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CHAPTER

3

Immunofluorescence and Robotic Surgery

Vivek Bindal, Anand Kumar Yadav

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INTRODUCTION

Information about the operative field is one of the most desired aspects of surgery in any given procedure from a surgeon's perspective. Minimally invasive surgery has increased the need for better visualization techniques because of the reduced amount of direct feedback from tissues that the surgeon usually gets in open surgery. Immunofluorescence imaging technology combines the three-dimensional high definition view, and many such details in robotic surgery, which are otherwise not available to the surgeon.

Immunofluorescence imaging enables the use of electromagnetic spectrum between 700 nm and 1,000 nm, which is invisible to the human eye, and along with fluorescent tracers produces fluorescence, thus helping in identification. Immunofluorescence spectrum has many advantages: it is invisible to the robotic camera, so it does not change the appearance of the surgical field unless the camera is activated to view the spectrum. It is nonionizing and has a decent tissue penetration (up to 5 mm), without any unfavorable effect on the tissue. As image acquisition takes only a few milliseconds, this technique provides real-time guidance during a procedure.[1]

Immunofluorescence imaging technique requires a contrast agent containing a molecule, called a fluorophore which on excitation by a immunofluorescence light source emits fluorescent light. Depending on the application, a targeting ligand directs the fluorophore to the structure under study. Visualization of the tissue is based on the signal of the contrast agent in the region of interest relative to the background signal, known as signal-to-background ratio (SBR). The only immunofluorescence fluorophores that are registered with the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical use are indocyanine green (ICG) and methylene blue (MB).[1]

On illumination with immunofluorescence light, protein-bound ICG emits light that peaks at approximately 840 nm **(Fig. 3.1)**.[2] This fluorescence property of ICG was revealed in detail back in the 1970s, and the clinical application of real-time fluorescence imaging using ICG started in the early 1990s in the field of ophthalmology with fundus angiography.[3] This application of imaging was extended to surgery as an intra-operative navigation tool of lymphatic flow, sentinel lymph node mapping, determining blood flow during coronary artery bypass grafting, and identifying cerebral aneurysms for clipping.[4-7] ICG is safe to use, with rare complications.[8,9]

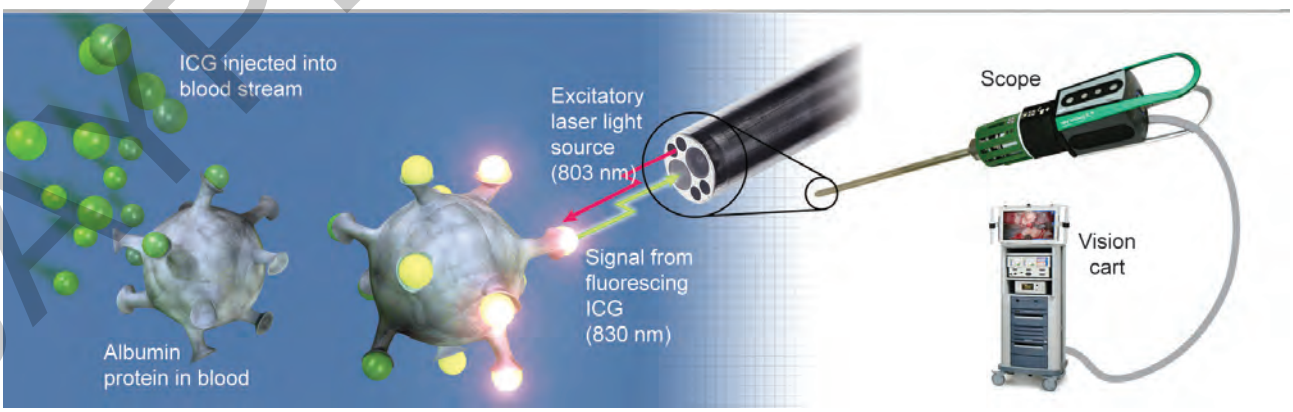


Fig. 3.1: The da Vinci Si surgical system integrated fluorescence imaging capability providing real-time, image-guided identification of key anatomical landmarks using near-infrared technology. Copyright © [2016] Intuitive Surgical, Inc

Clearance of the ICG in intravascular circulation happens rapidly by liver and much gets excreted in bile in particular. During laparoscopy procedure and robotic surgical procedures ICG is utilized mostly as fluorophore. By 2011 the Fluorescence Imaging Vision System got integrated in robotic platform like Da Vinci. After that there has seen a rapid expansion in most of the fields of the robotic surgery due to the critical role of immunofluorescence-ICG imaging. To date, there are many publications available as papers in scientific literature on various roles and responsibilities of fluorescence imaging in robotic procedures. However, there is limitation in experience as the technology is considered relatively new and few medical centers have initiated the use of this technique. The major operations where immunofluorescence-ICG is utilized are general surgery, urologic surgical procedures, thoracic and gynecologic robotic surgeries.[10-15]

Since 120 years methylene blue (MB) has been used for several medical procedures. When dilution happens to an extent which makes it nearly undetectable to normal human eyes, Methylene blue turns out to emit fluorophore at ≈700 nm.[16] This is known as 'unquenching' phenomena. At higher concentration, emission of fluorescence from methylene blue has intermolecular re-absorption which on dilutions, turns out unquenched. Methylene blue gets metabolized by both hepatic and renal tissues equally, allowing the imaging of both bile duct and ureters.

Identifying the cancers in real-time by utilizing fluorescence probes has recently become a very active field of research. Dye agents like methylene blue and indocyanine green (ICG) are considered as nonspecific immunofluorescence contrast agents. Novel immunofluorescence fluorescent probes are being developed to permit targeted imaging. To target a tumor cell the fluorophores are conjugated to any of the tumor specific ligand like tumor-specific antibody, nano body, small peptides. Alternatively they could be activated by enzymatic cleavage for fluorescence activity.[17]

CLINICAL APPLICATIONS OF IMMUNOFLUORESCENCE IMAGING IN GENERAL SURGERY

Following intravenous administration of ICG, in between 5 s and 60 s, initially arteries are seen following which veins can be visualized **(Fig. 3.1)**. This phase lasts only for few minimum seconds, but extra dose of ICG can be injected if required. The maximum recommended dose for an adult is 2 mg/kg with high toxicity margin.[8,9] Later, micro perfusion of organs and tissues gets more evident, followed by the lymphatic channels and neural anatomical structures. After nearly 45–60 minutes ICG gets accumulated in the hepatic tissues and secreted in bile, and hence can be used to assess liver and bile duct. Immunofluorescence imaging has many significant advantages in robotic surgery for various surgical operations. It can be used while dissecting a blood vessel or lymph node and it can help in identification of blood perfusion and hence viability of tissue before doing an anastomosis. It can help in identifying an anatomical structure or any lesion which needs resection (e.g. tumors) or protected (e.g. bile duct) **(Figs 3.2 and 3.3)**. Researchers are now making efforts to enable the targeted imaging, which in future will be able to change the way robotic procedures are performed.

Immunofluorescence imaging's used in various clinical applications are summarized in **Table 3.1**.

Biliary Imaging

In general surgery, ICG fluorescence can be used for identifying the biliary anatomy which is considered very

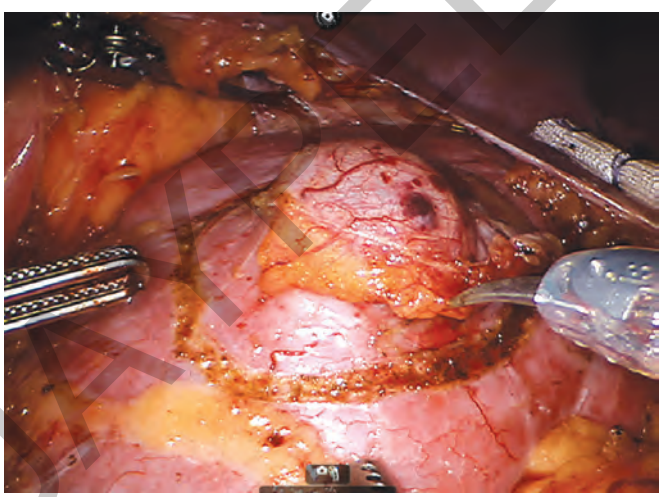


Fig. 3.2: Normal 3D HD illumination mode during da Vinci® Partial Nephrectomy. Firefly fluorescence imaging endoscope view before activation: Renal parenchyma. Copyright © [2016] Intuitive Surgical, Inc

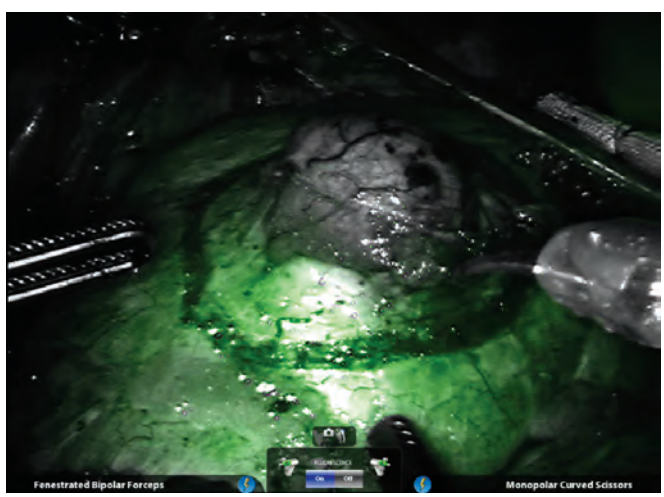


Fig. 3.3: Assessing parenchymal perfusion during tumor excision in FireFly™ fluorescence imaging mode during da Vinci® partial nephrectomy. Firefly fluorescence imaging endoscope view after activation: Renal parenchyma. Copyright © [2016] Intuitive Surgical, Inc

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Table 3.1: Clinical applications of immunofluorescence in robotic surgery

Imaging in robotic surgery	
Biliary imaging: Fluorescent cholangiogram	Example: Extrahepatic biliary ducts during cholecystectomy and hepatectomy
Vascular imaging: • Anatomy of blood vessels • Organ and tissue perfusion • Blood flow	 Example: Hepatic artery during hepatectomy, splenic artery and vein during distal pancreatectomy Example: Recognizing vital tissue during gastrointestinal anastomosis, renal perfusion after transplant Example: After vascular anastomosis
Lymphatic channels: Lymph node identification	Example: Sentinel lymph node in endometrial cancer
Specialized tissue: • Nerve identification • Parathyroid identification	 Example: Phrenic nerve during thymectomy, inferior hypogastric plexus during pelvic dissection, recognition of parathyroids during thyroidectomy
Identification of lesions, based on their vascular and metabolic pattern	Example: Well- vs poorly differentiated HCC, neuroendocrine lesions, angiomas

important application. Immunofluorescence imaging helps in reducing the risk of biliary duct injuries in cholecystectomy. ICG permits better visibility of the extrahepatic biliary ducts in real time, thereby allowing a constant monitoring by 'nonradiographic' cholangiogram. Using the immunofluorescence-ICG during a robotic cholecystectomy procedure had been described as to how the ICG fluorescence had capabilities to help in identifying an aberrant biliary side branch from hepatic segment VI to common hepatic duct during robotic procedure. The side branch could be preserved with success, and postoperative biliary leaks were avoided.[18] Other reputable authors have also published their experiences and confirmed the advantage of the technique.[19,20] Buchs et al. initially reported their assessments following 12 robotic cholecystectomies with immunofluorescence-ICG at single site. The cystic duct, common bile duct and common hepatic duct (CD, CBD, CHD) were visualized with success prior to dissections in Calot's triangle area among 91.7%, 50%, 33% of the cases, respectively. Following the surgical dissection, the visualization rates for the three anatomical structures were 100%, 83.3%, 66.7%.[19] Spinoglio et al. had published their evaluations after completion of 45 cases of robotic cholecystectomy with immunofluorescence-ICG at a single site, with successful visualization of three main biliary anatomical structures at a rate of 97% following dissection of Calot's triangle.[20] Daskalaki et al. had reported their results after 184 cases of robotic cholecystectomies where cholangiography had ICG fluorescent as an agent.[10] The overall rate of visualization for the cystic, common hepatic and common bile was 97.8%, 94%, 96.1%, respectively. Visualization rates of biliary structures in complex cases, like acute cholecystitis and patients with obesity were analyzed independently. In the first group, the CD, CBD, CHD were identified with success among 91.6%, 79.1%, 79.1% of patients respectively. In the second group of patients the visualization rates were 97%, 94%, 95%. The near-infrared light had the penetration ability of approximately 5–10 mm,[21,22] managing issues like presence of inflammation and obese patients that might potentially affect visualization of anatomical structures. It is important to note that, there were no biliary duct injury or any allergic reaction to ICG in any of these published series of articles. Immunofluorescence-ICG might improve the outcome of cholecystectomies, by replacing the usual X-ray method of cholangiograms, with no significant increase in operation time and with minimum risk of any adverse effect due to the dye.

Identification of the vascular and biliary structures during complex procedures such as hepatic resections and pancreaticoduodenectomies by immunofluorescence imaging allows a more precise and accurate dissection, potentially decreasing the risk of intraoperative complications.

Vascular Imaging

Identification of arteries and veins is one of the most interesting applications of the indocyanine green. Identifications of normal anatomical structures and/or recognition of the variations of anatomical structure in real-time decreases the risk of inadvertent injuries to nearby vascular elements. This can be considered as a substantial improvement, in any simple cholecystectomy operation (where vascular structures identification could reduce the risks of inadvertent injury of anomalous and accessory blood vessels), as well as in any complex situations of liver or pancreas resection (where identifying and dissecting any major blood vessel is a critical step to successful completion of the procedure).

Recently, immunofluorescence-ICG had been utilized in urology for clamping of selective arterial blood vessel (often referred to as 'firefly') in procedures like robotic partial nephrectomy **(Figs 3.4 and 3.5)**. Borofsky et al. published their first assessment in 34 patients of zero-ischemia robotic partial nephrectomies (RPNs) and opined that this imaging technique helps in super-selective arterial clamping methods, resulting in better functional outcomes.[23]

Organ and Tissue Perfusion

Evaluation of tissue perfusion is another important application of immunofluorescence imaging. Partial ischemia may lead to anastomotic leaks, which is a dreaded complication. Many a times the color demarcation is not precise and the Doppler scan may require specific probes which may show artifacts like poor contact or interphase. Adequately done ICG microperfusion assessment in colorectal operations and

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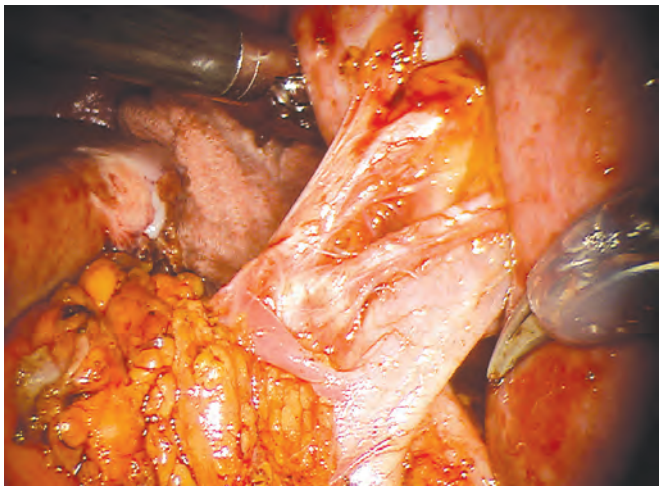


Fig. 3.4: Normal 3D HD illumination mode (view of renal hilum) during da Vinci® partial nephrectomy. Firefly fluorescence imaging endoscope view before activation: Renal hilum. Copyright © [2016] Intuitive Surgical, Inc

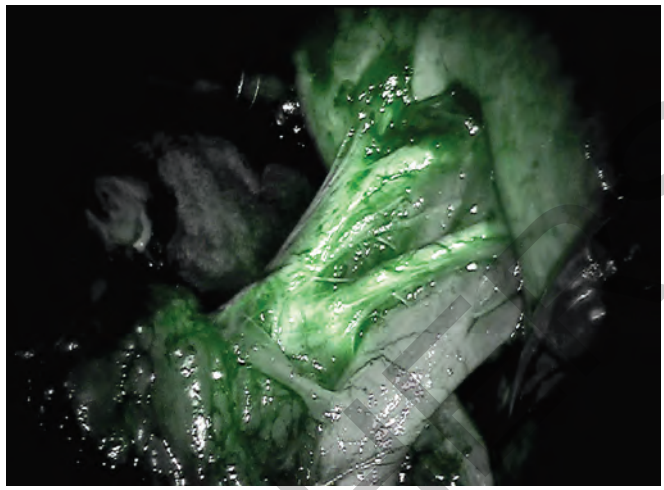


Fig. 3.5: Vessel identification in FireFly® fluorescence imaging mode (view of renal hilum) during da Vinci® partial nephrectomy. Firefly fluorescence imaging endoscope view after activation: Renal hilum. Copyright © [2016] Intuitive Surgical, Inc

esophageal surgeries provides real time recognition of viable tissue and operating surgeons may be able to revise their line of resection, if required, and therefore can perform safe anastomosis. Jafari et al. had first evaluated the anastomotic tissue perfusion at the time of robotic low anterior resection (RLAR).[24] The authors had carried out a retrospective case control analysis to find whether immunofluorescence-ICG can reduce the rate of anastomotic leaks following procedures like RLAR. A 12% decrease of overall risks for anastomotic leaks was reported in the immunofluorescence-ICG group.[24] Other relevant clinical studies also confirmed the effectiveness of the technique in evaluation of the bowel perfusion.[25-27] Hellan et al. had reported about 40% rate of changing the point of bowel or gastrointestinal tract transection following fluorescent imaging.[26] Many more studies are required to know whether this translates in a decrease of true incidences of anastomotic leaks, but the preliminary reports appear promising. It has been reported that a prospective multicentric trial is ongoing, in an effort to find out the real impact of immunofluorescence-ICG procedure in changing the point of bowel transection at the time of left-sided colorectal resections.[11] Immunofluorescence imaging with the help of ICG could be used to check blood flows and perfusion in solid organ. Example of this kind application could be used in robot-assisted renal transplant procedures. Following the completion of a vascular anastomosis, ICG can also assess the blood flow through the anastomosed blood vessel and the perfusion of the transplanted organ like kidney.[28]

Lymph Node and Nerve Identification

Immunofluorescence imaging helps in mapping of the possible of lymph nodes and identification of any sentinel lymph node. This application can be specifically useful in cancer surgery, allowing highly selective lymphadenectomy with better precision. In laparoscopic surgical procedure it is being successfully utilized in the cases of GI and colorectal cancers.[29,30] In robotic surgeries, it has recently been used for the purpose of mapping lymph nodes and sentinel lymph node identification in cancers of endometrium and cancer cervix. Rossi et al. had first reported their evaluation of 20 cases who received cervical injection of ICG.[13] They had reported success rate of 88% in procedures like detection of SLN. They had concluded that this type of imaging procedure is reliable for SLN detection and mapping of lymph nodes during early stages of endometrial cancer and cancer of cervix. The same authors had recently published that cervical ICG injection is of higher efficiency in comparison to the hysteroscopic endometrial injection in identifying SLN, with similar distribution of anatomical lymph nodes.[31] Holloway et al. had reviewed the identification rates of SLN in patients suffering from endometrial cancer who also had robotic staging, compared immunofluorescence-ICG procedure with colorimetric imaging procedure using isosulfan blue (ISB).[32] They had reported that immunofluorescence-ICG was much useful while identifying bilateral SLNs and metastasis of lymph nodes, with 77% versus 97% identification rates of bilateral pelvic or aortic SLNs utilizing procedures ISB and ICG respectively.

Few drawbacks of immunofluorescence-ICG in cases of colorectal surgeries, reported by some authors especially in patients of tattooed lesion and mapping of lymph nodes.[11] There were details of excess or inadequacy in diffusion of colorant around tumor location, spreading within the abdomen cavity and resulting in visualization failure of the lymph nodes, specifically after neoadjuvant radiotherapy of cancers of rectum.[11]

Identifying nerves could be one more application of interest for immunofluorescence imaging which can be explored in future as novel procedure when highly selective

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contrast agents are available. Detection of nerves and its plexuses may facilitate in better dissection and prevent inadvertent nerve injury, improving postoperative outcome. Surgical procedures like prostatectomy (prostatic nerve), colorectal surgeries (inferior hypogastric nerve plexus), thyroid surgery (detection of recurrent laryngeal nerve) and thymus surgery (phrenic nerve) are considered as the better examples.

Wagner et al. had reported their assessments with 10 cases of unilateral right robotic thymectomy utilizing immunofluorescene-ICG to detect the contralateral pericardiophrenic neurovascular bundle (PNB).[14] They had success rates of 80% while visualizing the PNB, mostly from left sided pleural view. No injury happened to the PNB. The authors reported that this technique can provide maximum tissue resection and reduce the nerve injuries.

Identification of Lesions

The normal and cancerous tissue parenchyma has many differences in their hemodynamic and metabolic physiological properties, also the difference in ICG excretions in biliary mechanism helps in identification of various lesions from the surrounding normal tissues.

Hepatocellular carcinoma (HCC) is an example for these kind of properties. There are many study groups who had reported the different varieties of fluorescent patterns in cases of well differentiated hepatocellular carcinoma, poorly differentiated hepatocellular carcinoma, colorectal cancers with metastases.[33-35] In cases of well differentiated hepatocellular carcinomas the ICG uptake is well preserved, but the biliary excretion is mostly impaired, hence they have appearance of uniform fluorescent lesions. Poorly differentiated hepatocellular carcinomas and colorectal cancer metastases, on the contrary, have the appearance of hypofluorescent lesions enclosed by a fluorescent ring, due to presence of biliary excretion disorder of the surrounding tissues which is getting compressed by tumor mass. In our total understanding, immunofluorescence-ICG had been more useful in identifying hepatocellular cancers, hemangioma, pheochromocytoma and small neuroendocrine pancreatic tumors.

Manny et al. reported a case series of three robotic partial adrenalectomies where immunofluorescence-ICG was used to confirm the identification of masses from normal adrenal tissues, which in turn will promote parenchyma sparing resections.[36]

OTHER APPLICATIONS IN ROBOTIC SURGERY

Ureteral Stricture Identification

Currently, the indications for immunofluorescence-ICG procedure have expanded to intraoperative localization of strictures in ureter. Lee et al. had published 7 cases of robotic ureteroureterostomy procedure for stricture.[37] ICG was given intraureterally to delineate the ureter and an extra intraureteral dose was administered to identify the healthy ureteral tissues from the devitalized tissues, allowing safe repair. Reconstruction of the upper urinary tract appears to be a likely newer indication for immunofluorescence-ICG procedure. In a separately conducted study on 40 patients who underwent robotic pyeloplasty surgery, ureteral implantation and ureteroureterostomy with ICG, radiological and symptomatic improvement following the procedure was 100%.[12]

ICG in Cases of Total Endoscopic Robotic-assisted Procedures for Coronary Artery Bypass

A probable future application of immunofluorescence-ICG had been reported by Hassan et al.[38] In a canine model they had identified internal mammary artery, characterized the coronary anatomy and checked patency of the anastomoses during robotic coronary bypass.

Segmentectomy in Early Stages of Lung Cancers

Pardolesi et al. reported a novel technique to identify the intersegmental planes at the time of robotic anatomic segmentectomy for smaller sized early stage lung cancers.[15] The ICG was administered intravenously following the division of bronchus, vein and artery of target segment. The results showed green coloration of the perfused parenchymal tissue where the target segment remained uncolored. The authors had also reported the excellent demarcation of the segment to be excised, facilitating the segment transection.

FUTURE APPLICATIONS

New technologies are emerging for improving immunofluorescence imaging procedures.[39] Scientists had been working on newer techniques to overcome the limitation of low tissue penetration and also to improve the fluorescent signals.[40,41]

The most revolutionary future applications can be *in vivo*, real time tumor identification and its characterization. Specific targeting ligand or monoclonal antibodies which are conjugated to near-infrared fluorophores may allow real time molecular diagnosis and identification of the cancer masses during surgical operation. Recently Metildi et al. reported a clinical study where the tumors of pancreas in orthotopic mice were labeled with fluorescent anticarcinoembryonic antigen antibodies. Fluorescence guided laparoscopy operation resulted in decreased local and distant recurrence rates and extended duration of median disease free survival as compared to bright light laparoscopic surgery.[42,43] It is expected that in future the major progress in immunofluorescence imaging will be with better software for image processing (subtraction and fusion imaging), estimate a quantitative evaluation of blood flow (speed) and patterns of perfusions (architectures).

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CONCLUSION

Near-infrared fluorescent imaging with ICG is one of the latest innovations in the field of surgery. This is a safe and non-invasive technique without any radiation hazard. Currently its main applications in the robotic general surgical procedures are real-time visualization of biliary anatomical structures and evaluation of anastomosis site perfusion during various gastrointestinal surgeries and colorectal operations. Although there are only a few clinical studies present, in the literature, the reported assessments are encouraging. In recent future, it is expected that these applications of technology will provide real time *in vivo* microscopy that can be a valuable asset during minimally invasive oncologic surgical procedures. This can be considered as initial phase of a new era in surgical field. As newer technological innovations grow, so will the multiple indications of immunofluorescence imaging procedure. Further clinical research and trials are required to understand the real value of immunofluorescence imaging procedure and expand its indications.

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A Practical Approach to Robotic Surgery



Ajit Saxena is a Senior Consultant Urologist and Andrologist, Indraprastha Apollo Hospitals, New Delhi, India. With an illustrious educational background and a vast gamut of experience, he has been serving the society for the last 30 years. An alumnus of Maulana Azad Medical College, New Delhi, he started his career as a urologist in the UK, and subsequently served as Consultant Urologist, Sir Ganga Ram Hospital, New Delhi, before accepting his role as Senior Consultant Urologist and Andrologist, Indraprastha Apollo Hospitals, New Delhi. He has been conferred a Fellowship of the Royal College of Surgeons (FRCS) from Edinburgh, and has a Diploma in Urology from the prestigious London Institute of Urology.

His passion for robotic surgery is exemplified by the first-ever robotic urology conference in Asia that he organized (2005) at Escorts Heart Institute, New Delhi. The same passion is visible in *A Practical Approach to Robotic Surgery*, which is one of its own kind.

Reviews



With telemedicine now being well established in India, it is robotic surgery that has come of age.
A must-have book for beginners as well as experienced robotic surgeons.

—Dr Prathap C Reddy, Chairman, Apollo Group of Hospitals



Robotic surgery is all set to change the way surgical procedures are done. From cardiac surgery to urology and many other specialties, it is the present and future. I strongly recommend this book for its contents, innovative approaches and simplicity.

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