

# Clinical application of indocyanine green-fluorescence imaging during hepatectomy

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**Abstract:** In hepatobiliary surgery, the fluorescence and bile excretion of indocyanine green (ICG) can be used for real-time visualization of biological structure. Fluorescence cholangiography is used to obtain fluorescence images of the bile ducts following intrabiliary injection of 0.025–0.5 mg/mL ICG or intravenous injection of 2.5 mg ICG. Recently, the latter technique has been used in laparoscopic/robotic cholecystectomy. Intraoperative fluorescence imaging can be used to identify subcapsular hepatic tumors. Primary and secondary hepatic malignancy can be identified by intraoperative fluorescence imaging using preoperative intravenous injection of ICG through biliary excretion disorders that exist in cancerous tissues of hepatocellular carcinoma (HCC) and in non-cancerous hepatic parenchyma around adenocarcinoma foci. Intraoperative fluorescence imaging may help detect tumors to be removed, especially during laparoscopic hepatectomy, in which visual inspection and palpation are limited, compared with open surgery. Fluorescence imaging can also be used to identify hepatic segments. Boundaries of hepatic segments can be visualized following injection of 0.25–2.5 mg/mL ICG into the portal veins or by intravenous injection of 2.5 mg ICG following closure of the proximal portal pedicle toward hepatic regions to be removed. These techniques enable identification of hepatic segments before hepatectomy and during parenchymal transection for anatomic resection. Advances in imaging systems will increase the use of fluorescence imaging as an intraoperative navigation tool that can enhance the safety and accuracy of open and laparoscopic/robotic hepatobiliary surgery.

**Keywords:** Indocyanine green (ICG); fluorescence imaging; intraoperative cholangiography; hepatocellular carcinoma (HCC); colorectal liver metastasis (CRLM); liver resection

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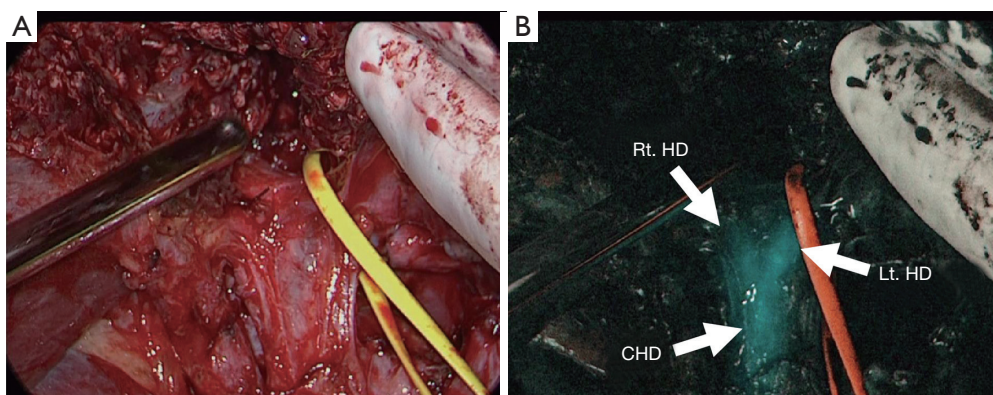
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## Overview of intraoperative fluorescence imaging using indocyanine green (ICG)

Since approval by the U.S. Food and Drug Administration (FDA) in 1954, ICG has been used clinically to estimate cardiac output and liver function. In the 1970s, protein-bound ICG was found to emit fluorescence, peaking at

about 840 nm, under illumination with near-infrared light (750–810 nm) (1). Because little light at 840 nm is absorbed by hemoglobin or water, fluorescence signals emitted by protein-bound ICG can be visualized through connective tissue 5–10 mm thick. ICG was first used clinically for fundus angiography in the early 1990s (2).

In the 21<sup>st</sup> century, fluorescence imaging using ICG has



**Figure 1** Fluorescence cholangiography following intraoperative intravenous injection of indocyanine green (2.5 mg) shows the confluence of the right and left hepatic ducts, enabling surgeons to determine the division point of the Lt.HD during left hepatectomy. Lt.HD, left hepatic duct; Rt.HD, right hepatic duct; CHD, common hepatic duct.

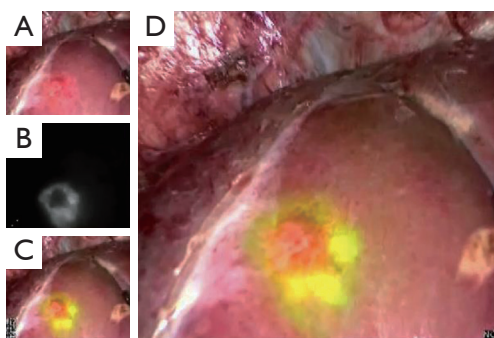
become widespread as an intraoperative navigation tool to detect lymphatic flow in the extremities (3); sentinel lymph nodes in patients with breast (4) and gastric (5) cancers; and blood flow during coronary artery bypass grafting (6) and clipping of cerebral artery aneurysms (7). In hepatobiliary surgery, however, little attention was paid to the fluorescence of ICG until, in the late 2000s, Japanese surgeons used ICG-fluorescence imaging to visualize hepatobiliary structures (8-11). This is probably because liver surgeons regarded ICG as a reagent for estimation of hepatic function.

Potentially, ICG-fluorescence imaging is highly suitable for hepatobiliary surgery, because the fluorescence of ICG and its biliary excretion can be used for the intraoperative identification of biological structures. In 2009, the first report of fluorescence cholangiography during laparoscopic cholecystectomy described using ICG excreted into bile following preoperative intravenous injection as the source of fluorescence (12). During the development of fluorescence cholangiography for hepatic tumors, it was found that ICG accumulated in cancerous tissues of hepatocellular carcinoma (HCC) and in non-cancerous hepatic parenchyma around adenocarcinoma foci (13,14). Recently, this technique has been used clinically to identify hepatic tumors during laparoscopic hepatectomy (15), and during open surgery. Refinements in imaging techniques have enabled the use of ICG-fluorescence imaging for visualization of hepatic segments, enabling more accurate anatomic resection of the liver (16,17).

### Fluorescence cholangiography

Because human bile contains proteins such as albumin and lipoproteins that bind ICG (18), fluorescent images of the biliary tract can be obtained by intrabiliary injection of ICG (11). The fluorescence intensity of protein-bound ICG was found to correlate with its concentrations to approximately 0.25 mg/mL, decreasing at higher concentrations because of the absorption of near-infrared light by ICG (9). Thus, to obtain clear fluorescence images of the bile ducts following intrabiliary injection of ICG, diluted ICG solution (approximately 0.025 mg/mL) should be used for imaging (11). It is also important to aspirate a small amount of bile into the syringe before injection to promote binding of ICG to proteins. When the intrahepatic bile duct anatomy and the extrahepatic biliary system must be identified, ICG should be diluted with radiographic contrast agents, enabling radiographic cholangiography easily and immediately following fluorescence cholangiography (19).

Fluorescence cholangiography could also be performed following intravenous injection of ICG, because ICG excreted into bile can act as a source of fluorescence (*Figure 1*) (7). This technique involves the intravenous injection of small amounts of ICG, usually 2.5 mg, diluted into 1 mL solution (12,20). Although biliary excretion of ICG begins within minutes after intravenous injection (21), ICG should be administered at least 15 minutes before imaging to obtain better signal-to-background contrast. Indeed, ICG fluorescence in the extrahepatic bile ducts continues up to 6 hours after injection (12). Intravenous



**Figure 2** Fluorescence imaging of hepatic tumor (CRLM). Fluorescence imaging following preoperative intravenous injection of indocyanine green (ICG), clearly showing a CRLM located in hepatic segment VIII as a rim-fluorescing lesion during laparoscopic hepatectomy. (A) White-light color image; (B) monochromatic fluorescence image; (C,D) pseudocolor fusion images of a fluorescence and a white-light color image.

injection of ICG has potential advantages over conventional radiographic cholangiography in saving time and avoiding bile duct injury associated with the catheterization required for injection of contrast materials. Although fluorescence cholangiography has a limitation in detecting small stones floating in the common bile duct, the present technique has recently gained attention as a novel and easy-to-use navigation tool that provides a roadmap of the extrahepatic ducts, enhancing safety (22) during laparoscopic (23-28) and robotic (29,30) cholecystectomy and reducing the need for intraoperative radiographic cholangiography.

### Fluorescence imaging of hepatic tumors

In a previous study of 37 patients with HCC and 12 with colorectal liver metastasis (CRLM), fluorescence imaging following preoperative intravenous injection of 0.5 mg/kg ICG identified all of the microscopically confirmed HCCs and CRLM on the cut surfaces of the resected specimens (13). The fluorescence patterns of these tumors could be classified into three types: total fluorescence, in which all tumor tissue showed uniform fluorescence; partial fluorescence, in which some tumor tissues showed fluorescence; and rim fluorescence, in which the cancer tissues were negative for fluorescence, but the surrounding liver parenchyma showed fluorescence. These fluorescence patterns were closely associated with the characteristics of the liver cancers. Total fluorescence-type tumors included all well-differentiated HCCs, whereas rim fluorescence-

type-tumors consisted only of poorly differentiated HCCs and CRLM.

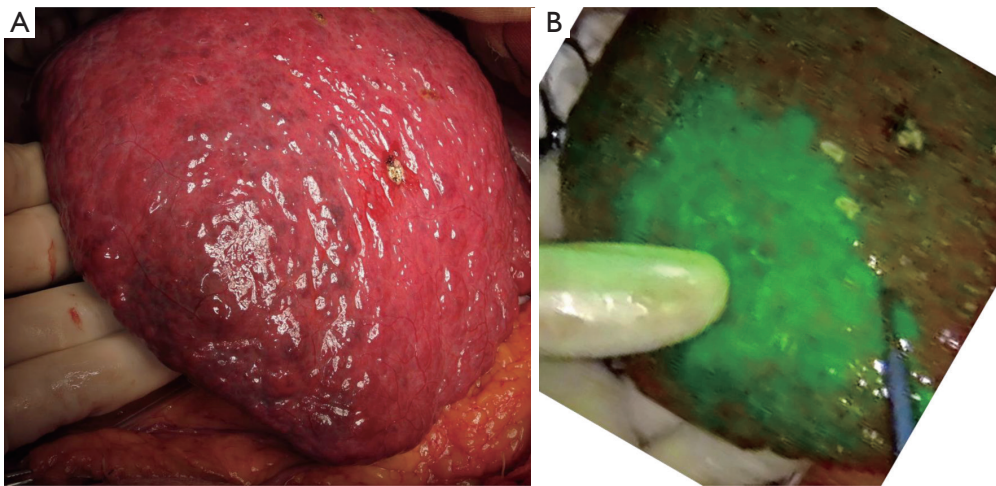
Recently, the mechanism of ICG-fluorescence imaging of HCCs was elucidated by immunohistochemical staining and gene expression analysis (14). In differentiated HCC tissues, the expression levels of portal uptake transporters of ICG [organic anion-transporting polypeptide 8 and Na<sup>+</sup>/taurocholate cotransporting polypeptide (31)] were well preserved, but functional or morphological biliary excretion disorders were present, leading to retention of ICG in cancerous tissues at the time of surgery, following preoperative intravenous injection. In poorly-differentiated HCCs, however, the portal uptake transporters were downregulated in cancerous tissues but biliary excretion of ICG by surrounding non-cancerous hepatic parenchyma was also disordered, resulting in rim-type fluorescence. The rim-type fluorescence signal in CRLM has been reported to be caused by immature hepatocytes with decreased bile excretion ability that surrounds the tumor (32). ICG fluorescence of HCC tissues was found associated with a risk of recurrence after hepatectomy (33).

Irrespective of their fluorescence patterns, subcapsular hepatic tumors can be identified on the liver surfaces by intraoperative fluorescence imaging, following preoperative intravenous injection of ICG. In this technique, ICG (0.5 mg/kg body weight) is administered intravenously, usually within two weeks before surgery. This method can also be used to detect biliary congestion caused by tumor invasion (34), micrometastases from pancreatic cancer (35), and extrahepatic spread of HCC (36). The intraoperative ICG-fluorescence imaging of hepatic tumors is simple and is especially useful for identifying subcapsular lesions for removal during laparoscopic hepatectomy, in which visual inspection and palpation are limited compared with open surgery (Figure 2) (15).

This technique has potential drawbacks, however, including a relatively high false-positive rate [around 40% (13,14)]. Lesions newly detected by ICG-fluorescence imaging should be resected only when other modalities, such as palpation and intraoperative ultrasonography, identify them as tumors to be removed. The incidence of false-positives can be reduced by not administering ICG on the day before surgery, especially in patients with decreased liver function due to cirrhosis or preoperative chemotherapy (13).

### Fluorescence imaging of hepatic segments

Anatomic segmentectomy is the essential surgical



**Figure 3** Identification of hepatic segments by dye-staining technique with concomitant use of indigo-carmin and indocyanine green. Fusion pseudocolor fluorescence image on color image (right), clearly showing the boundaries of hepatic segment VI. The blue stain on the liver surface is unclear because of fibrosis and the irregular surface of the liver due to cirrhosis. In this case, a laparoscopic fluorescence imaging system was used during open hepatectomy.

technique in hepatectomy, balancing cancer curability and postoperative hepatic function (37). Boundaries of hepatic segments prior to anatomic resection can be identified by a dye-staining technique, in which indigo-carmin solution is injected into the corresponding portal branch under ultrasound guidance, with positivity defined as blue staining of hepatic surfaces. In 2008, fluorescence imaging following portal injection of ICG was first used in the intraoperative identification of hepatic segments (10). This technique was later refined (16) by using a more diluted solution of ICG (2.5 mg) as a source of fluorescence and an imaging system enabling fusion of fluorescence images on color images. The concomitant injection of indigo-carmin solution with a small amount of ICG (0.25 mg) could enhance the success rate of hepatic segment identification, especially in patients with cirrhosis and/or those having livers covered by thick connective tissues owing to previous surgery (17) (*Figure 3*).

In addition to identifying hepatic segments for removal following portal injection of ICG solution (positive staining technique), hepatic segments can be identified as ischemic regions by intravenous injection of ICG (2.5 mg) following closure/division of the corresponding portal pedicle (negative staining technique) (16,38). The latter technique is especially useful in laparoscopic hepatic segmentectomy, in which injection of ICG solution into the portal vein is technically difficult (38,39). Intraoperative fluorescence imaging can also be used to estimate portal uptake function

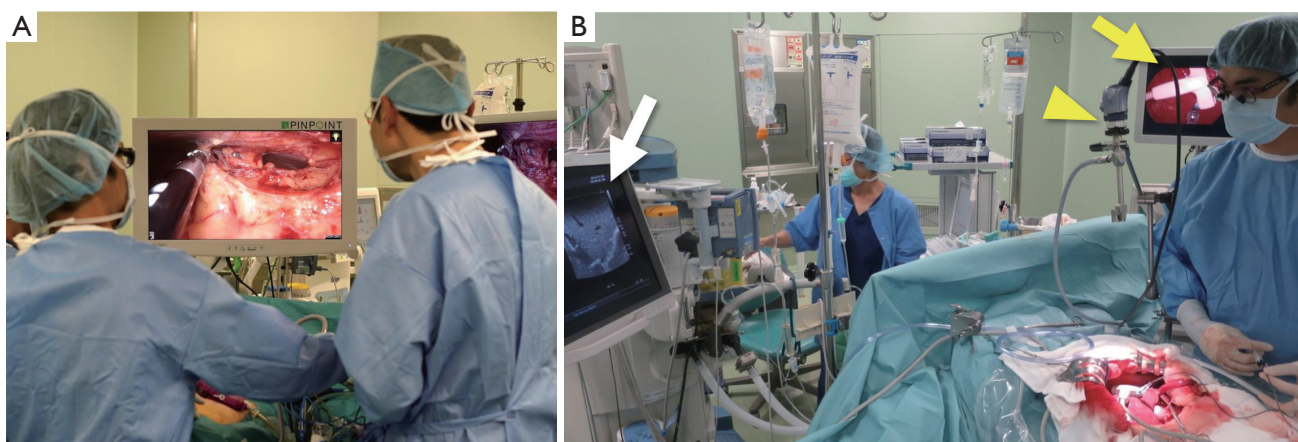
in veno-occlusive regions of the liver during hepatectomy or living-donor liver transplantation, by measuring trends of fluorescence intensity of the hepatic surfaces following injection of ICG (40,41).

### Future prospects of ICG-fluorescence imaging in hepatobiliary surgery

In laparoscopic and robotic surgery, surgical procedures are based on operation fields displayed on a monitor. Thus, it is not surprising that fluorescence imaging was rapidly applied to these minimally-invasive methods. Laparoscopic and robotic fluorescence imaging systems have become commercially available (*Figure 4A*). In the near future, three-dimensional and ultra-high definition imaging (4K) may be delivered to clinical settings. Further technological innovations, however, are needed to improve the feasibility of fluorescence imaging during open surgery, enabling surgeons to operate without having to switch from operation fields to a TV monitor (*Figure 4B*).

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**Figure 4** Fluorescence imaging systems in laparoscopic and open surgery. (A) In laparoscopic surgery, surgeons always watch a TV monitor to obtain white-light and fluorescence images, when needed; (B) during open surgery, surgeons must look from the field of operation to each monitor used for intraoperative diagnosis, such as intraoperative ultrasonography (white arrow) and fluorescence imaging (yellow arrow). Use of a laparoscopic imaging system equipped with a short-length laparoscope during open surgery (yellow arrowhead).

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### Footnote

**Conflicts of Interest:** The authors have no conflicts of interest to declare.

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