorescent guided surgery is intraoperative angiography; ICG injected into the bloodstream and excited by NIR light can give information about study vascular anatomy and parenchimal perfusion in various clinical situations.

Our group, at the beginning of our experience with ICG FGS, tested several possible “angiographic” applications (clarify vascular anatomy for dissection in delicate procedures or solid organ perfusion for transplants) but we decided to

focus our studies on the assessment of bowel microperfusion before anastomosis creation.

Anastomotic leak (AL) is a frequent and serious complication in gastrointestinal resective surgery and adequate bowel perfusion has been stressed as one of the key elements for suture healing. Currently, there is no widespread method to assess and quantify the perfusion of gastrointestinal anastomoses intra-operatively, besides the subjective evaluation by the surgeon.

Real time intraoperative ICG fluorescent angiography (FA) is a feasible technique and a promising tool for everyday surgical practice. Nevertheless randomized controlled trials are needed to standardize technique and determine if ICG-FA might have positive impact on the AL rate in gastrointestinal anastomoses.

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1. State of the Art

1.1 Fluorescence Guided Surgery – State of the Art

1.1.1. Definition and Basic Principles

The term **fluorescence guided surgery (FGS)** describes a medical technology based on real-time imaging intended to help and guide the surgeon during his operating practice.

In clinical practice, especially in surgery, the development of a new technology needs to meet these critical criteria: to represent a true clinical requirement, the possibility to solve an open issue and the peculiar ability not to interfere with normal clinical workflow [1].

This is the reason why, after many decades of improvements in medicine, there are few imaging techniques routinely employed in clinical practice (such as x-rays, computed tomography, magnetic resonance imaging etc) and only two of these (x-ray fluoroscopy and ultrasound) are used occasionally for image-guided surgery.

In recent years, technological progress in body imaging and diagnostics has enhanced patient's selection for surgical interventions. At the same time surgery has advanced substantially in order to decrease patients morbidity and trauma through innovations in endoscopic, laparoscopic and robotic technologies; nevertheless surgical practice is still based on anatomy as seen by the operating surgeon with white-light reflectance. This approach does not allow an accurate differentiation between different tissue: in the body they appear as various shades of white to pink (bone, nerves, cartilage, fat, connective tissue and muscle) or red to deep-red (blood vessels and more

easily visible organs such as the liver, kidney and spleen), thus leading to potential ambiguity during surgical dissection. Furthermore, open-field surgery depends on the direct visualization of patient tissues, whereas in minimally invasive surgeries (that is microscopic, endoscopic, laparoscopic and robotic surgery), patient's tissues are visualized through an interface (viewed through the ocular eyepiece or using cameras and digital displays). For the minimally invasive surgical procedures, the added hardware that is necessary for fluorescence imaging can be fitted into existing instrumentation. The emerging field of fluorescent surgical imaging promises to be a powerful enhancement to traditional low-contrast white-light visualization, offering real-time highlighted delineation of complex anatomic structures. Improved visualization will lead to more complete removal of disease, decreased inadvertent injuries to vital structures, and improved identification for repair of damaged tissues [2].

In addition working through an interface with cameras and instruments inserted into body cavities through narrow conduits has also sacrificed tactile feedback of directly working with tissue and dissection along tissue planes. Thus a medical speciality that is traditionally dependent on touch becomes even more dependent on vision.

Basically enhancing the visual differences between tissues by using fluorescent probes based on structure or disease could be equated to colour-coding the surgical field.

This is why FGS could represent a major contribution to intraoperative decision making during surgical procedures [3].

Fluorescence guided surgery is based on the mechanism that a molecule

(flurophore or dye), once excited with a specific wavelenght light, becomes bright.

Once the light energy is absorbed by the fluorophore's organic molecules, a promotion of delocalized electrons from ground state to a higher energy level occurs. Upon return from excited singlet state to ground state, energy is emitted in the form of photons [Fig. 1].

The "fluorescent" light emission from the dye may be of the same wavelenght of the exciting lightsource, may be higher or lower, it can be detected using specific scopes and cameras and then transmitted to a standard monitor allowing identification of anatomical structures where the dye is present [4].

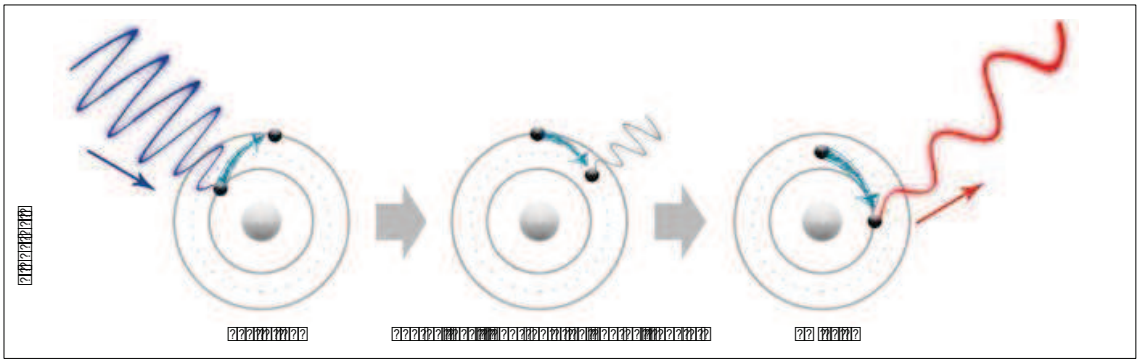


Fig. 1 - Mechanism of dye e□citation and fluorescence emission.

Nevertheless extrinsic contrast agents inevitably incur costs for the development and testing of efficacy versus potential side effect, and require regulatory approval, so they must greatly increase image quality and diagnostic accuracy to be justified [3].

Fluorescence imaging exhibits several favourable characteristics promoting its use in clinical practice which include [4-7]:

- an excellent safety record with clinically-used probes;
- the capability to be detected at low dye concentrations;
- a lack of ionizing-radiation exposure;
- real-time detection capabilities;
- lower costs and less complicated detection instrumentation compared with other intraoperative imaging modalities;
- high sensitivity of detection.

1.1.2. Cromophores in surgical practice

A cromophore can basically be defined as an “optically active” substance. Cromophore-containing molecules are widely used in conventional surgical practice because of their capability of enhance human vision; their application is demonstrated across several medical specialties, ranging from skin marking ink, sentinel lymph node biopsy (SLNB) to methylene blue for urinary tract lesions [8] [Table 1].

Many fluorescent compounds have already found a role into clinical practice, either as a free dye or a dye-functionalized targeting agent [9]. Based on their fluorescence emission spectra, clinically evaluated dyes can be separated into three groups.

Table 1 - Chromophores in current surgical practice.

Dye used	Uses in current clinical practice studies
Indigo Carmine	Indigo carmine: intravenous indigo carmine injection. Localize ureteral orifices, severed ureters and fistulous communications in radical retropubic prostatectomy and ureteral stenosis during robot-assisted ureteroureterostomy.
Indocyanine Green	Indocyanine green: localization of ureteral stenosis during robot-assisted ureteroureterostomy. Visualization of lens capsule in cataract surgery. Internal limiting membrane (ILM) of the retina green during pars plana vitrectomy. Intraoperative blood flow assessment of neurovascular system during angiography.
Methylene blue	Methylene blue: retrograde vaginal methylene blue testing for localization of urinary fistulae openings. Intravesical methylene blue identification of the diverticular neck during robot-assisted approach for bladder diverticulectomy. Methylene blue: stains the parathyroid glands for surgical visualization during parathyroidectomy.
Brilliant Blue Green	Brilliant blue green: macular hole surgery to stain the ILM.
Trypan blue	Trypan blue: measuring cell viability in-vitro. Visualization of lens capsule in cataract surgery. Macular hole surgery to stain the ILM. Visualization of lens capsule in cataract surgery.
Fluorescein	Fluorescein: prediction of breast flaps viability following reconstructive surgeries such as radical mastectomy or subcutaneous mastectomy.

The first group includes probes emitting fluorescence in the visible part of the light spectrum (400-650 nm) with fluorescein sodium being one of the most widely used visible dye in clinical care today. While its routine use lies in angiography, it has also been used successfully to identify ureters [10] and sentinel nodes [11-13].

The second group consists of dyes emitting in the far-red region of the light spectrum (650-750 nm). Here, an interesting development has been the clinical

introduction of the relatively bright cyanine dye named Cy5. Upon coupling to a c-Met receptor targeting peptide, this dye has proven its value for the visualization of polyps in the colon during colonoscopy [14].

The last group consists of near-infrared emitting cyanine dyes (750-1000 nm) such as indocyanine green (ICG). The clinical success of the use of ICG in angiography and lymphatic mapping applications [15] has stimulated the development of a wide range of near-infrared fluorescent tracers. Most of these new dyes (for example 800CW and ZW800) are based on Cy7 analogues that, in contrast to ICG, can also be coupled to a targeting moiety [16].

1.1.2.1. Visible versus ultraviolet and infrared applications

Surgeons increasingly demand for real-time sensory input to achieve a more accurate identification of tissues during their practice. Enhanced visual differentiation of anatomical structures becomes even more important, especially when considering new minimally invasive surgical technology where there is a loss of tactile feedback and everything the surgeon sees is “filtered” from the screen.

In a conventional operating setting, even if the use of some kind of cromophore in medical practice is already established, surgeons have always relied on which is visible to the naked eye (390–700 nm) [17]. Whilst this principle contributes the mainstay of conventional surgery, it suffers from several drawbacks including multiple light shadowing, dependence on the operating surgeon, and experience [18]. Furthermore, the unmet clinical need in surgery stems from the fact that visible light cannot penetrate into blood and tissue more than a few hundred microns, due to high photon attenuation from absorbance and

scatter. Thus, when a surgeon looks at the surgical field, he only sees surface features. The implications of this are significant since there are over 40 million surgical procedures performed in the United States (U.S.) each year, and even small improvements in outcome, or reductions in morbidity, will affect large patient populations. [1]. FGS can obtain a fundamental role in preservation of normal tissues such as lymphatics, ureters, bile ducts and blood vessels that are visualizable using untargeted tracers such as ICG [3].

The need to find new applications for chromophores in diagnostics and operative settings led to the development of specific dyes and probes that could be seen in target tissues beyond the visual spectrum, through the use of special light sources ranging from ultraviolet (UV) to near-infrared (NIR) wavelenghts.

UV light ranges in wavelength from 10 to 400 nm, but does not penetrate as deeply into human tissues as NIR. Fluorescein angiography (UV) was used for intraoperative assessment of microvascular perfusion, but its use was limited by long half-life (up to 18 h for clearance, preventing re-evaluation) and rapid leakage into the interstitium, enhanced by local ischemia, leading to false positive results. Fluorescein also suffers from a well-documented risk of anaphylaxis-type reactions [19].

In contrast, indocyanine green (ICG) is a cyanine class chromophore which is visible, but also fluorescent in the NIR range. IR frequencies penetrate deeper than UV, so their use in angiography facilitates imaging deeper patterns of circulation than fluorescein angiography. ICG has consequently experienced a wide range of novel applications, including measurement of cardiac output, hepatic function, and blood flow as well as ophthalmic angiography (Fig. 2).

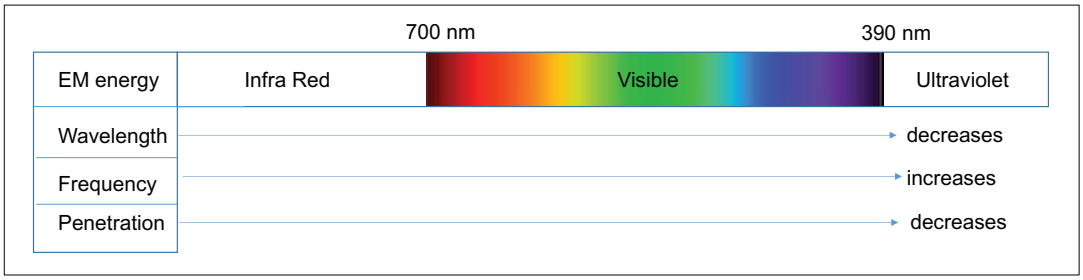


Fig. 2 - Electromagnetic spectrum and its relation to visible ultraviolet and infra red light avelength fre uency and tissue penetration. EM electromagnetic nm nanometer

Currently the only fluorescent probes that are used to assist intra-operative dissection are the untargeted dyes ICG and fluorescein sodium. Fluorescein sodium is approved for diagnostic angiography or angioscopy of the retina and iris vasculature. ICG is approved for determining cardiac output, hepatic function and liver blood flow and for ophthalmic angiography [1,4,8].

1.1.2.2. Fluorophores

There is a great variety of fluorophores; some of them are already available on the market, some others are still being tested and developped.

To be used *in vivo*, fluorophores should demonstrate fluorescence when stimulated by different wavelenght light source and should also have good solubility, photophysical characteristics (brightness, peak excitation and photostability). Owing to their hydrophobicity, fluorophores often demonstrate poor solubility and aggregation in solution, which can lead to changes in their excitation and emission ranges, as well as quenching of fluorescence [4, 20,21].

They can be distinguished based on the wavelenght they require to be excited and seen and can be classified in “targeted” and “untargeted” [3]. In current clinical practice non targeted fluorophores are the most common.

Usually the choice of a fluorophore must take into consideration the following specifics [1-3,22-24]:

- dose (quantity needed to achieve the desidered effect);
- time between administration and visualization;
- pharmacodynamics;
- way of administration (i.e. systemic versus local).

i. Clinically-Available NIR Fluorophores.

Due to multiple possible appications, many groups around the world are developing novel NIR fluorophores and targeted NIR fluorophores; nevertheless, the reality is that first-in-human testing of a new chemical entity (discussed below) has a minimum lag of ≈ 2 years and commercial availability of a new chemical entity has a minimum lag of 2 to 5 years. For the foreseeable future, there are only two NIR fluorophores approved for other indications by the FDA and thus clinically available for study: methylene blue (MB) and indocyanine green (ICG). Interestingly, both of these molecules have been used for the last decades as visible dyes and for this reason there is a large amount of clinical data regarding their safety when used at millimolar concentrations [1] [Table 2].

Table 2 - Non targeted dyes main features.

Name	Emission wavelength	FDA approv	Details
Non-targeted dyes			
ICG	820 nm	Yes	FDA and CE approved for injection, indicated for determining cardiac output, hepatic function and liver blood flow and for ophthalmic angiography. Off-label or research use to monitor fluid (for example, blood, lymph, cerebrospinal fluid or urine)-filled structures or as a vascular, renal or excretory pathway contrast agent
Fluorescein sodium	520 nm	Yes	FDA and CE approved for injection, indicated for diagnostic fluorescein angiography or angioscopy of the retina and iris vasculature. Off-label or research use to monitor fluid (for example, blood, lymph, cerebrospinal fluid or urine)-filled structures or as a vascular, renal or excretory pathway contrast agent
Methylene blue	680 nm	Yes	FDA approved for injection, indicated for drug-induced methaemoglobinaemia. Research use as an intraluminal gastrointestinal tract contrast agent
5-ALA	635 nm	Yes	FDA approved as a topical solution. 5-ALA is metabolized by certain tumours including glioblastoma into protoporphyrin IX, which is fluorescent.Mechanism of accumulation by tumors is unclear.

ii. Non targeted Fluorophores

Indocyanine Green (ICG)

Indocyanine green (ICG) is a tricarbocynanine dye that has been used clinically for over 50 years for hepatic clearance, cardiovascular function testing, and retinal angiography on the basis of its dark green color [15]. ICG dye was developed for near-infrared (NIR) photography by the Kodak Research Laboratories in 1955 and was approved for clinical use already in 1956 [25,26]. However, it took over ten years before ICG was used for angiography [27]. For retinal angiography it has been used from early 70s [28].

Structure and Stability. Indocyanine green is a tricarbocyanine dye having a molecular weight of 751.4 Daltons. It is a negatively charged ion that belongs to the large family of cyanine dyes [29]. Dry ICG is stable at room temperature. In aqueous solutions, ICG molecules tend to aggregate, which influences their optical properties [30]. The aggregation depends on concentration and time; The spectral stabilisation is fastest when ICG is dissolved in distilled water, and thus some authors do not recommend adding isotonic saline and/or albumin

to the injectate, when fast spectral stability is essential, for example, when using ICG for quantitative purposes [31].

Protein Binding and Fluorescence Life-Time. IGC is hydrophobic and, thus, is frequently bound to proteins in plasma (especially albumin), which confines ICG to the intravascular space and makes it especially suited for angiographic applications [20,21]. ICG-based angiography and lymphography have been used in a variety of clinical indications, such as perfusion-based imaging of the liver and blood vessels of the eye, and assessment of lymphatic vessel drainage [32,33]. The important property of fast binding to plasma proteins, especially lipoproteins, makes repeated intraoperational applications of ICG possible [34]. The binding to plasma proteins does not seem to alter protein structures, which is one sign of nontoxicity [35]. Binding to blood proteins also shifts slowly ,taking several minutes, the absorption peak at 780 nm towards longer wavelengths, to 805 nm [36]. The absorption peak maximum was observed at 810 nm in the epidermal cell cultures, and at 805– 810 nm in the human skin *in vivo* [37-38] [Fig. 3].

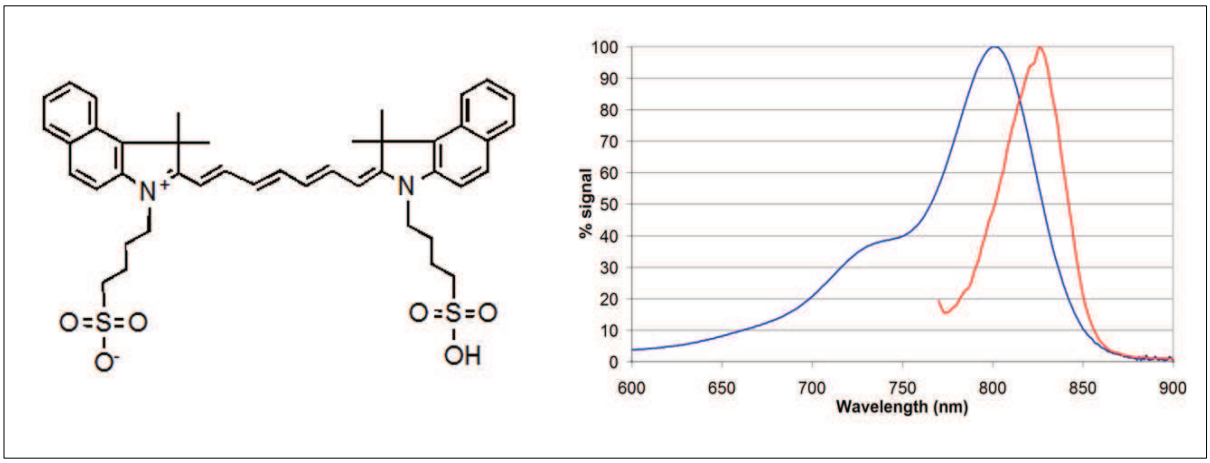


Fig. 3 - Chemical composition, excitation and fluorescence-emission spectra of Indocyanine Geen (ICG)

Physiology and Pharmacokinetics. ICG does not have any known metabolites, and it is quickly extracted by the liver into bile juice. The transport is done by a protein called glutathione S-transferase without modification [26]. The protein spectra of different liver diseases also affect ICG protein binding in blood [39,40]. It has a quick clearance rate of 18% to 24% per minute by the liver, which is the result of both the compound's confinement to the intravascular space and that the decomposition products of ICG are not metabolites. The dye is cleared from the system exponentially in the first 10 to 20 minutes after application, with a half-life of generally 3 to 4 minutes depending on the vascularization of the organ of interest [21,26,41].

The typical dye concentrations used for *in vivo* retinal angiography are in the range of 20–25 mg/mL of ICG applied by injection into a peripheral arm vein [36]. For studies of hepatic function an intravenous injection dose is calculated on the basis of 0.5mg/kg of body weight. In cardiac output and blood volume monitoring the total dose of dye injected should be kept below 2 mg/kg [15].

To□icity. Peculiar features are the low toxicity (LD_{50} after single IV dose of 50–80 mg/kg for animals). No significant toxic effects have been observed in humans with the high dose of 5 mg/kg of body weight [42]. ICG for injection contains sodium iodide and should be used with caution in patients who have an history of allergy to iodides because of the risk of anaphylaxis. ICG was not found to be mutagenic in the tests performed. No studies for reproduction, teratogenicity, or carcinogenic properties in animals are available but decades of experience in humans have not revealed any incidence of these properties [43].

To date ICG is the first and only clinically approved fluorophore that displays

NIR fluorescence [44]. Because ICG has no functional groups for conjugation to targeting moieties for molecular imaging application, it is a non-specific contrast agent. The commercially available instrumentation used for ICG detection is adjusted for the characteristics that ICG displays in plasma (peak excitation wavelength of 807 nm, and peak emission wavelength of 822 nm) [45,46].

Methylene blue (MB)

Methylene blue is an hydrophilic phenothiazine derivative that emits fluorescence at ≈ 680-700 nm wavelenght; it is a clinically approved dye with visible and NIR fluorescent properties. Its fluorescent performance it is inferior than ICG, due to a lower quantic energy (ratio between the number of electrons emitted and the number of incidents photons) and exhibiting an extinction coefficient and QY far below those of the indocyanines [1] [Fig 4-5].

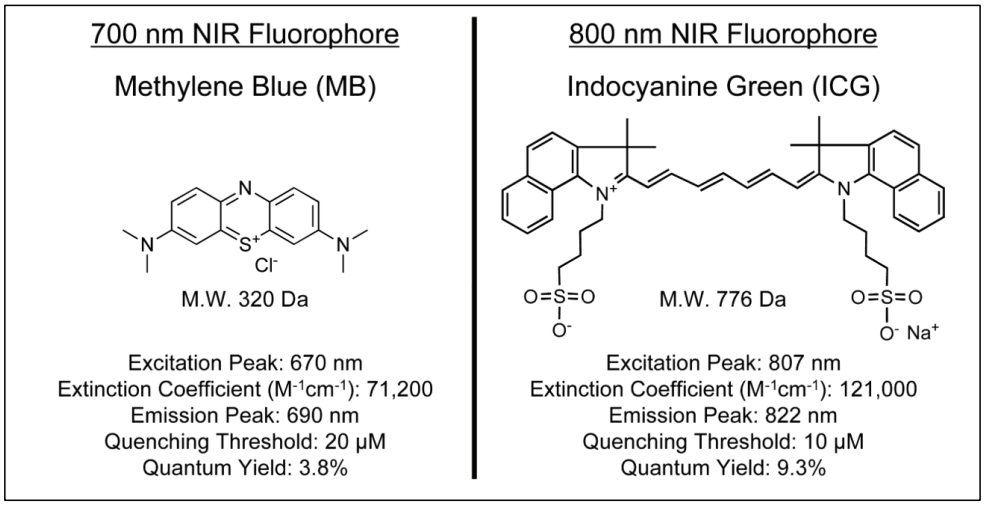


Fig. 4 - Chemical composition of Methylen Blue compared to ICG.

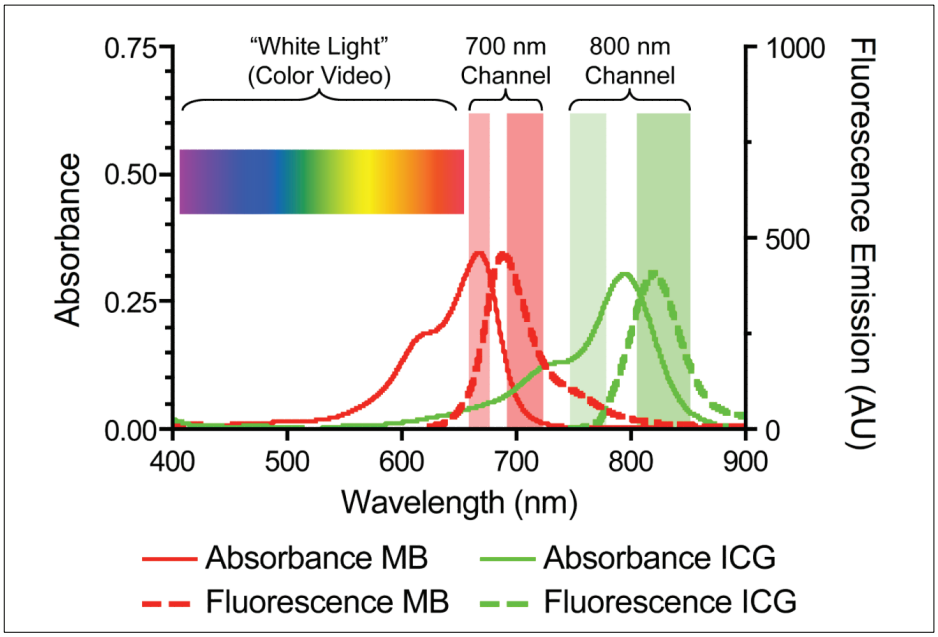


Fig. □ - Absorbance□Fluorescence □ avelenghts of Methylen Blue and ICG.

It is partially excreted through urines and, for this reason, it could be useful for ureters visualization in surgery [47]. Due to the unique biodistribution and fluorescence properties of MB, it has been demonstrated by several investigators its application in identifying vital structures, such as assessment of cardiac perfusion and detection of neuroendocrine tumors, fibrous pancreatic tumours, paragangliomas, and parathyroid adenomas [48-52]; however, the tumour targeting properties of methylene blue remain poorly understood and based on small studies and reports.

□□aminolevulinic acid □□□ALA□

5-ALA is an aminoacid that acts as a fluorophore processed by high metabolic active cells. It is used mainly for FGS for guided ablation of malignant glioblastomas based on the results of a phase III clinical trial [53].

It exhibits a fluorescence absorption peak of 405 nm, and an emission peak in the red visible light range (635 nm), permitting fluorescence imaging on the surface of tissues and in a depth-range of millimetres [54].

In addition 5-ALA has also been used in clinical settings for the photodynamic detection and photodynamic therapy of many superficial skin lesions, and its use is being investigated in several cancer types [55-57].

Fluorescein Sodium

Fluorescein is a manufactured organic compound that is FDA approved and widely used as a fluorescent tracer for many applications, especially in opthalmology and optometry (retinal angiography and angioscopy); it is the designated fluorophore to obtain images through confocal microscopy and its related applications, such as flow cytometry [58].

iii. Targeted Flurophores

Targeted fluorophores are designed in order to be able to link to specific sites (ligands); in this way optically active probes can specifically label intra- and extracellular biomarkers of cancer.

Targeted fluorophores actually under development aim mainly to identify cancer cells in order to early diagnose tumors and to verify that tumor dissection is complete.

Among the others, these are some of the targeted fluorescent agents that have been tested: Anti-CA 19.9, Anti-CEA, Anti-EGFR.

Although fluorophore-based contrast agents have been successfully used in small animal in vivo studies, clinical application is still under evaluation [59].

1.1.3. Image-Guided Surgery using Near-Infrared (NIR) Light and Indocyanine Green (ICG)

Fluorophores with excitation and emission spectra in the near-infrared (NIR) wavelength range (700–900 nm) have attracted the most attention owing to their improved depth-penetration range compared with fluorophores that emit electromagnetic radiation of shorter wavelengths. Within this NIR window, the absorption of most biomolecules (i.e., deoxyhemoglobin, oxyhemoglobin, water, and lipid) reaches minimal levels and scattering and autofluorescence are relatively low. Since NIR fluorescent light is essentially invisible to the human eye, special imaging systems are required to excite the NIR fluorophores within the surgical field and to collect emitted photons. Well-designed NIR fluorophores are needed to highlight the specific structures desired by the surgeon [1]. The commercial development of these agents for clinical application has synergized with concurrent improvements in detection instrumentation and software [4].

ICG becomes fluorescent once excited with NIR light or with a dedicated laser beam. The fluorescence can be detected using specific scopes and cameras and then transmitted to a standard monitor allowing identification of anatomical structures where the dye is present (i.e., biliary ducts, vessels, lymph nodes, etc.) [22, 60,61].

ICG is the only NIR fluorophore employed to date for human use; as described previously it had widespread uses in hepatic, cardiac, opthalmologic studies and its use is recently reported in analyzing tissue perfusion and identifying lymphnodes in cancer patients; it has several clinically excellent properties, which has been thoroughly verified during its long clinical use [15,41]:

- Patients safety (non toxic, non-ionizing);
- Ideal for angiography because it binds efficiently to blood lipoproteins and does not leak from circulation; ideal for bile ducts study because excreted selectively through the bile;
- Short lifetime in blood circulation allowing repeated applications;
- Good Signal to Background Ratio (SBR): there is not much INR autofluorescence in tissue where the exogenous dye is not present;
- Deep imaging with possibility to see beyond the surface, at a depth of several millimeters;
- Simple and relatively cheap imaging devices.

1.1.3.1. How does it work and currently available devices

Several companies have developed laparoscopic fluorescence imaging systems that are suitable for both near-infrared (NIR) fluorescence and white light (WL) imaging.

During surgical procedures the alternate exposure to from WL to NIR light (ICG mode) is used to identify anatomical structures, blood perfusion and other details.

The fluorescence imaging systems allow to obtain fluorescent images in real-time setting; they are quite unexpensive, if compared to some other technological equippments widely adopted in surgery.

The depth of penetration, that means how deep the surgeon can see into human tissues through these imaging devices, is approximately 1 cm with currently available technology [62].

Brielfy a FGS imaging system consists of a light source (xenon light, LED light or diode laser) that can emit both NIR light and WL, so it is able to excite a NIR fluorophore, like ICG.

The fluorescent signal emitted by the dye is then captured from a NIR transmitting telescope and a full HD 3 chip fluorescence imaging camera head. The different features that characterize each device (field of vision, zoom capability, type of light source, NIR wavelenght emitted and captured, etc.) will have an impact on system performance during surgery, in particular on SBR (signal background ratio); a good SBR would ideally be the lowest possible autofluorescence in tissue where the exogenous dye is not present [1] [Fig 6].

The system is usually equipped with a foot pedal or a dedicated button allowing the surgeon to easily switch from WL camera mode to ICG camera mode, and back.

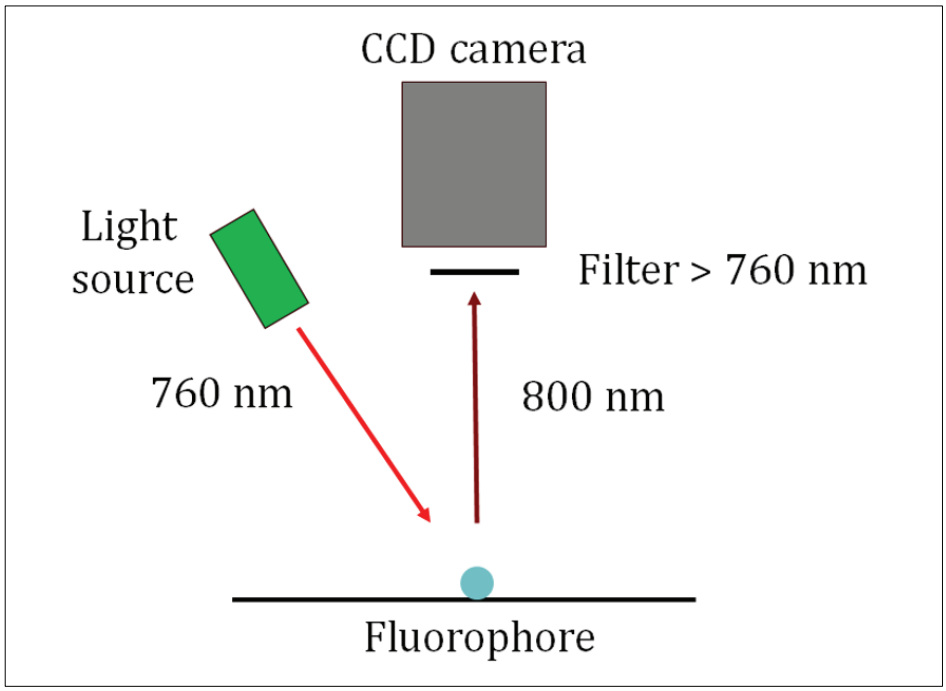
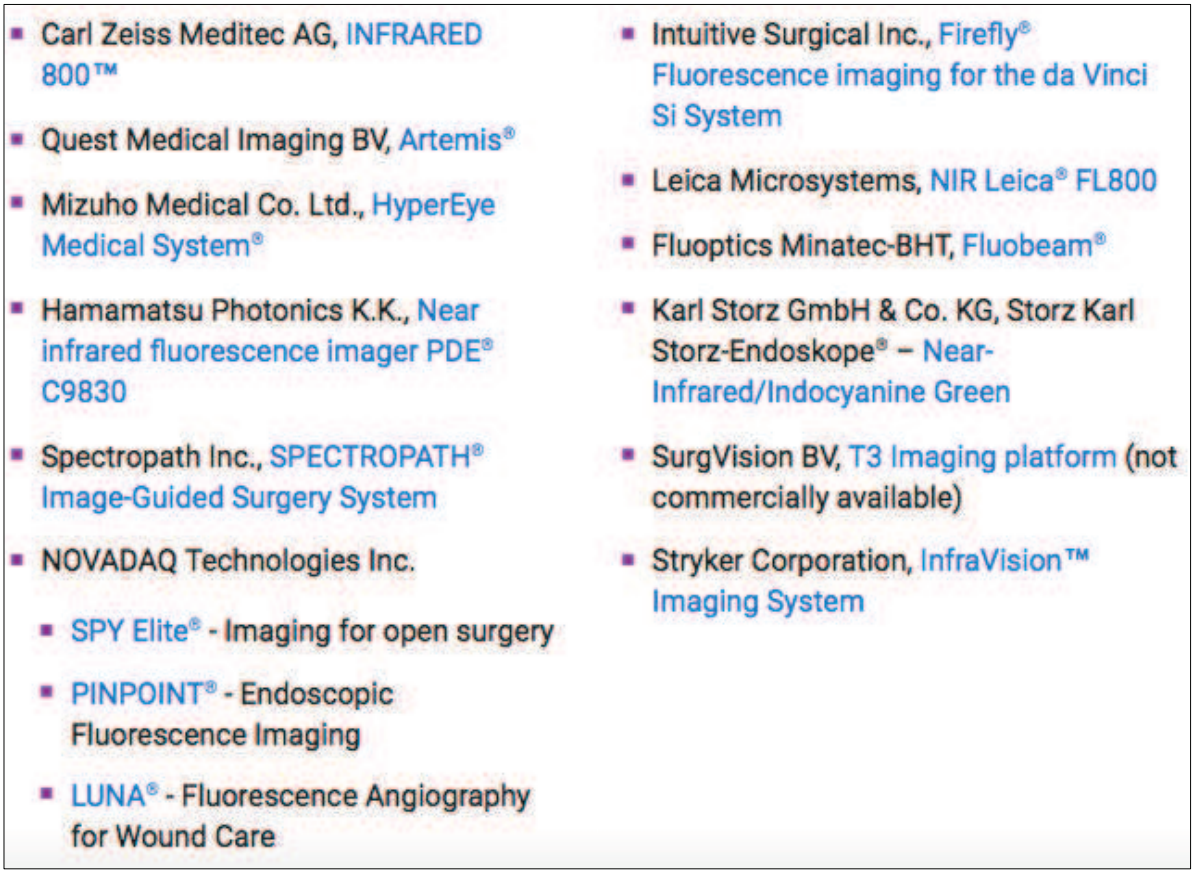


Fig. 6 - Operating principle of Fluorescence Guided Surgery (FGS)

Nowadays a growing number of companies are developping new systems for FGS (see above a list containing some of the most widely adopted devices both for conventional and minimally invasive/robotic surgery) [Table 3].

Table 3 - Clinical NIR Fluorescence Imaging Systems imaging systems that are appropriate for investigational use ith e citation and emission avelengths compatible ith ICG 800C



- Carl Zeiss Meditec AG, INFRARED 800™
- Quest Medical Imaging BV, Artemis®
- Mizuho Medical Co. Ltd., HyperEye Medical System®
- Hamamatsu Photonics K.K., Near infrared fluorescence imager PDE® C9830
- Spectropath Inc., SPECTROPATH® Image-Guided Surgery System
- NOVADAQ Technologies Inc.
  - SPY Elite® - Imaging for open surgery
  - PINPOINT® - Endoscopic Fluorescence Imaging
  - LUNA® - Fluorescence Angiography for Wound Care
- Intuitive Surgical Inc., Firefly® Fluorescence imaging for the da Vinci Si System
- Leica Microsystems, NIR Leica® FL800
- Fluoptics Minatec-BHT, Fluobeam®
- Karl Storz GmbH & Co. KG, Storz Karl Storz-Endoskope® – Near-Infrared/Indocyanine Green
- SurgVision BV, T3 Imaging platform (not commercially available)
- Stryker Corporation, InfraVision™ Imaging System

Each system differs from others on some key features:

- The “exciting” light source type (neon light, LED or laser beam)
- The system of signal detection
- The wavelenght emitted and captured
- The optimal distance to visualize fluorescence signal
- The strenght of SBR
- The possibility of directly overlay the NIR images to the WL ones

We started our experience in FGS few years ago with KARL STORZ GmbH CO. KG (Tuttlingen, Germany) with NIR fluorescence imaging system in its first prototype and since then we tested the equipment for preliminary studies and cooperated with the company in implementing and improving the technology itself [Fig. 7].

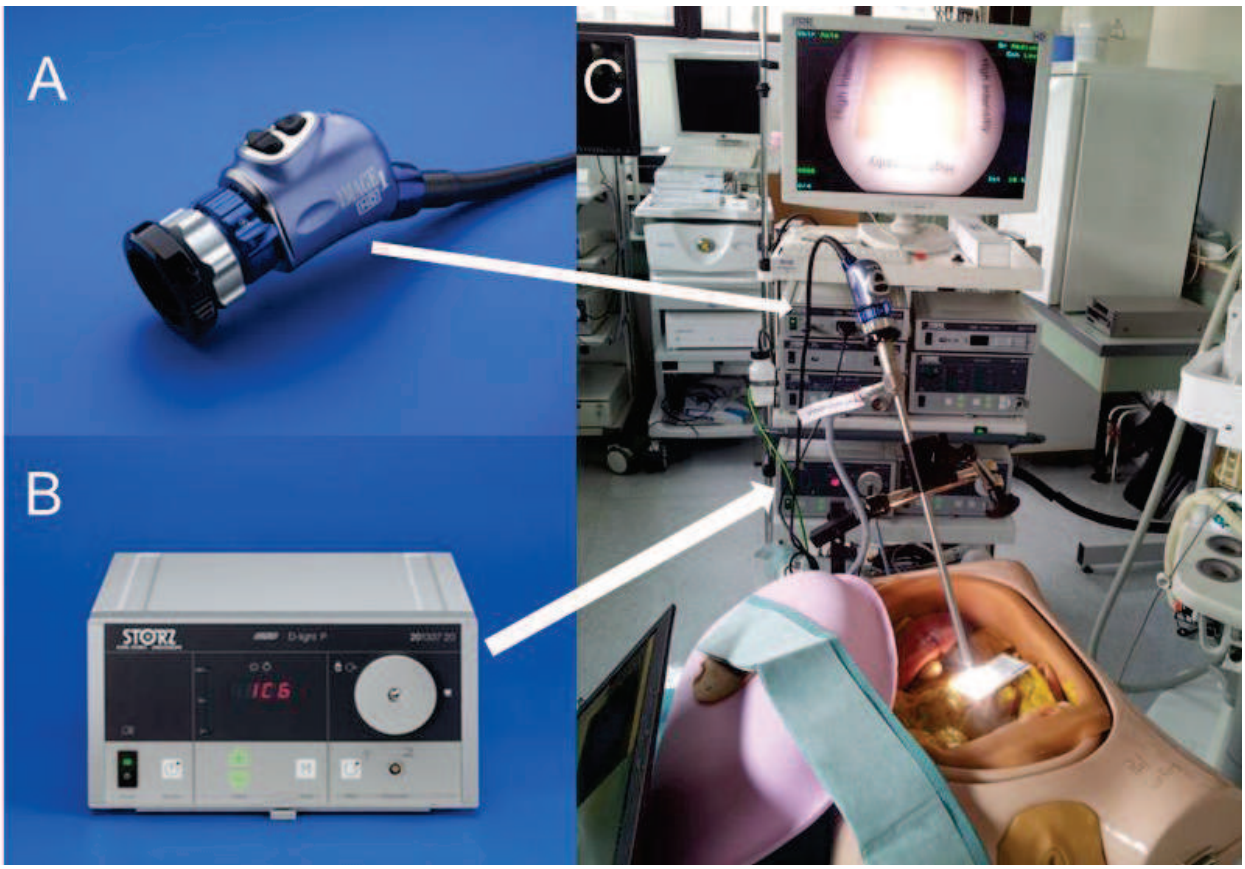


Fig. 7 - D-ight P System arl Stor Tuttlingen - Germany A Camera ead B NIR-emitting ight Source C Global vie of the system in lab setting.

1.2 Clinical Applications of ICG Fluorescence Guided Surgery in Laparoscopy

Recently ICG fluorescence guided surgery has increasingly been used as a tool for intraoperative diagnostics and has achieved the role of major contribution to intraoperative decision making during surgical procedures.

In conventional surgery ICG mediated fluorescence has been proposed for sentinel lymphnode biopsy in breast surgery and for melanoma using a specifically designated camera for “open” surgery [63,64].

Nevertheless this technique especially finds its ideal application in laparoscopic surgery, to improve the view and provide detailed anatomical information during the operation [65-66].

To date many different possible clinical applications have been tested and investigated, such as:

- Intraoperative cholangiogram for biliary ducts identification;
- Real time intraoperative angiography to check organ perfusion (for gastro-intestinal anastomosis creation, for skin flaps in plastic reconstructive surgery or to facilitate vascular dissection in unclear situations where anatomic variables can be expected, such as nephrectomies, liver resections or vascular surgery);
- Identification of intra-abdominal sentinel lymphnodes in metastatic melanoma, prostate or endometrial cancer;
- Lymphatic mapping in colorectal and gastric oncologic surgery;
- Liver metastasis identification and guided dissection;
- Ureter identification and preserving [65-70].

Some of them, such as the use of ICG mediated FGS for intra-operative cholangiogram in laparoscopic cholecystectomy and real time intra-operative angiography to check bowel perfusion, are quickly becoming popular and reported in literature; many case series and studies on small cohorts of patients have been published assessing that this technique works and has great potential. Nevertheless despite this rising excitement, clinical trials have barely begun, and much more translational effort will be required to harness this exciting technology. The complexity of fluorescent surgical molecular guidance necessitates a multidisciplinary approach as it progresses through development, testing, implementation, and widespread adoption. The future of fluorescence guided surgery depends on the parallel development of high-quality instrumentations and the achievement of large randomized controlled clinical studies.

1.2.1. Indocyanine Green Fluorescent Cholangiography (IFC) in Laparoscopic Cholecystectomy

1.2.1.1 Laparoscopic Cholecystectomy and Biliary Duct Lesion

i. Background

Laparoscopic cholecystectomy (LC) is one of the most commonly performed laparoscopic procedures in gastrointestinal surgery. Around 750,000 laparoscopic cholecystectomies are performed in the U.S. every year. This minimally invasive approach has demonstrated to have several advantages like

shorter hospital stay, less post-operative pain and prompt recovery, when compared to the open approach [71-74].

Since its first description, the main concern on laparoscopic cholecystectomy has been bile duct injuries (BDI), with an estimated incidence rate of between 0.4 and 1 % [75-78]. This is a rare but serious complication that could have a significant impact on quality of life and overall survival [79].

The high frequency of BDI with laparoscopic cholecystectomy was first considered to be a consequence of the initial learning curve of the surgeon, but it later became clear that the primary cause of BDI was misinterpretation of biliary anatomy (71%-97% of all cases) [80], in some cases influenced by lack of tactile feedback and anatomic variations [81,82]. Anatomic anomalies have been identified in 19% of patients [83,84] and hepatic accessory ducts in 8.4% [85,86].

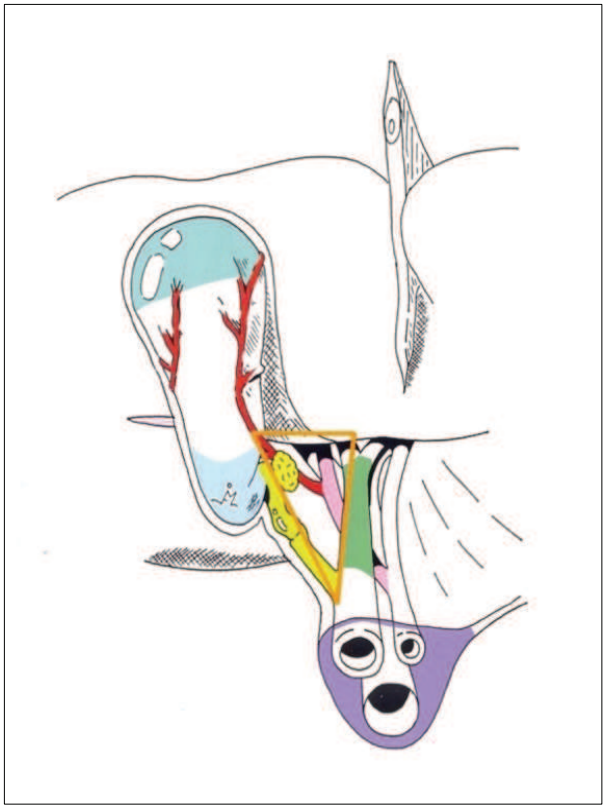


Fig. 8 - Calot's Triangle and Critical View of Safety.

Different approaches have been proposed to avoid a bile duct injury. Strassberg described the critical view of safety (CVS) approach, dissecting the Calot's triangle and identifying the cystic artery and extra-hepatic biliary ducts [87-90]. Even though the critical view approach of safety proved to be very useful and it is recommended by almost all surgical society and guidelines, it could not diminish the rate of bile duct injury [73] [Fig 8].

To establish CVS, two windows need to be created: one window between the cystic artery, cystic duct and gallbladder, another window between the cystic artery, gallbladder and liver [Fig. 9].

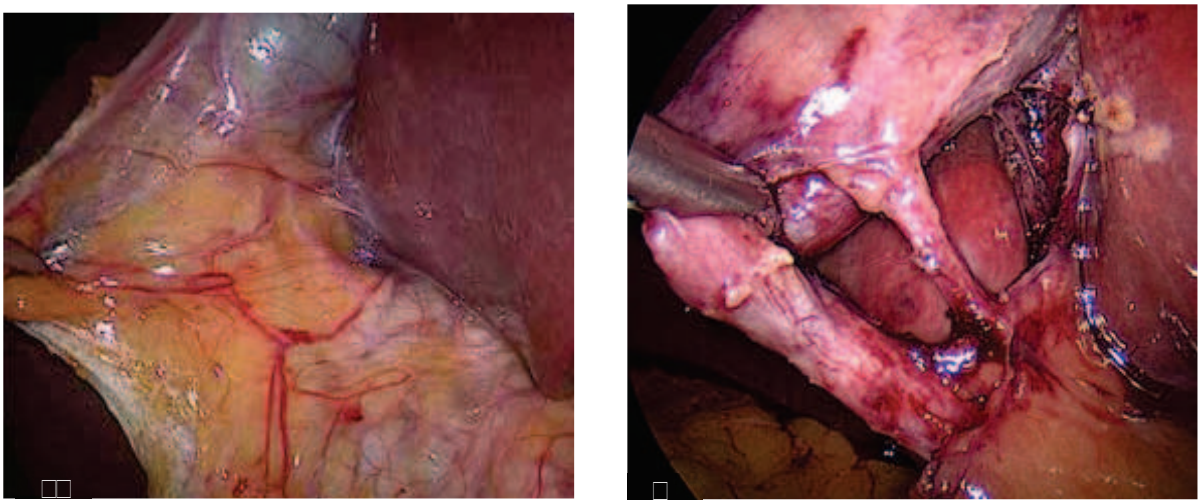


Fig. 9 - C□S in laparoscopic operative setting□a□before dissection□b□after dissection.

The CVS technique is especially aimed at mobilizing the gallbladder neck from the liver, in order to obtain a circumferential identification of the transition of the cystic duct into the gallbladder.

Pablo Mirizzi in 1931 developed the intraoperative cholangiography (IOC), an invasive method to map the intrahepatic and extra-hepatic bile ducts [91-94]. This consists in intraoperatively identify the cystic duct, isolate it and, through a small cut, put a cathether into the biliary duct and perform a iodate contrast x-ray imaging to detect bile ducts anatomy.

IOC has been advised to reduce the risk of bile duct injury, however, this radiological imaging of the biliary tree is only used selectively due to several disadvantages that includes:

- risk of bile duct injury during insertion of the trans-cystic catheter
- surgeon's skill to perform effectively the procedure

- radiation exposure for patient and OR staff
- time consuming procedure
- difficulty of moving bulky equipment into and out of a crowded OR (time delay and additional manpower needed).

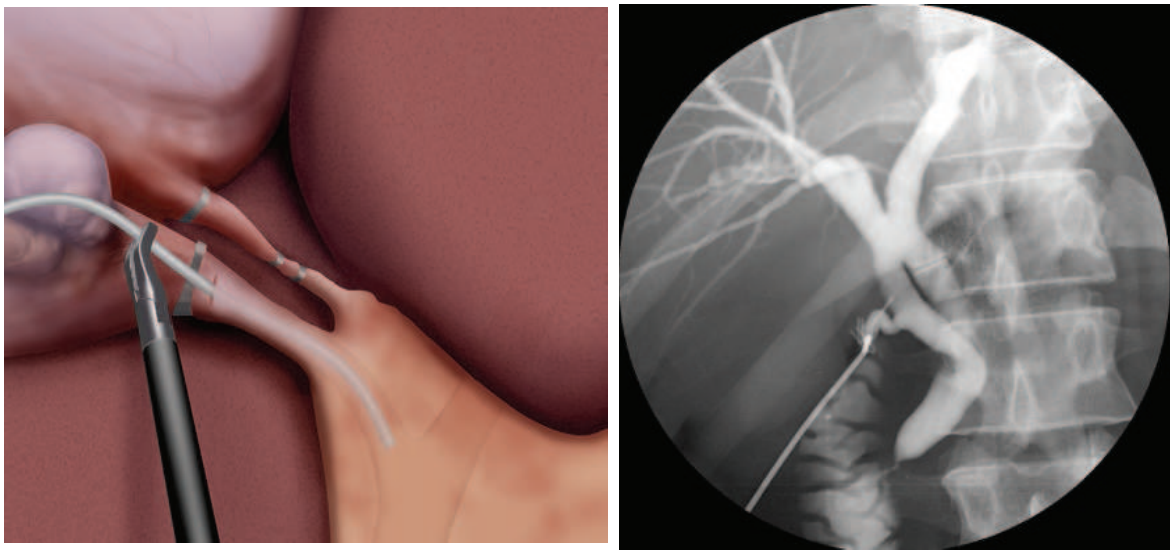


Fig. 10 - Intraoperative Colangiography [C]

Most important, IOC is an “operative” procedure, requiring a certain degree of dissection in potentially dangerous areas when the anatomy is not straightforward. The would-be accidental bile duct injuries cannot be prevented but only demonstrated by this exam. Therefore, worldwide consensus about implementation of intraoperative cholangiography is still lacking [95-97] [Fig 10].

ii. Rationale of the FGS application

Recently, a novel technique to visualize structures using fluorescent light and intravenous dye has been developed. Intraoperative ICG Fluorescent

Cholangiogram is a novel approach, which offers real-time intraoperative imaging to improve detection of biliary anatomic structures during surgery.

The Intraoperative ICG Fluorescent Cholangiogram (IFC) during LC has been evaluated in various animal models [98-100]. The first intraoperative use of this technique in humans was described by Ishizawa *et al* in 2010 [101]. As previously described, since ICG is excreted virtually unchanged by the bile, the most obvious application is the visualization of the biliary tree.

This dye can be used effectively to visualize the biliary system through a near-infrared (NIR) camera able to detect fluorescence. Preoperative injection of ICG can be used to image the biliary system in laparoscopic cholecystectomy and to prevent bile leaks after hepatectomy [102].

Fluorescence equipment and ICG dye are necessary in order to perform an IFC but neither radiological support nor additional intervention, such as opening the biliary tree, is required. The method involves the administration of indocyanine green (ICG) by either intrabiliary injection or intravenous injection at least 30 min before surgery. Optimal timing of ICG administraton is still under debate. From literature reports and from our preliminary experiences, it takes at least 20 to 30 minutes to injected ICG to be excreted into the bile. Nevertheless, since this dye stays stable in the bile for many hours (in part depending from patient's liver function) because it binds to lipoproteins, some authors suggest to inject ICG up to 12 hours before cholecystectomy and bile ducts surgery in order to obtain a better SBR because of the complete washout of the probe from the epatocites [68].

The excitation of protein-bound ICG by near-infrared light causes it to fluoresce and fluorescence is collected using optical filters that match the emission spectrum of the fluorophore. Imaging lenses and digital cameras are used to

produce the final image delineating components of the biliary system for the surgeon.

Surgical dissection, after initial visualization, may proceed switching from WL to NIR light every time the surgeon needs to clarify anatomical details. This might have a fundamental role, especially in case of acute disease, flogosis and distorted anatomy: all situations that can lead to misinterpretation of biliary structures [Fig 11-12].

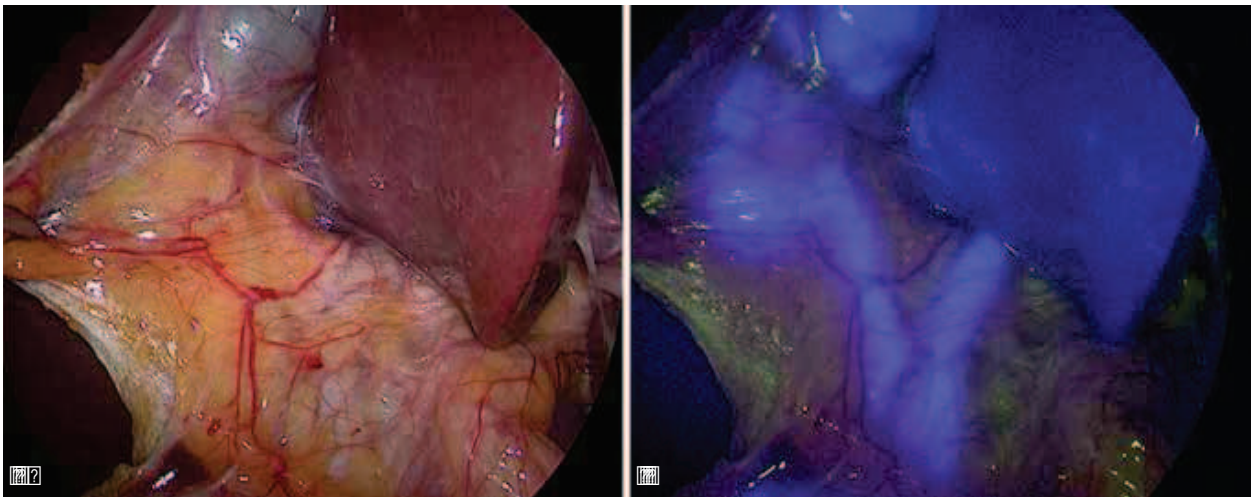


Fig. 11 - Calot's triangle before dissection: a) white light; b) ICG Fluorescent Cholangiography.

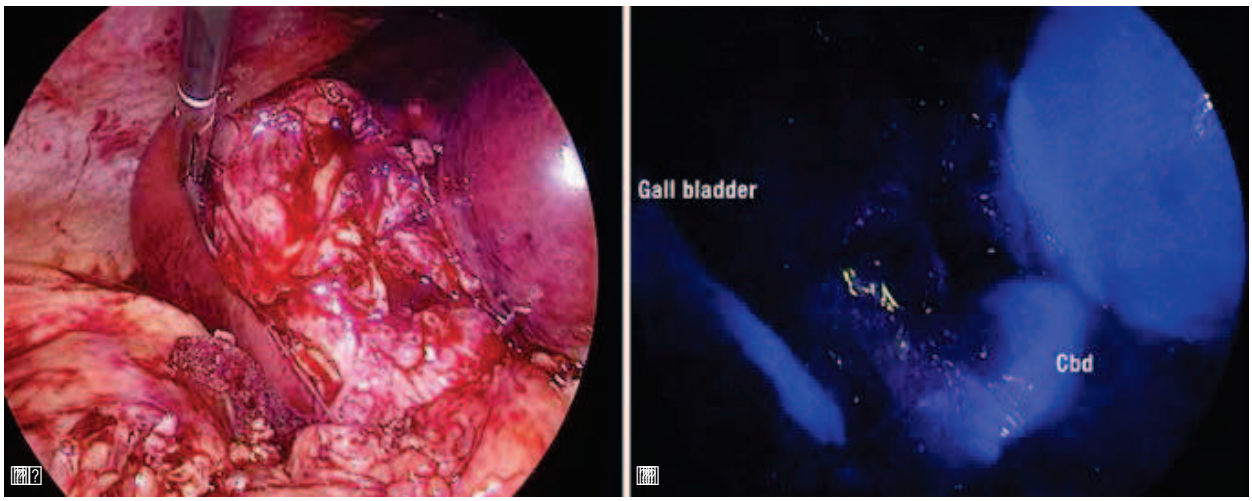


Fig. 12 - Acute cholecystitis: a) white light; b) ICG Fluorescent Cholangiography.

iii. Cost Effectiveness

It is intuitive that IFC is a cost-effective procedure, compared to standard IOC, to detect bile ducts and prevent BDI, however only few reports in literature focused on this issue. In particular Dip. et al tried to validate the economical aspect of this technique analyzing costs on a 43 patients series, evaluating costs both due to the operative time and to the procedure itself. They clearly reported that IFC was significantly less expensive than IOC [103].

In conclusion, intraoperative IFC has been successfully performed during mini-invasive cholecystectomies in various studies, including standard, single-incision and robotic cholecystectomies. [104].

Despite the encouraging results from preclinical feasibility studies, reporting that the rate of visualization of biliary anatomy with ICG was 95.1% to 99% [60, 105], wide clinical acceptance of the routine use of ICG fluorescence laparoscopy is still lacking due to the absence of reliable clinical data. Furthermore, it is important to assess whther IFC might improve the outcome in some subpopulation of patients for whom the evaluation dissection of Calot's triangle is difficult (e.g. obese patients or patients undergoing inflammatory processes) and the potential use of this technique as a training tool in laparoscopic surgery.

Therefore, multicenter randomized clinical trials are desirable to assess the potential added value of the fluorescence imaging technique during laparoscopic cholecystectomy in order to establish this methodology as a standard of care or at least as a recommendation.

1.2.2 Real Time Intraoperative Fluorescent Perfusion Assessment

Indocyanine green has been used for decades in ophthalmology for imaging retinal blood vessels, that is, in retinal angiography. However, sodium fluorescein operating in visual wavelengths has been much more popular in retinal angiography, partly because it is visible without any electronic cameras. Nevertheless ICG is peculiar because it gives information about deeper lying blood vessels because it works in NIR light, in which tissues are much more translucent than in visual wavelengths [15].

Based on this principle, it is intuitive that ICG fluorescent guided surgery can be used to study vascular anatomy and parenchimal perfusion in various clinical situations.

One of the main application in laparoscopic abdominal surgery surely is the assessment of bowel microperfusion before anastomosis creation. Nevertheless angiographic properties of fluorescence imaging can be used also to clarify vascular anatomy and facilitate vascular dissection in delicate procedures, such as nephrectomies, splenectomies or liver resections or to check parenchymal global perfusion for example for transplants or skin flaps in plastic reconstructive surgery.

For these procedures, ICG is usually injected in small boluses of 2–5 ml each (0.4 mg/ml/kg), and the real-time fluorescence signal is visible and can be recorded [67,68,106].

1.2.2.1 Anastomotic leakage in Colo-rectal Surgery

i. Background

Colorectal cancer (CRC) is the third most common cancer in the world and its incidence is increasing. According to Cancer Statistics Review the number of new cases each year in 2010-2014 period is 40,1 per 100,000 with an extimated growth of 8% in the next 4 years interval [107].

In conjunction with rising prevalence, novel technologic advances, better understanding of physiology, and improved surgical technical skills allow surgeons to offer patients curative surgery. For example, over the past 2 decades, long-term oncologic outcomes of rectal cancer have improved as a result of improved surgical technique and neoadjuvant treatment. Advances in surgical technique, technology, and neoadjuvant treatments currently allow surgeons to create lower anastomoses as an alternative to permanent colostomies [108-110].

The majority of patients suffering colorectal disease will require surgery to resect the diseased bowel and anastomosis to restore gastrointestinal continuity. The main, feared complication in colorectal surgery is an anastomotic leak with the associated immediate morbidity/mortality and long-term functional disorders. If the anastomosis fails to heal an anastomotic leak (AL) occurs, leading to sepsis and possible multi-organ failure [111].

One third of CRC involves the rectum, where surgical act is more technically demanding and the rate of AL reported increase to 10-15% of patients. AL is highest following rectal resection and increases as the anastomotic site

approaches the anal canal; rectal anastomoses within 10 cm of the anal verge are up to 6.5-times more likely to leak than at more proximal sites [112-114]. In addition to increase morbidity from ?20% to ?60% and mortality from ?5% to ?20%, AL is found to increase the risk of local cancer recurrence significantly from 10.1 to 17.5 % [115,116].

AL has a negative impact on patient recovery and consumes NHS resources for remedial interventions, extending the in-patient stay from an average of 7 to 19 days, dramatically rising the total clinical and economical burden on the healthcare system [117-119].

ii. Definition and Technical Aspects

The International Study Group of Rectal Cancer has published a universal definition and grading system for AL, defining AL as a defect of the intestinal wall at the anastomotic site leading to a communication between the intra- and extraluminal compartments [120]. In rectal cancer surgery, the current rate of AL is variously reported between 8% and 20% [121]. The most recent data, which takes into account new technologies, comes from the COLOR II and MRC/EME ROLARR studies. COLOR II was a large European randomised controlled comparison of laparoscopic versus open surgery for rectal cancer [122]. In the 1044 patients randomised, there was no significant difference in AL between laparoscopic and open groups. AL rate varied depending on the height of the anastomosis, being 11%, 15%, and 11% for anastomoses in the upper, middle, or lower rectum respectively. The ROLARR trial has recently reported the results of a randomised comparison of robotic versus laparoscopic surgery in 471 rectal cancers. There was no difference between the two arms, with an overall AL rate of 10.7% [123].

A variety of patient and tumour related factors can influence leak rates [124].

Literature has identified several patient's related factors connected with high-risk anastomoses [111,115,125-130]:

- male gender
- malnutrition
- cancer diagnosis
- renal failure
- immunosuppression
- level of anastomosis
- tobacco use
- preoperative radiation
- treatment with steroids or COX-2 selective non steroidal drugs (NSAID)

Most of these can not be modified or influenced by surgeons.

Regarding intraoperative and technical aspects that can influence anastomosis construction and healing, such as tension on the anastomosis, indaquate colonic mobilization and tissue approximation or technical failure of staplers, probably the most crucial and the one that the surgeon has some influence over, is the blood supply to the anastomosis. Ensuring that both ends of the bowel to be anastomosed are adequately perfused is essential for healing [131,132].

iii. Assessment of Colonic Perfusion

Currently, there is no widespread method to assess and quantify the perfusion of gastrointestinal anastomoses intra-operatively, besides the subjective evaluation by the surgeon during the operation performing a bowel inspection. For example, operator's assessment for the surrogates of a good blood supply is based on findings such as tissue colour, the presence of a palpable pulse within the mesentery and the manifestation of arterial bleeding following mesenteric division. Unfortunately this is a completely subjective and not completely reliable evaluation, especially in laparoscopic surgery where surgeon's visual perception may be distorted.

The current standard for intraoperative testing of rectal anastomotic integrity involves "air-leak" testing and assessment of completeness of anastomotic "doughnuts". Air-leak testing is easy and cheap and has been shown to more than halve the radiological AL rate [133]. In some centres, this is combined with intraoperative endoscopic assessment of the anastomosis [134].

Nevertheless while a variety of methods have been utilised to assess anastomotic integrity the "physical" integrity of an anastomosis (tested at the moment suture is created and the healing process has not yet begun) becomes less relevant when the bowel is hypoperfused. Thus, determining optimal perfusion of an anastomosis is essential.

There are several different techniques to assess intraoperative intestinal perfusion. They include:

- Real time intraoperative ICG fluorescent angiography (FA), one of the

application of FGS discussed above [108].

- Intraoperative assessment of anastomotic tissue oxygenation using white light spectroscopy; this technique uses white light emitted from a probe adjacent/in contact with tissues and detects the changes in scattering and absorption caused by oxy- and deoxyhaemoglobin; with this method Karliczek *et al* demonstrated that a reduction in bowel oxygen tension immediately after resection was predictive for AL, although the level of oxygen tension that led to irreversible necrosis was not defined (low sensitivity (41%) and specificity (59%) [135].
- Scanning laser Doppler flowmetry is a technique that employs a laser beam directed at the tissue in question. Subsequent shifts in frequency from the moving erythrocytes within blood vessels are detected as a means to determine perfusion. This technique has been applied to colorectal surgery and has demonstrated a reduction in perfusion following mesenteric division leading to revision in the form of further resection (few cases reported in literature) [136].
- Near infrared spectroscopy (NIRS) detects levels of haemoglobin concentration however using light in the NIR range. [137].
- Real time traditional angiographic study has been described, but is impractical to carry on in a routine clinical setting [137].
- Perfusion CT-scan: it is a radiological advanced technique that is able to assess cancer blood flow, blood volume, and permeability-surface area product. The ability of perfusion CT to quantify changes in rectal perfusion following radiotherapy makes it an attractive preoperative imaging tool for predicting anastomotic leak in rectal surgery. Its use in combination with CT angiography, to determine anatomical variations in rectal blood supply, should provide valuable information for

predicting anastomotic perfusion, however it is not acceptable as a real-time intraoperative tool [138].

Most of the methods described above are difficult to use in the operating room, not reproducible or too expensive.

iv. Rationale of FGS application in Rectal Surgery

Despite advances in surgery, with the introduction of stapling technology and laparoscopic/robotic techniques, there has been no progress in reducing the rate of anastomotic leak (AL) over the past 50 years. AL rates are particularly high following rectal cancer surgery, with the rate increasing as the level of the anastomosis approaches the anal verge; anastomoses below 10 cm from the anal verge have a 5.4-fold increased risk of AL [112, 114] whilst those below 5 cm from the anal verge have a 6.5-fold risk of AL [113]. The reason for this is usually attributed to poor blood supply to the rectal stump and the frequent use of preoperative radiotherapy in low rectal cancers.

In the hypothesis that assessment of microperfusion at the time of the creation of an anastomosis may influence the rate of anastomotic leak, a technology that would accurately predict perfusion may potentially improve outcomes in terms of morbidity/mortality for the patient, improve quality of life, and produce immediate cost-savings for the NHS.

As described above intraoperative fluorescent perfusion assessment with ICG it is a safe, easyly reproducible and cost-effective technology allowing real-time angiography of the bowel during surgical intervention [Fig 13].

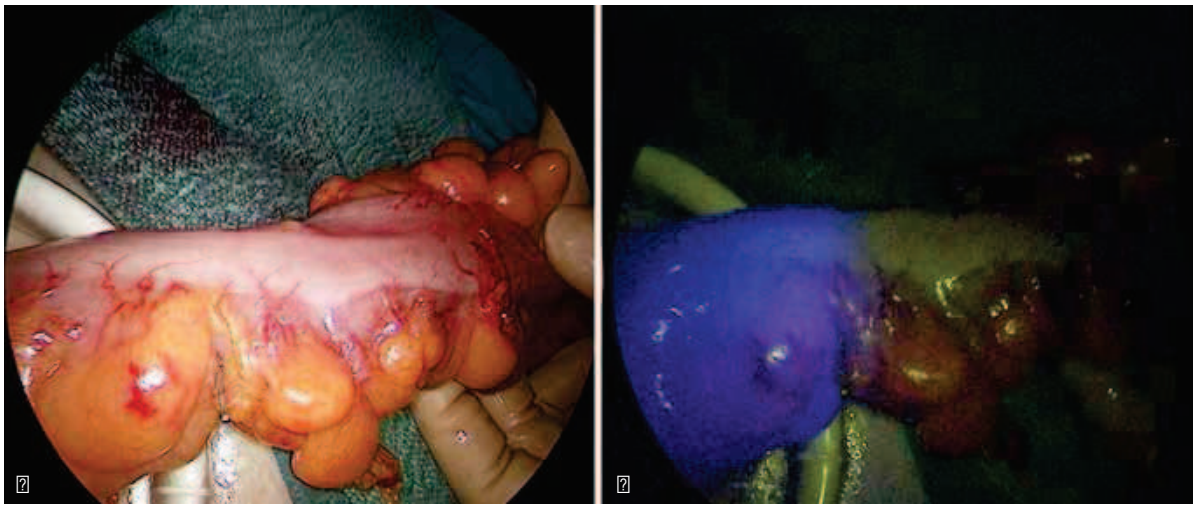


Fig. 13 - Intraoperative Fluorescent Angiography (FA) in colonic bowel resection.

Proof of concept for fluorescent angiography has been established, but the evidence is limited to a few case series and one multicentre non-randomized clinical study; for this reason if these encouraging results could be confirmed in a rigorous and large clinical randomized study, this techonology might become a standard of care in clinical practice, eliminating a major source of risk for patients, improving quality of life and producing immediate cost-savings for NHS.

v. Cost Effectiveness

AL, representing one of the most serious complications after gastrointestinal surgery, has a great impact on global patient care; an interesting study from Hammond et al in 2014 collected data on over 6000 cases of leakage and matched with equal number of comparable control patients; they clearly demonstrated to what extent AL causes an increase in overall postoperative morbidities and it leads to higher requirement of expensive therapies and procedures (including prolonged hospital stay and re-admission). As you can see from the graphic the total hospital cost for the group of patients that

developed a leak is almost double than control group [114] [Fig. 14].

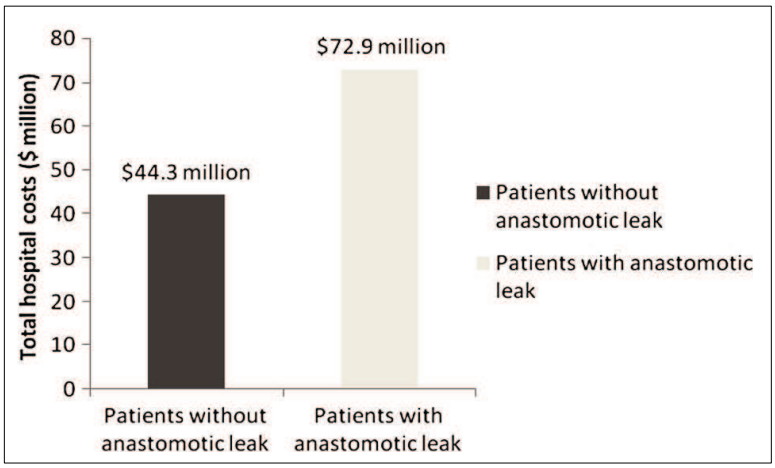


Fig. 14 □□ospital costs□Patients □ith□□ ithout anastomotic leak □□ammond □et al□

The prevention of postoperative AL must represent a priority for healthcare providers in order to ease a significant clinical and economic burden. For this reason FA, being a relatively unexpensive and easy procedure, might gain a fundamental role as a gold standard in gastrointestinal surgery.

□

2. AIM OF THE STUDY

The aim of this PhD research is to evaluate two of the main clinical application of ICG Fluorescence Guided Surgery through specifically designed clinical randomized controlled trials in order to validate these methodics.

The study focus on:

- Indocyanine green Fluorescence Cholangiography (IFC) to identify biliary anatomy and prevent bile duct injuries in laparoscopic cholecystectomy: **IFC trial.**
- Fluorescent Angiography (FA) for Bowel Perfusion Assessment in Laparoscopic Colorectal Resection: **Angio-core trial**.

□

3. PATIENTS, MATERIALS AND METHODS

Study procedures and data collection were conducted at General Surgery I Unit at Ospedale di Circolo of Varese (Department of Surgical and Morphological Sciences – University of Insubria, Varese).
During this PhD Research two studies on Fluorescence Guided Surgery have been conducted (patients enrolling is still ongoing).
Our center is a Principal Co-Investigator in both studies. Since recruiting is not completed, preliminary results will be shown.

3.1. Surgical Equipment and Technology

KARL STORZ GmbH & CO. KG (Tuttlingen, Germany) has developed a laparoscopic fluorescence imaging system that is suitable for both near-infrared fluorescence and xenon (white) light imaging. It is a CE approved system for clinical practice.
During laparoscopic cholecystectomies, alternate exposure from xenon white light (STD mode) to near infrared light (ICG mode) is used to identify the fluorescent structures during dissection.
During colorectal surgery procedure the NIR mode can be used to perform real-time intraoperative angiography on the bowel, before and after anastomosis contruction.

Fig. 1 - Karl Storz Laparoscopic NIR Light Tower

The fluorescent imaging system by KARL STORZ consists of a xenon light source (D-Light P) that can emit both near infrared (780 nm) and white light, NIR-transmitting telescopes and a full HD 3-chip fluorescence imaging (FI) camera head capable of capturing both white light and NIR images.

The system is equipped with a foot pedal, allowing the surgeon to switch from WL camera mode to ICG camera mode, and back [Fig. 15].

	Description
1	Image1 SPIES camera control unit
2	Image1 SPIES H3-Z FI camera head ICG endoscopes (0and 30)
3	D-Light P light source
4	Foot switch
5	Fiber optic light cable

For comparison (patients randomized in traditional imaging arm) the conventional white light mode of the laparoscopic imaging system will be used.

In all ICG fluorescence guided surgery procedures performed, Indocyanine Green (*ICG-Pulsion*, *Pulsion Medical Systems*Munich, Germany) is used in the diluted form either with saline solution or injectable water. The standard dose commonly administered in clinical practice (0.1–0.5 mg/ml/kg) is well below the toxicity level [15,43] [Fig 16].

Fig. 16- Indocyanine Green ICG-Pulsion Pulsion Medical Systems Munich Germany

Based on the standard protocol employed in the authors' clinical practice, a 25 mg-bottle of ICG is diluted using 10 ml of sterile water/saline solution [67].

To apply FGS in laparoscopic cholecystectomy, following injection (details and timing in paragraph 3.2), the dye is concentrated in bile, resulting in visual enhancement of the biliary tree anatomy, especially in Calot's triangle [Fig 17].

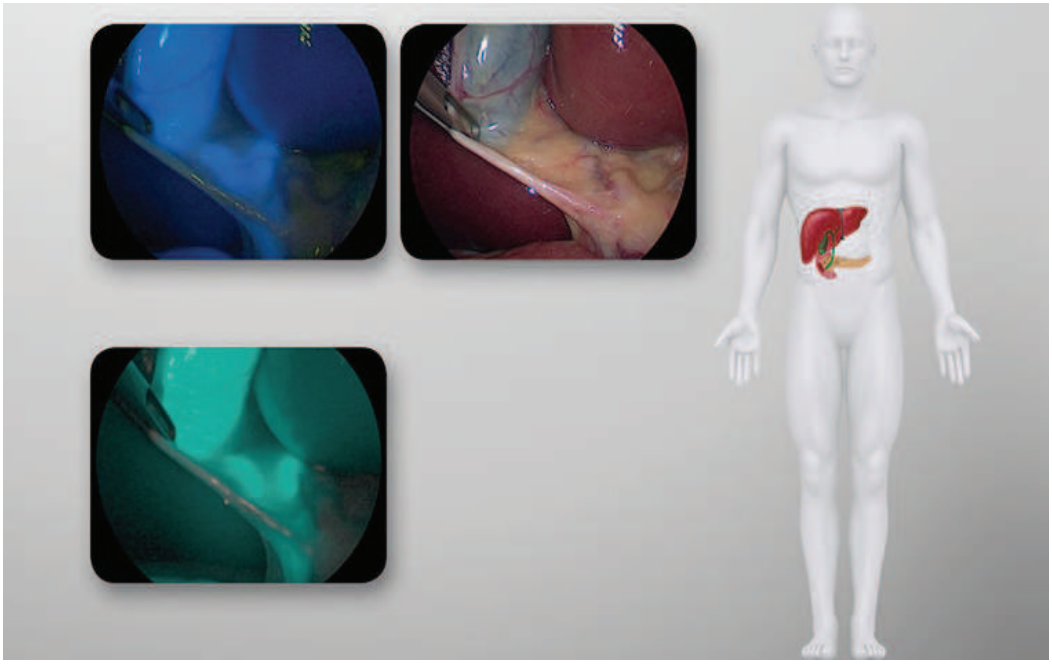


Fig. 17 ICG concentrated in the bile visual enhancement bile ducts anatomy.

During laparoscopic colorectal resection fuorescence imaging it is obtained

injecting intravenously small boluses of ICG (details and doses in paragraph 3.3) and as soon as the dye get to bloodstream providing real-time relevant information bowel perfusion [Fig. 18].

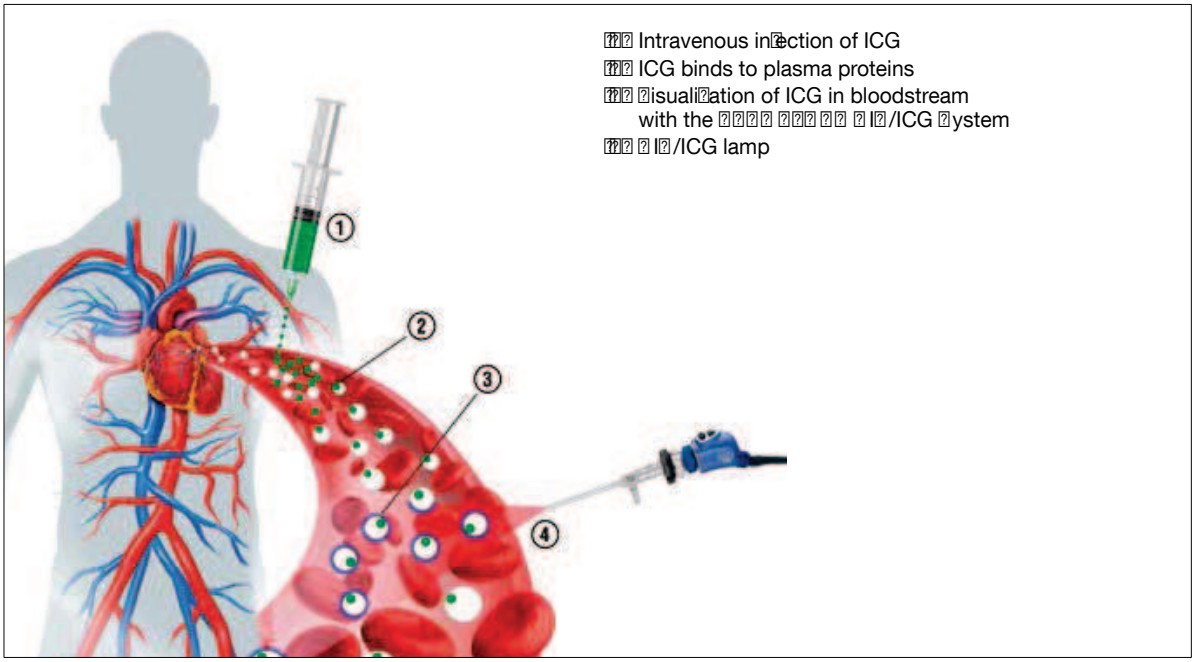


Fig. 18 ICG into the bloodstream for real time bowel perfusion imaging.

?.?. ?ndocyanine green Fluorescence Colangiography ??FC??rial

?.?.?. Study Overview

This study is a a multicenter randomized controlled trial with two study arms. The study is designed to compare the effectiveness (in terms of improved visualization and earlier identification) of ICG Fluorescence Cholangiography (IFC) with near-infrared (NIR) light to standard white light (WL) imaging in visualizing and identifying the main biliary and hepatic structures (Cystic Duct, Right Hepatic Duct, Common Hepatic Duct, Common Bile Duct, Cystic-CBD junction, Cystic-Gallbladder junction and any Accessory Ducts) during laparoscopic cholecystectomy (LC).

?.?.?. Study Endpoints

 Primary Endpoint

Time until establishment of Critical View of Safety (CVS).

 Secondary Endpoints:

1. Demonstrate that IFC performs better than standard WLI alone in visualizing and identifying extra-hepatobiliary structures (Cystic Duct, Right Hepatic Duct, Common Hepatic Duct, Common Bile Duct, Cystic-CBD junction, Cystic-Gallbladder junction, and any Accessory Ducts) before and after dissection during LC.
2. Total surgical time;
3. Incidence rate of intraoperative bile leakage from the gallbladder or cystic duct;
4. Incidence rate of bile duct injury;

5. Estimated blood loss;
6. Surgeon Confidence Level using NIFC vs WLI alone;
7. Postoperative length of hospital stay;
8. Complications due to the intravenously injected contrast agent;
9. Incidence rate of conversion to open cholecystectomy;
10. Postoperative complications (until 90 days after surgery);
11. Cost-minimization.

- *Additional observations (NIFC group only)*
  - "Reflux Maneuver"

According to previous publications in literature, in some cases the cystic duct may not be seen with IFC because the cystic cuct may not contain ICG at the time of viewing. In those cases, a "reflux maneuver" (defined as using an atraumatic laparoscopic instrument to push on the common bile duct in order to move more of the bile containing ICG from the common bile duct through the cystic duct into the Gallbladder – thereby increasing visualization of the cystic-gallbladder junction) can be performed.

We will evaluate the usefulness of the Reflux Maneuver to visualize the Cystic Duct.

Study Design

- *Study Population*

Participants will include patients aged 18-years old and above selected for laparoscopic cholecystectomy (LC). Patients scheduled for an elective LC will be recruited and randomized at the outpatient clinic (n = 262 total). Stratification is performed per participating center. A sample size of minimum 131 patients

in each randomization arm has been calculated to detect a reduction of at least 5 minutes in time until establishment of CVS (80% power and □ □ 0.05 (two-tailed)).

□□ *Subject Selection*

□□ *Inclusion Criteria*

i. Patients of both genders
ii. Minimum age: 18 years old
iii. Spoken and written command of the language spoken in the country's center
iv. Ability to understand and follow the study procedures and sign the informed consent

□□ *Exclusion Criteria*

i. Known allergies to iodides
ii. Known history of coagulopathy
iii. Known moderate or severe liver disease
iv. Women who are pregnant or breastfeeding, or for whom possibility of pregnancy was not ruled out

□□ *Setting*

Participants will be recruited by the Department of Surgery at the following study sites, all of which are recognized hepato-bilio-pancreatic surgery centers:

□□ Cleveland Clinic (Weston, FL, USA) - Co Principal Investigator

□□ University of Insubria (Varese, Italy) - Co Principal Investigator

□□ Hospital de Clínicas José de San Martín (Buenos Aires, Argentina)

□□ Tokyo Medical University Hospital (Tokyo, Japan)

Surgeons trained in laparoscopic and hepato-bilio-pancreatic (HBP) surgery will participate in the study. Principal and Lead Site Investigators of this Protocol are seniors and recognized HBP surgeons. Additionally, surgeons in training will be involved in the study. When a resident is present in the OR, he/she will perform the procedure under the supervision of the senior surgeon.

Enrollment Process

Eligible patients will be identified through clinical and test evaluation. Eligibility will be verified by the patient's primary surgeon. The surgeon will determine the indication and date of the surgery. Once a patient is confirmed as eligible, the surgeon will introduce the study in detail. If after being introduced to the study and having had the opportunity to ask questions, the patient is willing to participate, he/she will be asked to review and sign the informed consent document.

Upon entry in the clinical trial patients will be randomly allocated to the intervention arm. Data will be collected at enrollment time, during surgery, at the end of surgery and one month after surgery.

Randomization Process

Upon entry in the clinical trial, according to the study database patients will be randomly allocated to one of the study arms (1:1) within site (1:1) using a computer generated random sequence.

Patient will be blind to the intervention but surgeon blinding will not be feasible due to the nature of the intervention.

Basically two groups of patients will be compared:

1. Control: WLI

A laparoscopic cholecystectomy will be performed using Xenon white light imaging (WLI) only.

2. Experimental: IFC

   A laparoscopic cholecystectomy will be performed using both white light imaging and near infrared light (NIR) imaging with indocyanine green (ICG) intravenous dye.

?.?.?. Data collection and procedures

Data will be collected before, during and at the end of surgery; and 30 days after surgery [Form 1-3].

Form 1 – IFC Trial – Pre-operative case report form (CRF)

Indocyanine Green (ICG) Fluorescence and Radioactivity (Dual Modality Imaging in Sentinel Lymph Node Biopsy for Breast Cancer

INSTITUTION: _____ PATIENT ID: _____ PROCEDURE DATE:_____

SURGEON: _____ PERSON COMPLETING FORM: _____

1. PATIENT INFORMATION:

Age: _____ Sex: M / F Height: _____ (meter/centimeter) Weight: _____ (kg/g) BMI: _____

A. Previous surgery on the breast: No Yes Location: _____ (Quadrant)

B. Previous chemotherapy or radiotherapy: No Yes Location: _____ (Quadrant)

C. Lymph Node Disease: No Yes Location: Clinically palpable Confirmed by: _____

2. LABORATORY TESTS (within 1 month prior to surgery).

A. Documented laboratory findings prior to surgery:

a. Complete blood count – Normal / Abnormal – Evaluated: Reviewed / Not done
b. Hemoglobin – Normal / Abnormal – Evaluated: Reviewed / Not done
c. Platelet count – Normal / Abnormal – Evaluated: Reviewed / Not done
d. Coagulation profile – Normal / Abnormal – Evaluated: Reviewed / Not done
e. Urinalysis – Normal / Abnormal – Evaluated: Reviewed / Not done
f. Albumin – Normal / Abnormal – Evaluated: Reviewed / Not done
g. SGOT – Normal / Abnormal – Evaluated: Reviewed / Not done
h. SGPT – Normal / Abnormal – Evaluated: Reviewed / Not done
i. Alkaline phosphatase – Normal / Abnormal – Evaluated: Reviewed / Not done

B. PREGNANCY TEST: Not done Indicated: Negative Positive (Not applicable)

C. IMAGING STUDIES:

a. LITHIASIS: No Yes
Diagnostic tool: Ultrasound CT Scan MRCP EUS ERCP

b. ERCP/ES: No Yes
Date performed: _____ Stone Extraction: No Yes

c. SPHINCHTEROTOMY performed: No Yes

SURGERY STATUS: Elective Urgent Emergency

Form 2 – IFC Trial - Intra-operative CRF - Performing Surgeon

Indocyanine Green (ICG) Fluorescent Cholangiography (IFC)

INTRAOPERATIVE CASE REPORT FORM – IFC AND ICG ADMINISTRATION

INSTITUTION: _____ PATIENT ID: _____ PROCEDURE DATE: _____

SURGEON NAME: _____ _____
(PRINT) (SIGNATURE)

ACADEMIC TITLE: _____ Years in practice: _____ Approximate Lap Cholecystectomies Performed: _____

_____ ☐ IFC (STD choice in case of technical failure) ☐ **IFC** (using the STD and ICG combined)

☐ **For IFC group only**

A. Time of ICG Administration: _____ AM ☐PM

☐ Was there an Adverse Reaction to the ICG? No ☐ Yes ☐ If Yes please complete the following:

i. What was the reaction? _____

ii. How was it managed? _____

☐ _____

A. **Categorize the initial observation of the surgical field**:

i. Are the anatomical Can you see the Cystic Artery? ☐ Yes ☐ Maybe ☐ No

ii. Level of inflammation:

a. ☐ Minimal

b. ☐ Moderate (Mural anatomy can be visualized and acute or chronic scar tissue with acute or chronic edema and ascites)

c. ☐ Severe (Portal and Parenchyma)

iii. Were adhesions and/or scar tissue needed to be released prior to dissection? ☐ Yes ☐ No

☐ **Initial visualization of extrahepatic biliary structures**: (after release of adhesions and Calot's triangle and retraction of gallbladder and clearing the area BEFORE and/or after dissection)

i. Documented Time of Initialization: _____ AM ☐PM

ii. For each structure scope the Calot's triangle area (using STD (white light)) and label in the NIC area observed between STD and ICG images as needed. Check the appropriate boxes below based on visibility:

	Yes	Maybe	No
a. Cystic Duct	☐	☐	☐
b. Right hepatic Duct	☐	☐	☐
c. Common hepatic Duct	☐	☐	☐
d. Common bile Duct (CBD)	☐	☐	☐
e. Cystic CBD junction	☐	☐	☐
f. Cystic Gallbladder junction	☐	☐	☐
g. Accessory Ducts	☐	☐	☐

C. **For IFC arm only – Perforator – Retrograde maneuver**
This portion relates to visualization of the Cystic Duct using fluorescence dissection and transection of the ICG from the Common bile Duct in order to see the Cystic Duct continue to the Gallbladder where increasing visualization of the Cystic Duct to Gallbladder Union

☐ _____

□□

i. "Reflux Maneuver" Attempted? □ Yes □ No

ii. Check what is now newly visible:

	Yes	Maybe	No
a. Cystic Duct	□	□	□
b. Cystic/Gallbladder Junction	□	□	□
c. Cystic/C□□ Junction	□	□	□

□. **START DISSECTIO□□**

A. Hilar dissection start time□□□□□□□□□□□AM □□M

□. Document time dissection was completed and visualization documentation begun□□□□□□□□□□□AM □□M

C. **D□c□□** □□□□□□□□□□□□□□□ □□□□□□□□□□□□□**c** □□□□□ □□□□□□ □□

i. For both arms, scope the Calot's triangle area using □□□ (White light) mode. If in the N□□C arm, toggle between □□□ and □C□ modes on light source as needed. Check the appropriate boxes below based on what is visible:

	Yes	Maybe	No
a. Cystic Duct	□	□	□
b. Right Hepatic Duct	□	□	□
c. Common Hepatic Duct	□	□	□
d. Common Bile Duct (C□□□	□	□	□
e. Cystic/C□□ Junction	□	□	□
f. Cystic/Gallbladder Junction	□	□	□
g. Accessory Ducts	□	□	□

□. □□□□I□**C A**□□ **O**□□□ Repeat the Reflux Maneuver.

i. "Reflux Maneuver" attempted? □ Yes □ No

ii. Check the appropriate boxes based on what is visible:

	Yes	Maybe	No
h. Cystic Duct	□	□	□
i. Cystic/Gallbladder Junction	□	□	□
j. Cystic/C□□ Junction	□	□	□

□. **T**□□ □ □□ **C**□□ □□**T**□□ Time the Cystic Duct was clipped or tied: □□□□□□□□□□AM □□M

□. **E**□□□ □□□□ □□□□□ □□□□□□ □□□□□□□□ m□ in the Bucket/Bag or from anesthesia chart at the conclusion of the procedure

□. □□□ □□□□□□□□□

A. Was there bile leakage? No □ (If no, go to □□) Yes □ (If yes, go to □□□)

□. Was the source of bile leak seen? No □ (If no, go to □□) Yes □ (If yes, complete the following)

i. Bile leak source(s): □

a. □ Liver bed □

b. □ Right hepatic duct □

c. □ Common hepatic duct □

d. □ Common bile duct □

e. □ Other□□□□□□□□□□□□□□□□

ii. Did N□□C help identify the existence of a leak? No □ Yes □

iii. Did N□□C help identify the source of the leak? No □ Yes □

□ □□□□□□□ □□□ □□ □□ □□ □□□□ □□□ □□ □□□□□□□□
□

Indocyanine Green (ICG) Fl□ore□cen□C□olan□io□ra□□y (IFC)

I□□□□□□□□□□□I□□ C□□□ □□□□□□□ F□□□ □ □□□F□□□I□G □□□G□□□

7. **□na□o□ic □no□alie□□** No □ Yes □ If yes, describe: ______________________________

If yes, do you feel NIF□ □el□ed you ide□ify □is □□o□ □ly□ Yes □ No □ N□□ □□ □l □r□ □□

□□ **□a□I□C □er□or□ed□** Yes □ No □

□□ **□ere C□□ □□one□ □re□en□□** Yes □ No □ No□□ □ec□ed □

□□. **□a□ □□e □roced□re con□er□ed □o □ □en□** Yes □ No □

If yes, e□□l□i□ □□y□ ______________________________

____

□□. **□a□ □□ere a □ile □□c□In□□ry (□□□l) d□rin□ □□e □□r□ery□** Yes □ No □

If yes, co□ □le□e □□e □□□l For□ □s described i□ □□e □□udy □ro□ocol.

□□. **□□□G□□□ F□□□□□□C□ a□□er □IFC□**	□efi□i□ely □ore	□o□e□□□□ □ore	□□□e	□o□e□□□□ □ess	□efi□i□ely □ess
□. □id you feel NIF□ □ □de your □rocedure □□F□□□	□	□	□	□	□
□. □ere you □ore □□NFI□□N□ usi□□ NIF□ □s □ □l □lo□e□	□	□	□	□	□
□□ □ould you □□□F□□ □o use NIF□ □□□i□ □s □ □l □lo□e□	□	□	□	□	□

Form 3 IFC Trial - Post-operative form

Indocyanine Green Fluorescence Cholangiography

INSTITUTION: _____ PATIENT ID: _____ PROCEDURE DATE: _____

SURGEON: _____ FORM COMPLETION DATE: _____

FORM COMPLETED BY: _____ _____
(PRINT) (SIGNATURE)

1. Length of operation: _____ (hours)

2. Was there any intraoperative problem: No Yes If Yes please specify:

   a. Dose dose administered: ____ (NOTE: Dose of Surgeon to Dose)

   b. Reason dose not administered:

   _____

   c. Type of problem encountered:

   _____

   d. Length of delay: _____ (hours)

3. Dose (dose): No Yes If Yes please specify: _____

4. Cholangiography: No If No please specify: Yes If Yes please specify:

   a. Intraoperative green staining: No If No please specify: Yes If Yes please specify:

   a.a. Filling No Yes . If yes please specify:

   a.a.1. Type of problem No Yes

   a.a.2. If Yes please specify: _____

   a.b. Ultrasound No Yes . If yes please specify:

   a.b.1. Type of problem No Yes

   a.b.2. If Yes please specify: _____

   a.c. Other intraoperative staining No Yes If Yes please specify:

   a.c.1. Describe: _____

   a.c.2. Once (dose dosage): _____

   a.c.3. Duration (hours): _____

   a.c.4. Type of problem No Yes

□□

a.iii.5. If Yes, what treatment:

____

b. Hemorrhage? □o □ Yes □ If Yes, how manage□? □he□□ a□that a□□□: □

b.i. □ transf□sion □

b.ii. □ re□o□eration □

b.iii. □ □onger hos□ita□i□ation

□. □b□omina□□ain or ten□erness re□□iring inter□ention? □o □ Yes □ If Yes, how manage□? □he□□ a□□ that a□□□:□

□.i. □ □□traso□n□□

□.ii. □ □ □ □ □□

□.iii. □ □ □ □ □□

□.i□. □ □ranshe□ati□ □ho□angiogram□

□.□. □ □er□□taneo□s as□iration of □o□e□tion□

□.□i. □ □er□□taneo□s □rainage □atheter □□a□ement□

□.□ii. □ □□□orator□ s□rger□□

□.□iii. □ □ther: ________________________________

□. □i□e □ea□ □witho□t bi□e □□□t in□□r□? □o □ Yes □ If Yes, □□ease □om□□ete: □

□.i. □ □er□□taneo□s □rainage□

□.ii. □ reo□eration□

□.iii. □ treate□ with □er□□taneo□s □rainage on□□□

□.i□. □ treate□ with trans□a□i□ar□ stent□

□.□. □ treate□ with □er□□taneo□s □rainage an□ trans□a□i□ar□ stent

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e. □i□e □□□t in□□r□? □o □ Yes □ If Yes, □om□□ete se□arate □□l form□

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□

Study procedures

Informed Consent

Prior to admission, the surgeon will review the Informed Consent Form with the patient and, if he/she desires to participate, both the patient and the Researcher will execute and date the form. The laparoscopic cholecystectomy procedure itself will not be performed differently than usual. Next, there will not be any radiation involved for the patient. Neither are any psychological or psychiatric investigations involved. Patients are not asked to undergo additional testing after the surgical procedure, they are also not requested to fulfill any questionnaire [Form 4].

Form 4 IFC Trial - Subject informed consent form

Subject informed consent form IFC trial
University of Insubria - Varese- ITALY
Vers. 1.0 del 1.01

Informativa e Modulo di Consenso Informato

Colecistectomia Laparoscopica con Colangiografia a Fluorescenza con Verde di Indocianina versus Colecistectomia Laparoscopica Convenzionale

Titolo originale

Near-infrared ICG Fluorescence Cholangiography Laparoscopic Cholecystectomy versus Conventional Laparoscopic Cholecystectomy.

Gentile Signora

Le viene gentilmente richiesto di partecipare ad uno studio medico-scientifico. La partecipazione è volontaria e richiede la compilazione e firma di un modulo di consenso informato.

Prima della Sua eventuale adesione a questo studio Le verranno illustrate le caratteristiche e le finalità dello studio stesso.

La preghiamo di leggere attentamente questa informativa e di chiedere allo sperimentatore eventuali ulteriori chiarimenti o spiegazioni. Il paziente può discutere dello studio in questione con i propri familiari persone di fiducia. Qualora avesse delle domande o delle richieste di chiarimento dopo aver letto l'informativa è possibile contattare il coordinatore dello studio Prof. Luigi Bonini Centro Ricerche Chirurgia Mini-invasiva - Università degli Studi dell'Insubria Varese alla fine di questa informativa troverà i relativi contatti.

Le viene consegnata questa informativa in quanto Le è stato proposto l'intervento chirurgico di colecistectomia laparoscopica per calcolosi della colecisti. Il Comitato Etico locale ha approvato questo studio.

La colecistectomia laparoscopica è una delle procedure chirurgiche addominali più frequentemente eseguita nell'ambito della chirurgia addominale. Tra le possibili complicanze di questo intervento chirurgico la lesione della via biliare extraepatica ha una bassa prevalenza ma può condurre a conseguenze gravose tali lesioni richiedono spesso procedure addiuntive quali la colangio-

pancreatografia retrograda (ERCP) o il drenaggio biliare transepatico (PTBD) e talvolta la necessità di reinterventi chirurgici.

La causa principale condizionante una lesione della via biliare è la non appropriata visualizzazione dell'anatomia biliare. Utilizzando una telecamera chirurgica dedicata e iniettando un colorante specifico il Chirurgo può ottenere una migliore percezione dell'anatomia biliare e di conseguenza incrementare il grado di sicurezza e di efficienza dell'atto chirurgico stesso.

Alcuni studi già pubblicati in letteratura scientifica hanno mostrato come questa nuova tecnologia (una telecamera dedicata con possibilità di imaging a fluorescenza) possieda un valore aggiunto rispetto alle tecniche di visualizzazione convenzionali. Tuttavia gli studi condotti sino ad ora si sono basati su piccoli gruppi di pazienti. Con questo progetto si intende ampliare lo studio su questo nuovo strumento tecnologico su un gruppo di pazienti più numeroso ed in diversi ospedali europei, al fine di dimostrare se la la tecnica di imaging a fluorescenza possa fornire al chirurgo un reale vantaggio rispetto alla tecnica convenzionale.

Per condurre questo studio i pazienti verranno divisi in due gruppi: la metà dei partecipanti verranno operati con l'ausilio della tecnica di imaging a fluorescenza mentre l'altra metà verrà sottoposta ad intervento chirurgico con metodica di visualizzazione tradizionale.

La scelta dell'una o dell'altra tecnica di visualizzazione verrà determinata con procedura di randomizzazione casuale, pertanto né Lei, in quanto paziente che aderisce allo studio, né il medico che eseguirà la procedura chirurgica potrà scegliere quale metodica di visualizzazione adottare (imaging a fluorescenza versus imaging convenzionale).

[illegible]

Un'azienda di dispositivi medicali e strumentario chirurgico (Karl Storz, Tuttlingen, Germania) ha recentemente dotato i propri sistemi di telecamere chirurgiche (già ampiamente utilizzati e diffuse nel mondo) di nuovi filtri e fonti luminose in grado di poter fornire imaging a fluorescenza; con questi presidi è possibile visualizzare con maggior precisione, rispetto alle tecniche convenzionali, l'anatomia dell'albero biliare e l'anatomia vascolare distrettuale, previa somministrazione al paziente di una sostanza colorante. Questo

colorante denominato erde di Indocianina IC e viene utili ato in dia nostica medica da molti anni revalentemente in ambito oftalmolo ico e er uantificare la fun ionalit e atica dia nostica re o eratoria

Se ei acconsente a arteci are a uesto studio un rocesso di scelta randomi ata casuale determiner se l intervento di colecistectomia la aросco ica a cui ei verr sotto osto verr ese uito con tecnolo ia di ima in standard conven ionale o con tecnolo ia di ima in a fluorescen a In tal caso e verr iniettato il colorante so ramen ionato IC almeno minuti rima dell intervento c irur ico urante l intervento sar ossibile utili are la modalit di ima in a fluorescen a er me o di una telecamera ed una fonte di luce dedicata ed ottenere una visione a fluorescen a dell anatomia biliare erranno raccolti dati sui re erti intra o eratori e la rocedura verr re istrata su su orto com uteri ato er essere successivamente anali ata all interno dello studio

utti i arteci anti verranno se uiti sino al iorno dall arruolamento nello studio

er uanto ri uarda le abitudini di vita taba ismo ti olo ia di alimenta ione assun ione di farmaci etc non ven ono im oste limita ioni al a iente iorni do o la rocedura il a iente verr contattato telefonicamente er racco liere alcune informa ioni com lican e ost o eratorie effetti avversi della rocedura ese uita etc

a rocedura c irur ica a cui il a iente verr sotto osto colecistectomia la aросco ica sar la medesima a rescindere dal ru o in cui il a iente verr randomi ato e da uale ti o di ima in verr uti li ato ll ru o di a ienti sele ionati er l utili o dell ima in a fluorescen a ricever due somministra ioni endovena re o eratoria e intra o eratoria di IC

utili o del colorante IC consentito e a rovato nell essere umano

□urante l□intervento c□irur□ico verranno effettuate re□istra□ioni della □rocedura nel momento della visuali□□a□ione ed identifica□ione dell□anatomia biliare□
□o s□erimentatore dic□iara di non essere a conoscen□a di □uale delle due □rocedure □reviste dallo studio sia la mi□liore□

□□□ □ □□□□ □□□□□□□□□□□□□ □□□□□□□

Il colorante verde di Indocianina □IC□ □ □otrebbe causare rea□ioni aller□ic□e□ tuttavia □ un evento molto raro□la □ercentuale ri□ortata □ infatti inferiore a □ caso su □□□□□□□In caso di □a□ienti aller□ici allo iodio□la □ossibilit□ di rea□ione aller□ica □ □i□ elevata□motivo □er cui □uesto □ un criterio di esclusione dallo studio□
I □ossibili sintomi con cui □otrebbe manifestarsi una rea□ione aller□ica all□IC□ sono□ irre□uiete□□a□ sensa□ione di calore□ □rurito□ tac□icardia□ i□otensione□ dis□nea□ broncos□asmo□ larin□os□asmo□ edema del volto□ nausea□ arrossamento cutaneo□ Se si dovesse verificare uno di □uesti sintomi□ il □a□iente deve rivol□ersi al □ro□rio Curante e nell□eventualit□ in cui una rea□ione aller□ica ven□a confermata verranno somministrate le cure necessarie□
□a □ossibilit□ c□e si verific□i una rea□ione aller□ica viene incrementata dalla condi□ione di insufficien□a renale cronica□□er tale motivo □a□ienti affetti da tale condi□ione □atolo□ica non sono arruolabili nello studio□

□□□ □ □□□□□□□□□ □□□□□□ □□□□□□□□□ □□□□□□□□□□□□□□□□□□

□ □ossibile c□e la visuali□□a□ione delle strutture anatomic□e sia □i□ a□evole con l□utili□□o dell□ima□in□ a fluorescen□a□ tale fatto □u□ influen□are □ositivamente la durata dell□intervento c□irur□ico□ diminuendola□ □l di l□ di □uesto non vi sono condi□ioni di vanta□□io n□ di svanta□□io □er il □a□iente a □arteci□are a □uesto studio

□□□ □ □□□□ □□□□□□□□ □□□ □□ □□□□□□□□ □□□□ □□□□□□ □□□□□□□□□□ □□ □□□□□□ □□□□□□ □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□

In □uanto □a□iente□□ una Sua libera scelta □arteci□are o meno a □uesto studio□ □a □arteci□a□ione □ volontaria□ Se il □a□iente non volesse acconsentire a □arteci□are allo studio□ verr□ sotto□osto alla □rocedura c□irur□ica di colecistectomia la□arosco□ica standard□

□

Se il paziente acconsente a partecipare a questo studio, potrà in ogni momento cambiare idea ed interrompere la partecipazione allo studio stesso; in tal caso verranno utilizzati solamente I dati raccolti prima del momento dell'abbandono dello studio.

Nel caso ci fossero informazioni supplementari o modifiche riguardanti il protocollo di studio, lo sperimentatore avrà il dovere di informarLa, ed in tal caso Lei potrà decidere se procedure o meno alla partecipazione allo studio.

[illegible]

La Sua partecipazione allo studio si conclude [illegible] giorni dopo l'adesione. Lo studio verrà considerato completo quando tutti i partecipanti avranno completato i [illegible] giorni di osservazione post-operatoria.

[illegible]

Per questo studio è necessario raccogliere ed archiviare I Suoi dati personali e sanitari. Ciascun partecipante allo studio riceverà un codice che verrà in seguito utilizzato su tutti i dati raccolti; il nome del paziente non comparirà sui dati archiviati. Tutte le informazioni ed i dati raccolti rimarranno confidenziali. Lo sperimentatore ed i revisori dello studio saranno i soli a sapere a quale nominativo corrisponde ogni codice; la chiave di lettura dei codici assegnati ai pazienti rimarrà in possesso dello sperimentatore. Nella raccolta e utilizzo dati a scopo scientifico e di ricerca verranno utilizzati solo i codici assegnati ai pazienti. Lo sperimentatore conserverà i dati per una durata di [illegible] anni.

[illegible]

Il paziente non riceverà alcun compenso per la partecipazione allo studio né verrà richiesto al paziente alcun contributo per poter partecipare.

[illegible]

In caso aveste dubbi o domande, siete pregati di contattare lo sperimentatore principale (Prof. Luigi Boni – Università degli Studi dell'Insubria, Varese) o il coordinatore della ricerca (Dott.ssa Cassinotti Elisa - Tel [illegible]; email: cassinotti.elisa@gmail.com).

Grazie per la Sua Attenzione

Io sottoscritto ____

Affermo di aver preso visione dell'informativa riguardante questo studio.

Affermo inoltre di aver potuto porre eventuali domande e aver ricevuto risposte soddisfacenti.

Affermo di aver avuto sufficiente tempo a disposizione per decidere se partecipare/non partecipare allo studio.

Comprendo che la partecipazione è volontaria e che posso decidere in ogni momento di non partecipare o di ritirare la mia partecipazione dallo studio senza il bisogno di dover fornire una motivazione specifica.

Comprendo che alcune persone (sperimentatore/coordinatore dello studio) avranno accesso ai miei dati personali e sanitari.

Acconsento che i miei dati vengano utilizzati a scopo scientifico, come riportato nella lettera informativa.

Acconsento che i miei dati vengano archiviati per __ anni dopo la partecipazione a questo studio.

Acconsento ad essere contattato circa __ giorni dopo la procedura chirurgica per follow-up.

Acconsento a partecipare a questo studio.

Firma ________________________________ Data ___/___/___

Io sottoscritto (Sperimentatore/coordinatore) ________________________________

Dichiaro di aver informato in maniera esaustiva il paziente per arruolarlo nello studio.

Nel caso ci fossero informazioni supplementari o modifiche riguardanti il protocollo di studio, sarà mio dovere informare il paziente per permettergli di decidere se proseguire/non proseguire nella partecipazione allo studio stesso.

Firma ________________________________ Data ___/___/___

Patient randomization

Each surgeon will follow the standard laparoscopic surgical protocol and will comply with the following additional steps according to which arm of the study the patient is assigned:

Control arm – WLI only procedures

1. After inserting the scope in the abdominal cavity, once other standard operatory procedures (e.g., taking down adhesions if necessary, or releasing scar tissue) are completed using white light imaging (WLI) only, the Researcher will record the time when Calot's triangle is first visualized. From this moment on, the surgery will be recorded.

2. After removing the fat from the gallbladder area, lifting the gallbladder over the liver, and grabbing the Hartman's pouch to visualize the Calot's triangle, but PRIOR TO DISSECTION, the Researcher will determine what extra-hepatic biliary structures can be specifically identified. Of note: because one of the goals of the Study is to evaluate NIFC as a teaching tool, residents and juniors should record what structures they were able to identify by themselves before any intervention of the attendant surgeon.

3. Then, AFTER DISSECTION, a similar assessment of what extra-hepatic biliary structures can be specifically identified using WLI only will be performed.

4. Anatomic anomalies of the biliary system will be documented on the case form and if an anatomic variation is found, a snap shot photo will be taken in addition to the video.

5. Immediately before clipping/tying or proceeding with open surgery, the total time from Step 1 will be recorded, known herein as the “Time to Clip or Tie”;

6. Before leaving the operation room, the surgeon will complete the Intra-Operative Case Report Form.

E perimental arm IFC procedures

1. Patient randomized to the experimental arm, at least 45 mins before surgery, will receive a weight-scaled dose of indocyanine green (0.05 mg/kg) intravenously and the time of administration and any adverse reactions will be recorded.

2. After inserting the scope in the abdominal cavity, once other standard operatory procedures (e.g., taking down adhesions if necessary, or releasing scar tissue) are completed using white light imaging (WLI) only, the Researcher will record the time when the Calot’s triangle is first visualized. From this moment on, the surgery will be recorded.

3. After removing the fat from the gallbladder area, lifting the gallbladder over the liver, and grabbing the Hartman's pouch to visualize the Calot's triangle, but PRIOR TO DISSECTION, the Researcher will use IFC to determine what extra-hepatic biliary structures can be

specifically identified. This involves toggling the light source back and forth between STD mode (WLI) and ICG mode (IFC).

4. Then, AFTER DISSECTION, a similar assessment of what extra-hepatic biliary structures can be specifically identified using IFC will be performed.

5. Anatomic anomalies of the biliary system will be documented on the case form and if an anatomic variation is found, a snap shot photo will be taken in addition to the video.

6. Immediately before clipping/tying or proceeding with open surgery, the total time from Step 1 will be recorded, known herein as the “Time to Clip or Tie”;

7. Before leaving the operation room, the surgeons will complete the Intra-Operative Case Report Form

Sample size calculation

Based on the primary objective of this study, a sample size is calculated with a power of 80% and □ of 0.05 (95%-confidence). This has been done in consultation with a statistician.

A sample size of 131 patients in each randomization arm has been calculated to detect a reduction in “time to identification of CVS” of at least 5 minutes (80% power and □ □ 0.05 (two-tailed)). It is expected that this time reduction can possibly be up to 10 minutes.

Statistical Analysis

Patient clinical history (including: previous surgery, history of cholecystitis / pancreatitis, etc.) baseline characteristics (including: age, length, body weight, BMI, etc.) indications and results of the procedure, intraoperative findings (including primary and secondary endpoints), as well as hospital course and postoperative follow-up evaluation will be prospectively recorded and computerized in a database.

In general, descriptive statistics including frequencies and means will be used to characterize patient's baseline characteristics in both study arms.

Categoric variables will be compared by the Chi-Square test. Numerical variables will be compared by the independent sample T- test or the Mann-Whitney U test, depending on distribution. All p-values will be 2-sided. A P-value of less than 0.05 will indicate a statistically significant difference. All data will be analyzed on an intention-to-treat principle.

- Primary study parameter(s)

Primary outcome parameter of “time until establishment of CVS” will be given in minutes, with a mean and standard deviation.

A linear regression analysis will be applied for determination of possible significant differences between the time measurements, therewith comparing the IFC-LC group to the standard WL- LC group. This concerns the time measurements as recorded during the operation, indicated by the surgical team.

This will be conducted to determine whether a reduction in time can in fact be achieved using IFC technique compared to standard WL technique.

- Secondary study parameter(s)

All numeric secondary outcomes such as time until visualization of cystic duct and cystic artery will be analyzed with a linear regression analysis.

All categorical secondary outcomes such as bile duct injury and conversion to open surgery will be analyzed with a logistic regression analysis.

3.3. Fluorescent Angiography (FA) for Bowel Perfusion Assessment in Laparoscopic Colorectal Resection (Angiocore Trial)

3.3.1. Study Overview

This study is a multicenter randomized controlled trial with two study arms. The study is designed to investigate and validate the effectiveness of a new technology (ICG Fluorescence intraoperative Angiography) to assess bowel perfusion and reduce anastomotic leak rate in colo-rectal surgery. The comparator will be standard white light laparoscopic surgery technique.

3.3.2. Study Endpoints

- *Primary Endpoint*

Anastomotic leak rate within 30-days post-operation after left-sided colectomy and rectal resection.

- *Secondary Endpoints*

i. Intraoperative changes in planned anastomosis, including
   - the decision to undertake a permanent stoma rather than an anastomosis;
   - the site of proximal bowel used for anastomosis;
   - the site of rectal remnant used for anastomosis;
   - the decision to undertake a diverting temporary stoma (protective stoma).

ii. Operative and post-operative complications (Clavien-Dindo score)

iii. Rate of re-interventions within 30 days

iv. Length of post-operative hospital stay

v. Death within 30 days of operation

3.3.3. Study Design

- Study population

208 patients suffering from colorectal disease will be randomised prior to surgery, on a 1:1 computer basis, to either surgery with fluorescent angiography (FA) or surgery without FA.

All patients will undergo standard preoperative work-up and standard post-operative hospital care. The trial will not be blinded to participants, medical staff, or clinical trial staff.

- *Subject Selection*

  - *Inclusion Criteria*

v. Patients of both genders

vi. Minimum age: 18 years old

vii. Ability to understand and follow the study procedures and sign the informed consent

viii. Surgical candidates to elective laparoscopic left side colectomy or anterior rectal resection both for benign and malignant disease

  - *Exclusion Criteria*

v. Recurrent rectal cancer

vi. Locally advanced rectal cancer requiring extended or multi-visceral excision

vii. Known allergies to iodides

viii. Known moderate or severe chronic kidney disease (defined as eGFR 40mmol/l)

ix. Women who are pregnant or breastfeeding, or for whom possibility of pregnancy was not ruled out

Setting

Participants will be recruited by the Department of Surgery at the following study sites, all of which are recognized high volume laparoscopic colorectal surgery centers:

- University of Insubria (Varese, Italy) – Co-Principal Investigator
- Università Vita Salute San Raffaele (Milano, Italy) – Co-Principal Investigator

Surgeons highly trained in laparoscopic colorectal surgery will participate in the study.

Enrollment Process

Eligible patients will be identified through clinical and test evaluation. Eligibility will be verified by the patient's primary surgeon. The surgeon will determine the indication and date of the surgery. Once a patient is confirmed as eligible, the surgeon will introduce the study in detail. If after being introduced to the study and having had the opportunity to ask questions, the patient is willing to participate, he/she will be asked to review and sign the informed consent document [Form 5].

Upon entry in the clinical trial patients will be randomly allocated to the intervention arm. Data will be collected at enrollment time, during surgery, at the end of surgery, one week after surgery and one month after surgery.

Patients are not asked to undergo additional testing after the surgical procedure, they are also not requested to fulfill any questionnaire.

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Randomization Process

Upon entry in the clinical trial, according to the study database patients will be randomly allocated to one of the study arms (1:1) within site (1:1) using a computer generated random sequence.

Patients, surgeond and medical staff will not be blinded.

Basically two groups of patients will be compared:

1. Control:

   A standard laparoscopic colorectal resection and anastomosis construction will be performed using Xenon white light imaging (WLI) only.

2. Experimental:

   A laparoscopic colorectal resection and anastomosis construction (with standard surgical technique) with use of intraoperative Fluorescence Angiography with indocyanine green (ICG).

Data collection and procedures

Data will be collected before, during and at the end of surgery, one week after surgery and 30 days after surgery [Form 6].

Form 6 Angio-Co-re Trial - Case Report Form CRF

Protocollo **Codice Paziente**

Dati anagrafici del paziente

Data di Nascita _ _ / _ _ / _ _ _ _ Età _ _ Sesso

Peso _ _ _ Kg Altezza _ _ _ cm _ _ _

Dati clinici Procedurali

ASA _ Creatinemia _ _ _ mg/dl

Diabete Mellito Ipertensione Arteriosa Precedente PTCA

Consenso informato

Data di acquisizione _ _ / _ _ / _ _ _ _

Criteri di inclusione

Età maggiore di 18 anni

Intervento chirurgico di resezione colorettale per neoplasia laparoscopica

Criteri di esclusione

Insufficienza renale di grado

Allergia all' ICG o ai farmaci contenenti iodio

Stato di gravidanza o allattamento

Intervento

Data del ricovero _ _ / _ _ / _ _ _ _

Data dell'intervento _ _ / _ _ / _ _ _ _

Data della dimissione _ _ / _ _ / _ _ _ _

Patologia oggetto di studio

Tipo di Patologia _ ENDOMETRIOSI _ ADENOMA

Chemioterapia neoadiuvante

Radioterapia neoadiuvante

Braccio di assegnazione

Braccio di assegnazione _ SPERIMENTALE _ CONTROLLO

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Study procedures

Patient randomization

Each surgeon will follow the standard laparoscopic surgical protocol and will comply with the following additional steps according to which arm of the study the patient is assigned:

Control arm – SI only procedures

For participants randomised to the surgery with no FA arm, the laparoscopic colorectal resection - left colectomy or rectal anterior resection (high or low) - will be performed according to the surgeon's usual technique, using standard laparoscopic approach, with white light assessment of bowel perfusion. This, otherwise specified, is conventionally based on surgeon's subjective visual evaluation.

Colo-rectal/anal anastomosis will be performed according to surgeon's preference (hand-sewn, stapled, end-to-end, end-to-side, colo-pouch etc.)

The level of colonic transection, formation of colo-rectal/anal anastomosis, and defunctioning stoma will be performed according to normal practice.

Experimental arm – FA procedures

For participants randomised to the surgery with intraoperative FA, the colorectal resection - left colectomy or rectal anterior resection (high or low) - will be performed according to the surgeon's usual technique, using laparoscopic approach. The specifics of each operation, including the decision to make a change to the planned anastomosis following FA assessment, will be at the discretion of the operating surgeon.

The left colon and/or rectum will be mobilised and distal transection will be performed. Distal point of transection will be on high rectum or rectal-sigmoid junction in left colectomy, on medium-low rectum in rectal anterior resection.

The proximal colon will be assessed under white light, with standard surgeon's subjective visual evaluation, and the point of planned transection marked.

0.1-0.3mg/kg of 5mg/ml ICG (reconstituted as per the manufacturer's instructions) will be administered intravenously and proximal colonic and rectal stump perfusion assessed using near-infrared light.

Any change in the planned colonic transection level or revision of the rectal stump as a result of FA assessment will be recorded and registered on CRF indicating the extent of the revision in centimeters.

The intensity of fluorescence, that corresponds to bowel perfusion, will be assessed subjectively by the surgeon as "good perfusion", "borderline/reduced perfusion", or "no perfusion" (ischemia).

Colo-rectal/anal anastomosis will be performed according to surgeon's preference (hand- sewn, stapled, end-to-end, end-to-side, colo-pouch etc.).

Use of a defunctioning stoma will be at the discretion of the surgeon, with the reason for defunctioning and the relation to IFA assessment will be documented.

Another dose of ICG will be permitted (e.g. extracorporeal assessment, or immediately prior to anastomosis) should the surgeon feel this to be beneficial, the dose and timing of any additional ICG dose should be recorded on the CRF.

Post□operative Care and Clinical Assessments

Post-operative care will be as per institutional protocol and this can include enhanced recovery after surgery pathways. There will be no difference in post-operative treatment based on randomization arm.

Participants will be reviewed in an outpatient clinic on post-operative day 7 (if they have already been discharged) and on post-operative day 30.

Sample Size Calculation and Statistical Analysis

Based on the primary objective of this study, a sample size is calculated with a power of 80% and □ of 0.05 (95%-confidence). This has been done in consultation with a statistician.

Anastomotic leak (AL) following rectal cancer resection is reported between 8% and 25%. Recent evidence, which takes into account innovation in laparoscopic technique and stapler technology, documents a leak rate between 10% and 15%. We will assume an overall AL rate of 12.0%, as a value that most colorectal surgeons would accept.

An estimated sample size of 208 patients (104 in each randomization arm) has been calculated to detect a significative difference between the “null” hypotesis that AL rate is 15% in both groups and the alternative hypotesis that in FA group the incidence of AL will be possibly reduced to 3%. A □2 test with p □□ 0.05 will be used.

For the statistical analysis SPSS Windows (22nd edition) will be used.

Secondary endpoints with binary measures (change in planned anastomosis, defunctioning stoma, complications, anastomotic leak, rate of re-intervention and death) will also be analysed using multi-level logistic regression adjusting for the stratification factors, incorporating random effects with respect to surgeon.

Secondary endpoints with continuous measures – length of stay, LARS score, other quality of life scores – will be analysed using multi-level generalised linear models incorporating random effects with respect to surgeon and assuming Normal errors at the patient level.

?. ?ES? L?S

?.? ?ndocyanine green Fluorescence Colangiography ??FC??rial

From May 2016 to ?une 2017 a total of 171 patients (85 in the Indocyanine green Fluorescent Cholangiography group and 86 in White Light control group) were enrolled overall in partecipating centers.

According to the study design, as previously mentioned, the expected number of cases is 152 patients per study arm; the trial is still ongoing, so results presented are preliminary.

□ *Preoperative Data*

Patients preoperative demographics (age, sex, BMI) were comparable between the two groups.

Cholelitiasis was the main indication for surgery: in IFC group 94% of patients and 95% in the control group respectively. In remaining cases the indication was adenoma or benign polyp. 21 patients of IFC group (25 %) and 26 in WL group (19 %) underwent laparoscopic cholecystectomy for acute cholecystitis in urgent setting [Table 4].

Table 4 - Preoperative Data

	IFC Group (n = 85)	WL Control (n = 86)	p
Age (mean)	58 ± 12	56 ± 11	NS
Sex (M/F)	39/46	32/54	NS
BMI (mean)	25,8	26,4	NS
Surgery Status			
Elective	64 (75%)	70 (81%)	NS
Urgent	21 (25%)	26 (19%)	NS
Lithiasis			
Yes	80 (94%)	82 (95%)	NS
No	5 (6%)	4 (5%)	NS

- *Intraoperative Data*

No side effects or allergic reaction related to the injection of ICG have been reported. To date our primary endpoint (Time of establishment of CVS) has not achieved statistical significance, although the mean value (in minutes) is lower in IFC group.

The other intraoperative findings showed no significant difference between the two groups [Table 5].

Table 5 - Intraoperative Data

	IFC Group (n = 85)	WL Control (n = 86)	p
Time Establishment CVS (mean value)	**[illegible] min**	**[illegible] min**	**S [illegible]**
Operative Time (mean value)	58 min	56 min	NS
Blood Loss (mean value)	27 ml	29 ml	NS
Bile Leakage	3 (3.5%)	4 (4.6%)	NS
Conversions	1 (1.2%)	2 (2.3%)	NS

Rate of biliary structures visualization, before and after dissection of the Calot's triangle, showed significant advantage in structure identification for the IFC study group [Table 6-7].

Table 6 - Intraoperative Data. Biliary ducts identification before dissection.

	IFC Group (n = 85)	WL Control (n = 86)	p
Visualization BEFORE dissection			
Cystic Duct	80 (94%)	51 (59%)	<0.05
Common Hepatic Duct	31 (36%)	11 (13%)	<0.05
Common Bile Duct (CBD)	81 (95%)	49 (57%)	<0.05
Cystic-CBD junction	49 (58%)	29 (34%)	NS
Cystic -Gallbladder junction	57 (67%)	32 (37%)	<0.05
Accessory Ducts	1 (1%)	0 (0%)	NS

Table 7 - Intraoperative Data. Biliary ducts identification after dissection.

	IFC Group (n = 85)	WL Control (n = 86)	p
Visualization AFTER dissection			
Cystic Duct	82 (96%)	81 (94%)	NS
Common Hepatic Duct	39 (46%)	14 (16%)	<0.05
Common Bile Duct (CBD)	82 (96%)	58 (67%)	NS
Cystic-CBD junction	78 (92%)	41 (48%)	<0.05
Cystic -Gallbladder junction	78 (92%)	76 (88%)	NS
Accessory Ducts	1 (1%)	1 (1%)	NS

Post-operative outcomes and complications

Post-operative outcomes and complications are described in Table 8.

To date only one case of bile duct injury (BDI) has been reported in the WL control group. Hospital stay, postoperative complication rate and mortality were comparable between groups.

Table 8 - Postoperative outcomes.

	IFC Group (n = 85)	WL Control (n = 86)	p
BDI	0 (0%)	1 (1.1%)	NS
Hospital stay (days)	1.6 ± 2	1.9 ±2	NS
Postoperative complications	5	6	NS
Mortality (30 days)	0 (0%)	0 (0%)	NS

?.? Fluorescent Angiography ?FA?for Bo? el Perfusion Assessment in Laparoscopic Colorectal ?esection ?AngioCo?e ?rial?

From March 2016 to ?une 2017 a total of 152 patients (76 in the Fluorescent Angiography group and 76 in White Light control group) were enrolled altoghether in both partecipating centers.

According to the study design, as previously mentioned, the expected number of cases is 104 patients per study arm; the trial is still ongoing, so results presented are preliminary.

?□ *Preoperative Data*

Patients preoperative demographics were comparable except for statistically significantly higher incidence of cardiovascular medical history in the FA group. Resection for malignancy was the main indication for surgery: in FA group 24% of patients underwent surgery for benign disease (mainly diverticular disease)

while 76% of patients were operated for colorectal cancer. In control group 21/76 patients (28%) were admitted for benign disease while the other 55/76 (72%) for cancer. There is no statistically significant difference between the groups [Table 9].

Table 9 - Preoperative Data.

	FA Group (n = 76)	WL Control (n = 76)	p
Age (mean)	69 ± 13	67 ± 15	NS
Sex (M/F)	44/34	38/40	NS
BMI (mean)	28 ± 7	29 ± 6	NS
ASA score			
ASA 1	24 (32%)	22 (29%)	NS
ASA 2	32 (42%)	34 (45%)	NS
ASA 3	20 (26%)	20 (26%)	NS
Cardiovascular Disease	41/76 (54%)	37/76 (48%)	NS
BPCO	12/76 (16%)	8/76 (10%)	NS
Diabetes	11/76 (14%)	12/76 (16%)	NS
Benign Disease	18/76 (24%)	21/76 (28%)	NS
Malignant Disease	58/76 (76%)	55/76 (72%)	NS

Intraoperative Data

No side effects or allergic reaction related to the injection of ICG have been reported.

In FA group 34 patients (44%) underwent laparoscopic left colectomy while 42 (56%) underwent laparoscopic rectal resection; the type of surgery in control group was laparoscopic left colectomy for 31 patients (41%) and laparoscopic rectal resection for 45 (59%); also in relation to this data there is no statistical difference between the two arms.

The mean operative time for FA group was a mean value of 144 ± 49 minutes versus 162 ± 42 min in the control group. Even if it is not significant, this might

suggest that the use of fluorescence imaging and technology is definetely not time consuming.

A diverting stoma was created in 8 patients of FA group and in 17 of the WL arm; considering that the two groups are homogeneous in terms of type of surgery and disease, this is a relevant fact supporting the potential benefit of real-time intraoperative fluorescent angiography in colorectal surgery. The two groups were comparable in terms of blood transfusion [Table 10].

Table 10 - Intraoperative Data.

	FA Group (n = 76)	WL Control (n = 76)	p
Type of operation			
Left Colectomy	34	31	NS
Anterior Rectal Resection	42	45	NS
Conversion	1	2	NS
Stoma	8	17	
Lateral ileostomy	8	16	NS
Lateral colostomy	0	0	NS
Terminal Ileostomy	0	0	NS
Terminal colostomy	0	1	NS
Abdominal Drain			
Yes	47	68	
No	29	8	
Operative Time (mean)	144 ± 49	162 ± 42	NS
Blood Transfusion (Y/N)	11	16	NS

FA and change of surgical strategy

No changes in surgical plan are described before performing FA. In all cases (both groups), the surgical team judged the perfusion of the colon “adequate” on standard white light, according to the subjective evaluation and surgeons experience, and no visible sign of ischemia was evident at visual inspection.

ICG enhanced fluorescence was detected in 100 % of the cases where it was used; there have been no reports of failure of the FA technique. A mean value of 12,5 mg were administered to each patient.

In FA group ICG fluorescence technique showed inadequate perfusion of the colic stump in 8 cases (10,5%). In all the cases (8/8) was the distal part of proximal bowel to be ischemic or hypoperfused while in any case it was on the rectal stump.

In 3/8 cases the inadequate perfusion was classified by the surgeon as “Reduced perfusion” while in 5/8 cases as “No perfusion”. All these patients (8 cases) had an extended resection, from 2 to 7 cm (mean value: 3 cm), until a viable well-perfused colon segment was obtained [Table 11].

Table 11 - FA group intraoperative data.

	FA Group (n = 76)
ICG adminstered (mean)	12.5 mg/pt
Evaluation of Perfusion	
Good perfusion	68 (89.5%)
Reduced perfusion	3 (4%)
No perfusion (ischemia)	5 (6.5%)
Change of Strategy	
Proximal bowel	8 (10.5%)
Distal bowel (rectum)	0
Both	0
Extended Resection	
n cases	8 (10.5%)
cm (mean value)	3

Post-operative outcomes and complications

Post-operative outcomes and complications are described in Table 12.

In study group 11/76 (14%) patients had complications, while in the control group the complication rate was 17/76 patients (22%). No difference in overall postoperative morbidity was observed in the two groups.

In particular anastomotic leak developed in 7 (9,2%) patients of the control group and in 3 (3,9%) patients of the study group (p: 0.05).

Table 12 - Postoperative outcomes.

	FA Group (n 76)	WL Control (n 76)	p
Postoperative leak	3 (3,9%)	7 (9,2%)	NS
eak treatment			
Conservative tp	2	2	NS
Percutaneous Drain	0	1	NS
Reintervention	1	4	NS
Severity of complication			
Mild (Clavien Dindo 1)	6	6	NS
Moderate (Clavien Dindo 2)	2	3	NS
Severe (Clavien Dindo 3–5)	3	8	NS
Mortality (30 days)	0	0	NS
Lenght of hospital stay (days)	6 4	7 4	NS

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ndocyanine green Fluorescence Colangiography FC rial

Bile duct injuries (BDI) represents a rare but severe complication of laparoscopic cholecystectomy (LC), with a reported incidence of 0.4-0.8%; it is the major cause of severe post-operative morbidity after this very common surgical procedure. Misperception and misinterpretation of the biliary anatomy is the main recognized cause of BDI: fluorescence guided surgery may help surgeons identfying and preserving biliary structures [75-78].

Since BDI rate is very low, to demonstrate the advantage of indocyanine green fluorescent cholangiography (IFC), we based our study on the hypothesis that this new technology might significantly reduce the time necessary to complete Calot's triangle dissection and achieve the Critical View of Safety (CVS).

Several authors have already described the use of IFC both in laparoscopic and robotic cholecystectomies, reporting that this methodic is safe and feasible. To date no randomized controlled trials have been conducted [96, 101, 105, 139-141].

Regarding our primary outcome (time to obtain CVS), so far we could demostrate an advantage in IFC group (21.3 minutes vs 25.3 minutes in WL group), still with no statistical significance, although *p* it is 0.060, so we believe that at the end of recruitment our endpoint will be achieved.

Many reports and patients series (including our first experience on 52 patients published in 2015) already demonstrated IFC to be an effective instrument to identify biliary anatomy, reporting very high rate (90%) of cystic duct

fluorescence identification [60,61,67,105,141].

Our preliminary data are comparable to existing literature with IFC allowing visualization of at least one biliary structure in ≥99% of cases.

In our trial we compare biliary ducts detection rate in IFC group to a randomized control group performed with white light and we can clearly demonstrate, even in preliminary data, that there is a significant difference in biliary structures identification, especially before surgical dissection of Calot's triangle. Additionally, as predictable, the visualization rates increase even more after dissection.

It has to be noticed that in 5 (6%) of cases in the IFC group, we reported that CD was not detectable (before dissection manouvers) and in 3 (3.5%) cases it remained not fluorescent even when dissection was completed and CVS achieved. This was due to severe inflamation of gallbladder and surrounding tissues and/or when CD was "excluded" (in these cases common bile duct and common hepatic duct were clearly visible under fluorescence). The same reason for lack of CD visualization have been reported in other studies, especially those including acute cholecystitis cases as the large series from Daskalaki et al. in 2014 [60, 141].

As regards mean operative time, we could not demonstrate yet a difference between IFC study group and WL controls. This might be explained by an "extended" operative time for the second part of the procedure (gallbladder dissection from liver bed, emosthasis, specimen extraction and closure). Nevertheless, in agreement with literature, our findings demonstrate that IFC is not a time-consuming additional procedure [61,67,142,143].

Our data regarding post-operative outcomes are comparable to literature in terms of lenght of hospital stay, postoperative complications and mortality; in particular we reported no cases of BDI in sperimental group (0/85 patients) and 1 case in control group (1/86 patients - 1.1%), however the number of patients included in the trial to date is still too low to make a statement [104].

Fluorescent Angiography (FA) for Bowel Perfusion Assessment in Laparoscopic Colorectal Resection (AngioCore trial)

Anastomotic leak (AL) is a significant complication of colorectal resection and leads to increased length of stay, cost, local recurrence, and mortality rates [111,121]. Despite advances in surgery, there has been small progress in reducing the rate of AL over the past 50-years.

It has been demonstrated that one of the crucial factor in anastomosis healing is good bowel blood perfusion [131,132]; as previously explained this is an area in which improvement may be achieved with the introduction of a new technology.

Fluorescence Angiography (FA) with ICG allows to real-time assess intraoperative perfusion. It has already been reported in literature that FA is a safe and feasible technique and we supported these findings in our previous published series [108, 144-148].

Nevertheless most of published studies had a small population, no control group, and none of the studies were randomized controlled trials. It is notable that none of the studies had a prospective calculation of the study size, which mean a low statistical power.

Our aim is to support existing evidence and validate the methodic through a randomized controlled trial.

The largest published study, to date, is PILLAR II where this technique was tested on 147 colorectal resections: they reported no adverse events and successful fluorescent imaging in 98,6% of cases [149].

Preliminary results of Angio-Co-Re trial confirm that FA is a safe and effective technique (no adverse reaction and fluorescent imaging obtained in 100% of study group cases).

A disadvantage of ICG-FA is that the assessment of fluorescence intensity is subjective. This means that, according to technology currently in use, we can not provide an objective measurement of fluorescence signal.

In our study we ask surgeons to “quantify” FA intensity with a three-step score trying to achieve a more objective perfusion assessment. The same method, a five-step score, was adopted by Sherwinter et al. [147], while two other studies quantified the light intensity by intraoperative pixel brightness analysis [144, 148]; unfortunately the quantification did not lead to a cutoff value in any of the two studies.

As we show in our preliminary results, most of the studies on colorectal anastomoses report changes in surgical strategy if FA detect unsatisfactory or insufficient perfusion (□ischemia).

In six studies, resection of the colon or rectum was extended into more vital tissue if ICG-FA detected an insufficient perfusion at the site intended for construction of the anastomosis [108, 145, 148, 150-152]. So far Angio-Co-Re

trial results on change of strategy in case of hypoperfusion (10.5% of FA group patients) is alligned to largest clinical prospective studies such as Jafari et al. in the PILLAR 2 (change in surgical plan 7.9% of cases) [149]. In three studies, no changes were done in the surgical procedure due to the results of ICG-FA but they were conducted few years ago, on small cohort of patients and FA was used only to check perfusion after anastomosis completion [147, 153, 154].

Regarding anastomotic leak (AL) rate, a recent review conducted on 13 studies on fluorescence bowel perfusion assessment, reported a mean leak rate of 3.8% in patients undergone FA, regardless of intraoperative changes in surgical procedure [155]. In our preliminary analysis we report AL rate of 3.9% for the FA study group (compared to 9.2 % in the controls); still there is no statistically significant difference between the groups but data are encouraging and further enrollment and results may support our primary endpoint.

Once more our data are comparable with the large series of PILLAR II, especially considering that both studies focus on left colectomy and rectal resection with anastomosis construction in the last 15 cm of the bowel. This is undoubtely an "high risk" group in terms of anastomotic leakage: a recent systematic review and meta-analysis of 98 prospective studies on rectal surgery, found no difference in leak rate between those studies published after 2003 compared to earlier investigations [156, 157]. Since AL rate in rectal resection remains unmodified over the years (10-12 % mean), our result on 76 patients undergone FA (3.9%) is certainly a promising reduction.

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The emerging field of fluorescent surgical imaging promises to be a powerful enhancement to improve surgical guidance.

In less that a decade the number of fluorescence guided surgery systems available commercially has considerably grown; it is well demonstrated that this technology has a great potential to become a standard in everyday clinical practice due to the multiple different possible applications and the ease of employ.

The challenge now is to validate main potential clinical applications with large randomized controlled study in order to establish FGS as a recommendation or a standard of practice in clinical care.

In last years we tested near infrared fluorescence techonology in several fields of general surgery. In this study we focused our efforts on two of the most promising applications of FGS: indocyanine green fluorescent cholangiography (IFC) in laparoscopic cholecystectomy and fluorescent angiography (FA) to assess bowel perfusion in colorectal surgery.

In conclusion it has been demonstrated that IFC is a safe and effective procedure to enable real-time visualization of biliary anatomy; it may become a standard of care in order to prevent bile duct injuries and it might replace standard intraoperative cholangiogram allowing a more accurate and less invasive identification of bile ducts. Once the RCT will be concluded, we hope that our final results will strongly support and validate this technique.

As regards to FA in colorectal surgery, from our preliminary results we can conclude that it is a safe and feasible tool to guide the surgeon in intraoperative decision-making process. The evidence of FA clinical benefit has not be

demonstrated yet and we designed our trial in order to to assess the impact of this technology on colorectal surgery. In preliminary data we reported the effectiveness of this technique in reducing the incidence of anastomotic leakage, although only final results could define if FA might be a standard of care.

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