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Evolving applications of fluorescence guided surgery in pediatric surgical oncology: A practical guide for surgeons

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ABSTRACT

Fluorescence-guided surgery (FGS) is an increasingly available and popular method of visual field augmentation. The basic premise of FGS entails injection of fluorescent indocyanine green (ICG) and subsequent detection with a near-infrared (NIR) camera. For pediatric surgical oncologists, FGS remains experimental but is a promising modality for identifying tumor margins, locating metastases, performing sentinel lymph node biopsies, protecting peritumoral structures of interest, and facilitating reconstruction. Familiarity with basic ICG pharmacokinetics and NIR detection optics is critical for surgeons wishing to judiciously use FGS, as its success is firmly grounded in a thorough understanding of its capabilities and limitations. In this practical guide, we outline several well-described and innovative FGS applications by disease type, including their methods of administration, modes of detection, and typical ICG dosing paradigms.
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Contents

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Fluorescence-guided surgery (FGS) is the use of fluorescent markers to identify structures of interest during an operation. The structures or tissues of interest can be visualized with instruments that detect near-infrared (NIR) light, which is not visible to the human eye, as an adjunct to a surgeon's visual field or white-light (e.g., standard digital laparoscopic) images. FGS does not necessarily yield stand-alone pictures or images but rather offers real-time intraoperative augmentation of a surgeon's visual field. In this review, we highlight the contemporary applications of FGS in pediatric surgical oncology, with examples and practical guidance organized by indication.

The sine qua non of FGS is the generation of an NIR signal. Indocyanine green (ICG) is the only NIR fluorescent agent currently approved for human use. ICG is a safe, water-soluble compound that received FDA approval in the 1950s and is currently indicated for determining cardiac output and hepatic blood flow, and performing ophthalmic angiography. All of the applications we discuss herein are off-label. Despite the bright green color of the reagent to the human eye, its use as a contrast agent is based on its robust fluorescence in the NIR range. ICG is widely available and relatively inexpensive, and its side effects are uncommon [1].

ICG can be delivered by at least five routes of administration corresponding to at least eight distinct modes, and this list is rapidly expanding (Table 1). When intravenously administered, ICG remains largely protein bound in the intravascular space and is cleared by the liver, with an intravascular half-life of approximately 3 min. During this time, arteriogram, venogram, or general perfusion assessments can be made. Common examples of the latter include intestinal perfusion before anastomosis and soft tissue pedicles during complex plastic surgery.

From the vasculature, ICG undergoes uptake by hepatocytes and hepatic clearance, which can be detected within minutes, peaks over the course of hours, and continues for over a day after typical dosing. This exclusive hepatic clearance with biliary excretion led to the use of fluorescence cholangiography as an adjunct for laparoscopic cholecystectomy [2,3]. Uptake by hepatocytes is also helpful for locating hepatoblastoma and hepatocellular carcinoma metastases within lung parenchyma, as these tissues will sequester ICG in a similar manner as that of native liver parenchyma.

The enhanced permeability and retention (EPR) effect permits systemically administered small molecules to passively accumulate in tumors because of increased vascular permeability and impaired lymphatic drainage [4]. Accordingly, when an agent such as ICG is administered, it will be retained in pathologic tissues supplied by abnormal vasculature after clearance from the circulation and healthy tissues. In some instances, this can be used to distinguish tumor tissues from healthy organs and to delineate margins because the EPR effect differentially identifies parenchyma supplied by healthy blood vessels from diseased tissues supplied by leaky vessels. The ICG dose required to identify nonhepatic tumors with the EPR effect is 8- to 10-fold higher than that required for hepatic tumors, which selectively accumulate ICG because of their hepatic clearance properties.

When administered as an intradermal, subcutaneous, or other interstitial injection, ICG is similarly protein bound and confined to clearance via the lymphatic system. This can be leveraged for FGS because NIR emission can be tracked noninvasively with an NIR camera through the regional lymphatic basin to facilitate sentinel lymph node detection and biopsy. Additionally, direct injection into a lymph node can be used to visualize larger lymphatic channels, such as the thoracic duct.

ICG can be directly injected around a lesion with imaging guidance and be subsequently used to locate this area after creating surgical access to the appropriate body cavity. For example, lung lesions that are deeper than visceral pleura may be difficult to locate for resection with minimally invasive techniques. However, ICG injections around these lesions permit minimally invasive visualization of nodules approximately 1 cm below the pleural surface. Other modes that do not have well-described indications or technical considerations to date are also included in Table 1 [5,6].

1. Available platforms, imaging modalities, and best practices

1.1. Available platforms

FGS depends on the availability of equipment with NIR excitation and detection capabilities. These systems are available from several companies for open, endoscopic, and robotic platforms. Features vary by platform but include the ability to overlay NIR images over standard white-light pictures, as well as options for qualitative or quantitative assessments of fluorescence (Table 2).

The SPY™ and Pinpoint™ systems by Novadaq Technologies were among the first commercially available devices for NIR surgical imaging in North America. Since purchasing Novadaq Technologies in 2017, Stryker has released new iterations of this technology. The current 1688 AIM 4 K platform includes both laparoscopic and portable handheld imager (SPY PHI) fluorescence imaging technology. The laparoscopic fluorescence imaging modes include SPY overlay, with fluorescence overlaid on white-light images, and SPY contrast, with maximal fluorescence contrast in black and white. The open SPY PHI also has a semiquantitative color-segmented fluorescence mode,

Table 1
Routes of administration, modes of detection, and dosing examples for fluorescence-guided surgery with indocyanine green.

Route of administration	Mode of detection	Examples	ICG Dosing (alternative)[a]
Intravenous	Immediate organ perfusion	Angiography, bowel perfusion, flap perfusion	2.5 mg IV immediately before assessment (0.05–0.1 mg/kg)
	Hepatic clearance	Cholangiography	2.5 mg IV 1 h before assessment (0.1 mg/kg)
		Hepatoblastoma/HCC metastasectomy	0.5 mg/kg IV 24 h before surgery
		Primary hepatic tumor resection	0.5 mg/kg IV 72–96 h before surgery
	Enhanced tumor permeability and retention	Sarcoma resection & pulmonary metastasectomy	4–5 mg/kg IV 24 h before surgery
Interstitial/lymphatic injection	Lymph node	SLNB, retroperitoneal lymph node biopsy	2–4 mL peritumoral 20–40 min before assessment
	Lymphatic channel	Thoracic duct imaging	0.5 mg/kg into inguinal lymph node
Inhaled	Pulmonary imaging	Bronchial tree visualization	TBD
Direct renal pelvis injection	Urinary	Ureter identification	TBD
Intraparenchymal (imaging guided)	Lesion directed	Pulmonary nodule	0.1–0.2 mL of 20× dilution (0.125 mg/mL)

Abbreviations: HCC, hepatocellular carcinoma; ICG, indocyanine green; IV, intravenous; SLNB, sentinel lymph node biopsy; TBD, to be determined.
[a] Alternative weight-based dosing used for children weighing less than approximately 25 kg.

Table 2
Fluorescence-guided surgery imaging features available on commercial platforms available in the United States.

	Endoscopic NIR camera	Open NIR camera	White-light overlay	Fluorescence quantification	NIR-emitting catheter or stent
Novadaq SPY Elite		✔		✔	
Stryker 1588	✔	✔			
Stryker 1688 AIM 4 K with SPY PHI	✔	✔	✔	✔[a]	✔
Medtronic EleVision IR	✔	✔	✔	✔	
Intuitive Firefly	✔		✔[b]		
Karl Storz OPAL1/IMAGE1 S	✔	✔[c]		✔	

Abbreviations: NIR, near-infrared; PHI, portable handheld imager.
[a] Semiquantitative color-segmented fluorescence mode.
[b] Partial overlay with blue-light image.
[c] Via adapter on laparoscopic camera.

providing heat maps based on fluorescence intensity. The platform also includes an NIR-emitting IRIS stent for ureteral placement and identification during abdominal and pelvic surgeries.

The EleVision IR system by Medtronic can be used for both open and endoscopic surgeries. It combines real-time infrared imaging with white-light overlay and high-definition imaging. Additionally, this system provides qualitative and quantitative assessments of infrared intensity. The DaVinci robot by Intuitive includes an integrated fluorescence imaging capability. This Firefly imaging system initially used technology licensed from Novadaq, but newer generations use their own proprietary systems, incorporating illumination with an NIR laser and blue light. The NIR laser produces the fluorescence images, and the blue light reflects sufficiently to construct a black and white image of nonfluorescent tissues. Karl Storz Endoscopy offers fluorescence imaging on its IMAGE1 S RUBINA imaging platform through its OPAL1(R) technology. For this platform, an adapter on the endoscopic camera permits fluorescence visualization during open surgeries. Many of these companies have indicated that they will add new IR imaging features to their visualization platforms in the near future including Olympus with its Visera Elite II system.

1.2. FGS best practices

Regardless of which systems are used, generally applied best practices can maximize success with fluorescence imaging. Ambient light can create substantial artifacts and must be minimized during fluorescence visualization by reducing room lights and even creating a tent or shield of towels around the operative field. Thus, surgeons must take great care to remain aware of instrument and tissue movements without typical visual cues when a mode without white light is employed more than briefly. Frequently toggling between light sources is advantageous because surgeons can use the fluorescence to augment visualization without detracting from basic surgical principles.

A thorough understanding of the basic pharmacokinetic concepts described herein is critical for surgeons wishing to judiciously use FGS because the timing of ICG administration is of paramount importance. Both dose and preoperative timing can facilitate or preclude a desired mode of visualization. Additionally, the penetrance of the ICG fluorescence is limited to approximately 1 cm. False negatives will occur if attempting to identify deeper structures. This limits the ability to perform transcutaneous lymphoscintigraphy during sentinel lymph node biopsies (SLNBs) for larger patients and illustrates the importance of meticulous lung isolation for thoracic cases to ensure complete lung collapse. Additionally, establishing a clear line of sight while minimizing interposing structures will facilitate visualization of fluorescence. To this end, achieving relative proximity of the camera to the targets of interest is beneficial. There is rapid diminution of signal intensity by solid objects or instruments, as well as at air/fluid interfaces such as aerated lung.

2. Applications in tumor identification, resection, and margin delineation

ICG fluorescence is an increasingly frequent adjunct for several applications in pediatric surgical oncology to augment tumor identification, resection, and margin delineation. Although these applications are still experimental, they are discussed in general order from the most well established to more evolving and novel uses.

2.1. Pulmonary metastasectomy for primary hepatic tumors

Approximately 10% to 20% of children with hepatoblastoma present with pulmonary metastases, and these children have an overall survival rate of less than 50% [7]. Clearance of pulmonary disease by chemotherapy and/or surgical resection is critical for achieving cure. In the current Pediatric Hepatic Malignancy International Therapeutic Trial (NCT03017326), pulmonary metastasectomy is required for patients whose pulmonary disease fails to resolve after induction chemotherapy. It is performed after local control for resectable primary tumors but before transplantation for tumors not amenable to partial hepatectomy. Surgical exploration with biopsy is also recommended for patients if there is any question of residual disease versus scar prior to consideration of transplant. Thoracoscopy is associated with lower morbidity and faster postoperative recovery than is thoracotomy but requires reliable techniques for identifying nodules because most are not visible and tactile sensation is lost. This can be achieved with computed tomography (CT)-guided wire, coil placement, or dye injection. However, fluorescence imaging with intravenous ICG is uniquely suited to identify hepatic metastases without requiring adjuvant localizing procedures.

Because ICG is concentrated in hepatocytes and metastatic hepatic tumors, it facilitates thoracoscopic identification and removal of lesions, particularly upon delayed imaging. Lung isolation is essential to ensure the target lung is fully collapsed, since ICG penetration is limited to approximately 1 cm. If thoracoscopy is planned and any nodules are more than 1 cm below the pleural surface, a secondary localization with a coil or wire should be considered for the deeper lesions. Intravenous ICG (0.5 mg/kg) is given approximately 24 h before surgery. This timing allows ICG concentration in tumor hepatocytes and appropriate clearance from background lung tissue. Contrast mode (black and white) is used for initial inspection to allow the greatest sensitivity for identifying potential nodules. Overlay mode is then used to guide wedge resection (Fig. 1). As reported in case series from single institutions, ICG-guided pulmonary metastasectomy was demonstrated to be safe and feasible [8]. However, more robust data on the sensitivity and specificity of this localization technique is required. This approach is also safe and feasible for identifying pulmonary metastases from hepatocellular carcinoma [9].

2.2. Primary hepatic resection

Fluorescence imaging with ICG can assist in primary hepatic resection, both for identifying multifocal lesions and ensuring negative

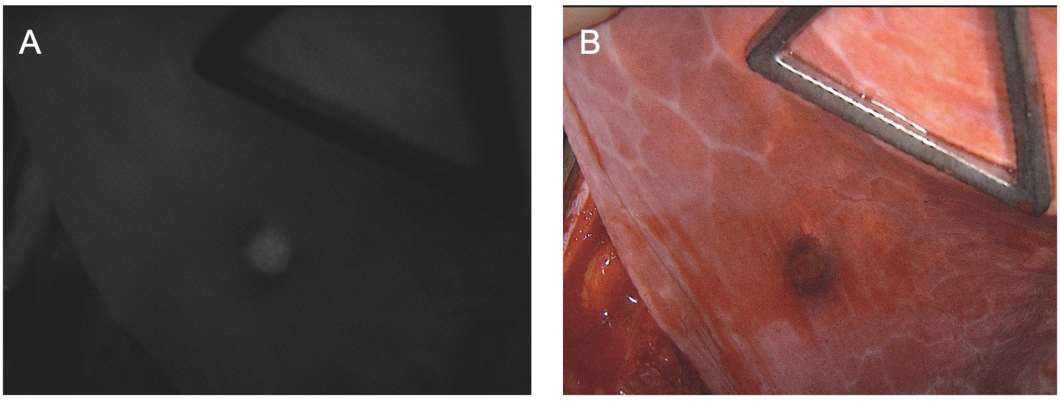

Fig. 1. Pulmonary metastasectomy for hepatoblastoma with (A) fluorescence imaging contrast mode and (B) white-light view, demonstrating the metastatic lesion.

margins with resection (Fig. 2). In these scenarios, a small dose of ICG is given far enough in advance to allow biliary excretion from healthy liver tissue but selective retention in tumor cells, which tend to be cholestatic. To maximize tumor-to-background fluorescence, an ICG dose of 0.5 mg/kg intravenously administered 72 to 96 h before surgery is optimal. This requires careful coordination of care and a clinic visit for ICG administration. This can often be combined with preoperative blood draws. Alternatively, when patients are unable to come to the clinic that far in advance, a dose of 0.3 mg/kg can be given 48 h before surgery, but this may cause higher levels of background noise.

For adult patients, ICG imaging was demonstrated in a systematic review to have high sensitivity (0.96–1) for identifying superficial liver tumors but lower sensitivity (0.75) for deeper tumors [10]. This review also found that ICG identified additional malignant hepatic tumors in 11.6% of patients. Data on the utility of ICG for enhanced delineation of margins or identification of additional malignant lesions in children, particularly for hepatoblastoma, is thus far limited to a small case series [11]. Patients with underlying hepatocellular disease (e.g., Fanconi anemia) or cholangiopathic disorders (e.g., biliary atresia) can sequester ICG, making underlying tumor identification and margin assessment challenging.

An alternative application of ICG in hepatectomy is delineating segmental boundaries. An ICG dose of 1.25 mg given by intravenous injection after division of the portal vein and hepatic artery branches to the specimen will rapidly produce fluorescence in the remaining liver. The contrast between fluorescence in the remaining liver and non-fluorescence in the specimen can guide parenchymal transection [12].

2.3. Pleural and pulmonary metastasectomy for nonhepatic metastases

ICG can also be useful for identifying superficial pleural and pulmonary metastases of other cancers, including Wilms tumor and a variety of sarcomas (Ewing sarcoma, synovial sarcoma and osteosarcoma, among others) (Fig. 3). Because of the EPR effect, high doses of ICG lead to its accumulation in these tumors, even tumors which are initially hypofluorescent [4]. Similar to operations for hepatic metastases in the lung, these procedures can be accomplished by thoracoscopy or thoracotomy. Secondary localization with coils or wires should be considered for nodules more than 1 cm below the pleural surface. For this technique, ICG (4 mg/kg) is intravenously administered approximately 24 h before surgery. To reduce the injection volume, a concentration of 5 mg/mL (rather than the standard 2.5 mg/mL) can be used. Although this dose is higher than that approved by the FDA, reactions or adverse events are extremely rare [13,14]. At the time of surgery, contrast mode (black and white) is used for initial inspection to allow the greatest sensitivity for identifying potential nodules, and then overlay mode is used to guide wedge resection.

In a study of 45 adult patients, Newton et al. found that 9 of 9 other malignant neoplasms in the lung (e.g., thoracic metastases, thymoma, or mesothelioma) exhibited fluorescence at doses of 4 to 5 mg/kg, and 13 of 14 exhibited fluorescence at doses of 1 to 3 mg/kg. The tumor-to-background ratio was optimal at a dose of 3 mg/kg [15]. In a separate study of 30 adult patients given 5 mg/kg ICG, Predina et al. found that 89% of sarcoma metastases displayed fluorescence [13]. This approach for nodule identification relies on the EPR effect in viable tumor cells. Therefore, false-negative results may be more likely to occur for extremely small nodules or those with extensive necrosis and minimal viable tumor, which can occur in heavily pretreated patients. Further investigation is required to determine the correlation between treatment effects and fluorescence levels. In these cases, it may be advisable to perform direct intraparenchymal injections under CT guidance. This approach is similar to the use of methylene blue injections and can likewise be combined with the placement of a wire or coil. The advantage of using ICG over methylene blue is the ability to inject a few additional millimeters below the pleural surface to avoid accidental staining of the diffuse pleural surface, which can impair precise nodule

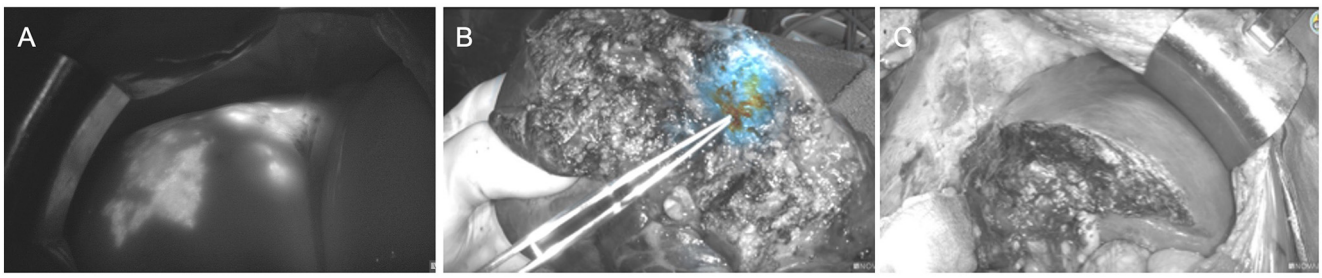

Fig. 2. Partial hepatectomy for hepatoblastoma with ICG margin delineation. (A) Initial view of the right lobe of the liver, (B) color-segmented fluorescence view along cut surface of the specimen, and (C) cut surface of the remaining liver showing no evidence of residual fluorescence.

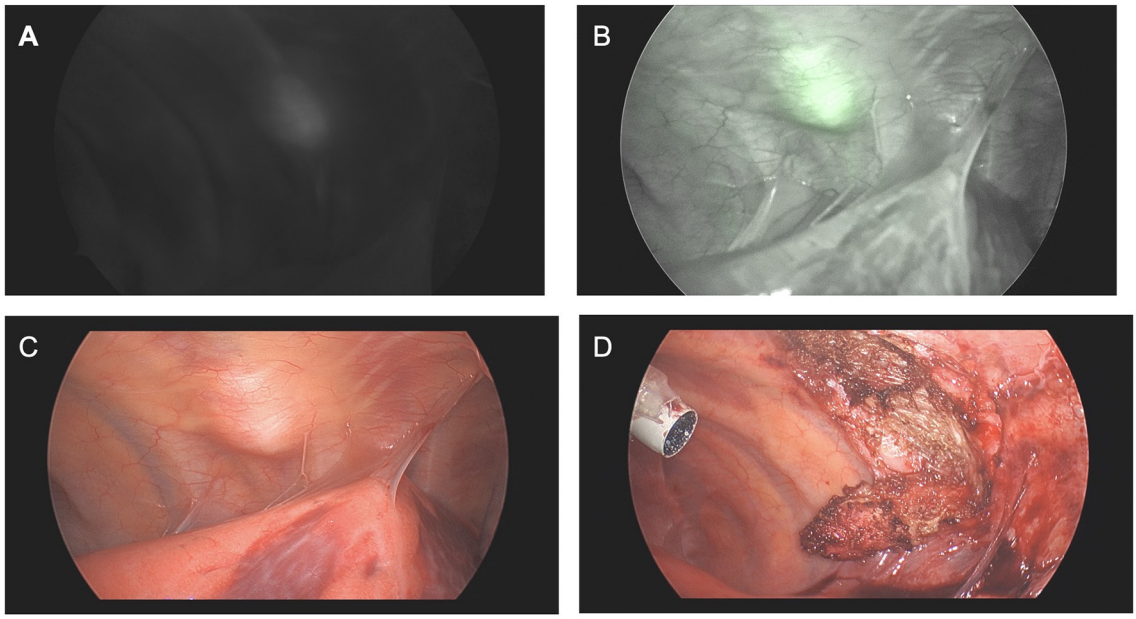

Fig. 3. Thoracoscopic excision of a DSRCT pleural metastasis with ICG identification. (A) The mass is identified along the chest wall in contrast mode and confirmed in (B) overlay mode and visualized with (C) white-light imaging. (D) Further intraoperative view during dissection of a metastasis of the chest wall.

localization. For this indication, a dose of 0.1 to 0.2 mL of a 20x dilution (0.125 mg/mL) is injected under CT guidance immediately before the resection procedure [16].

2.4. Sentinel lymph node biopsy

SLNB plays an important role in regional lymph node assessment for children with malignant melanoma, rhabdomyosarcoma, and some nonrhabdomyomatous soft tissue sarcomas (e.g., synovial, epitheliod, and clear cell sarcomas). Technetium lymphoscintigraphy (TcL) is the gold standard for mapping and identifying sentinel lymph nodes, allowing both whole-body imaging and on-table localization of lymph nodes. However, intraoperative gamma probes have poor resolution and do not permit direct visualization during dissection. TcL is often augmented by the use of blue dyes for visual identification (i.e., methylene blue or lymphazurin). Blue dyes can facilitate visual identification of the sentinel lymph nodes but cannot be well-visualized through layers of tissue and are somewhat limited by the risk of hypersensitivity. Recently, fluorescence lymphatic mapping with ICG was shown to be equivalent to TcL for SLNBs in breast cancer and has the added benefit of direct visual identification that can be followed through to 1 to 2 cm of tissue [17].

Although an investigation to determine whether ICG lymphoscintigraphy can obviate the need for TcL (NCT02910726) is currently underway, surgeons who use ICG now do so as an adjunct. For extremity and trunk tumors, 4 mL of ICG at a concentration of 1.25 mg/mL is subcutaneously injected in four distinct quadrants (1 mL per quadrant) around the tumor in the operating room. In small children, ICG is often visible through the skin with a handheld NIR camera, allowing for real-time lymphoscintigraphy. This is best performed using the black and white contrast mode. Once the sentinel lymph nodes are visualized and the operation commences, toggling between the various modes of fluorescence imaging is helpful. In particular, the color-segmented fluorescence mode allows identification of the regions with high fluorescence intensity (Fig. 4).

Sentinel lymph node techniques may also be beneficial for regional lymph node identification in locations outside of the trunk and extremities. Recently, fluorescence lymphatic mapping with ICG has demonstrated benefit in adults with cervical and uterine cancer by using direct cervical injections and laparoscopic or robotic NIR imaging for pelvic sentinel lymph node removal, with minimal lymphedema and lymph leak complications [18]. This strategy can be extrapolated to boys aged ≥10 years with paratesticular rhabdomyosarcoma who require ipsilateral retroperitoneal lymph node assessment. Current protocols require ipsilateral template dissection or random sampling of 6 to 10 lymph nodes (NCT02567435). Sentinel lymph node techniques may allow optimization of regional lymph node assessments without the morbidity caused by template dissection [19]. For this experimental technique, 2 mL of 1.25 mg/mL ICG is directly injected into the spermatic cord under ultrasound guidance or laparoscopic visualization. Laparoscopic, open, or robotic fluorescence imaging platforms are then used to identify and dissect the draining lymph nodes, which may extend up to the renal hilum (Fig. 5). Lymph node mapping with this technique is reported to be successful; however, given the limited sample size in this study, the precision of sentinel lymph node detection is not yet determined [19].

Data on the sensitivity and specificity of ICG lymphoscintigraphy in children are currently limited. However, in a trial of adult women with breast cancer randomized to SLNB with either radioisotopes alone or combined with ICG fluorescence, both groups had excellent rates of sentinel lymph node identification, but ICG permitted transcutaneous visualization of the lymphatic drainage in 65.5% (38 of 58) of cases [20].

2.5. Nonhepatic primary tumor resection, including parenchymal-sparing approaches

ICG accumulation and retention in nonhepatic primary tumors by the EPR effect may distinguish tumor from background tissues and enhance margin delineation during tumor resections. The accuracy of ICG in delineating the margins of adult pancreatic tumors was assessed in a prospective feasibility trial [21]. Although the association between histopathology and fluorescence was encouraging, a powered study is required to examine ICG precision for margin identification to guide resections. A prospective trial is also currently investigating the feasibility of fluorescence margin identification as a guiding tool for pediatric tumor resections. (NCT04084067).

The relative hypofluorescence of some tumors compared to their background healthy tissues may aid in organ-sparing procedures, including partial adrenalectomy and partial nephrectomy. In a study of 100 adult patients given a 5-mg dose of ICG after retroperitoneal

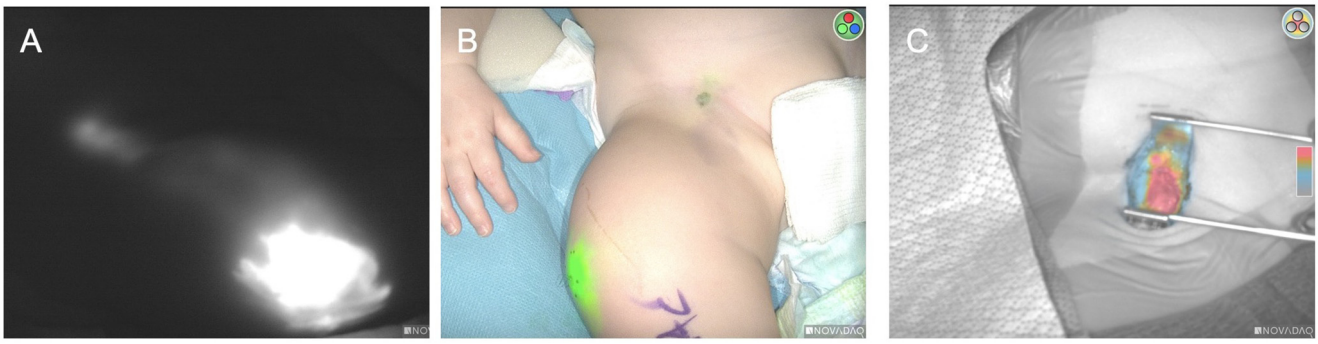

Fig. 4. Sentinel lymph node biopsy for a patient with a rhabdomyosarcoma on the right thigh, showing (A) initial lymphoscintigraphy in contrast mode, (B) external identification of the positive lymph node, correlating with a black dot made in nuclear medicine, and (C) color-segmented fluorescence view of the positive lymph node.

exposure, tumors of adrenocortical origin were hyperfluorescent, as compared with that of background tissues, whereas those of medullary origin were hypofluorescent [22]. This distinction may aid in partial adrenalectomies, particularly for cortical-sparing procedures in patients with pheochromocytoma. In the case of nephron-sparing surgery, 83 of 86 solid kidney tumors in adults were hypofluorescent compared to background kidney [23]. Further work is required to define the fluorescence patterns of Wilms tumors compared to background kidney and to determine the utility for guiding margin delineation in nephron-sparing surgery. Other potential areas for future investigation include margin delineation and metastasis identification in neuroblastoma, peritoneal implant identification in peritoneal surface malignancies such as desmoplastic small round cell tumor, and margin identification in sarcoma local control operations.

3. Protection of adjacent healthy structures

In addition to identifying tumors, FGS can highlight adjacent healthy structures to aid in their identification and protection during tumor resection procedures.

3.1. Ureter

The ureters are at risk of injury during many tumor resection procedures, and ureteral stents may minimize the risk of injury. The development of NIR instruments, such as lighted ureteral stents, represents an exciting addition to FGS that does not rely on ICG. Rather than relying on systemic or local administration of contrast agents, such

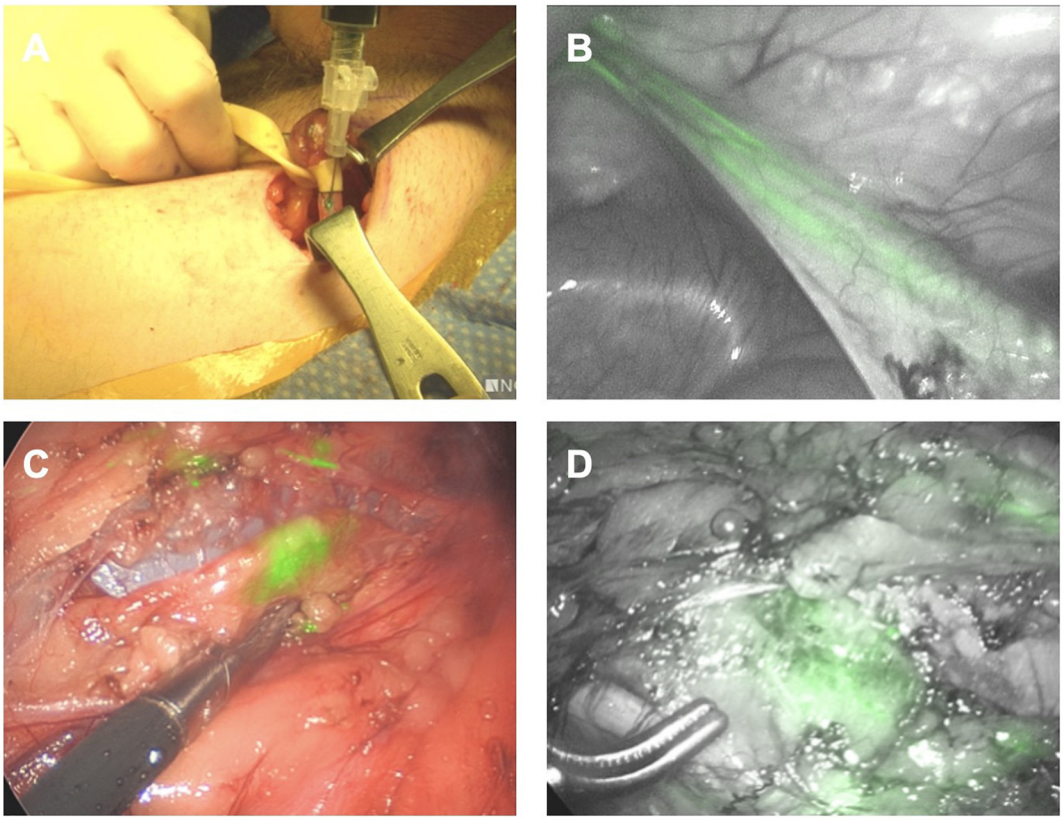

Fig. 5. Retroperitoneal lymph node sampling in paratesticular rhabdomyosarcoma. (A) ICG was injected into the cord in an open manner. (B) ICG was visualized traveling along the spermatic cord. (C, D) Fluorescence is present in the aortocaval sentinel lymph nodes.

NIR instruments are mechanical apparatuses with light-emitting filaments in the NIR range that are detected by the same imaging platforms. The Stryker IRIS product includes sterile 6 Fr close-ended ureteral catheters that can be placed by cystoscopy in the same manner as other standard and lighted ureteral stents. The NIR-emitting 0.75-mm filaments thread into the stents, and when activated, they emit a steady or pulsating light that transilluminates the ureters as red via a video tower. In contrast with standard white-light emitting stents, which require elimination of background light, these NIR images are superimposed on standard white-light laparoscopic or open images (Fig. 6).

Another approach to ureter identification involves injection of ICG into the renal pelvis under ultrasound guidance. This is an alternative approach for FGS systems that do not include a NIR-emitting ureteral stent but is limited by the duration of fluorescence time in the ureters before washout.

3.2. Thoracic duct

Fluorescence imaging of the thoracic duct can help protect this structure during excision of paraspinal masses (or during esophageal surgeries) [24]. For this application, 0.5 mg/kg of ICG at a concentration of 5 mg/mL is injected into an inguinal lymph node under ultrasound guidance. Lymphatic drainage results in fluorescence of the thoracic duct approximately 45 to 60 min later. This approach can also be used to identify and control thoracic duct leaks after tumor resections. This experimental technique is reported in adults but requires validation for pediatric patients.

3.3. Biliary system

Fluorescence cholangiography is a well-established adjunct in biliary surgery, including laparoscopic cholecystectomy. The same approach can be used to highlight the bile duct during excision of tumors adjacent to the porta hepatis. ICG is typically administered in the preoperative holding area, approximately 1 h before incision at either a weight-based dose of 0.1 mg/kg for smaller children or a standardized 2.5-mg dose for older children and adults. The timing of this injection is largely driven by factors related to patient convenience, to avoid the necessity of patients to be present several hours before surgery. However, the highest bile duct-to-liver fluorescence ratio is achieved when ICG is administered 3 to 7 h before surgery, and this timing should be considered whenever practical [25]. If the dose is given too close to the time of surgery, background fluorescence from the liver considerably impairs bile duct visualization.

The utility of this modality for reliably identifying the bile duct is well established in adult benign hepatobiliary surgeries. A randomized trial of 120 adult patients demonstrated that fluorescence cholangiography was noninferior to intraoperative cholangiograms for visualization of the bile ducts [3]. Another randomized study found that fluorescence cholangiography was superior to white-light imaging for pre-dissection identification of extra-hepatic biliary structures [2]. Further efforts are required to determine whether fluorescence cholangiography is equally effective in children, particularly for indications other than laparoscopic cholecystectomy.

4. Reconstruction and perfusion assessment

ICG is also a helpful adjunct for reconstructive procedures after tumor resections. For these indications, fluorescence imaging is performed immediately after ICG injection to assess early tissue perfusion. These techniques for perfusion assessment are similar regardless of indication. For older children and adults, a dose of 2 mL at a concentration of 2.5 mg/mL is intravenously administered a few moments before assessment. For smaller children, weight-based doses of 0.05 to 0.1 mg/kg can be employed. Administration of such small ICG doses requires dilution to much lower concentrations (i.e., 0.125 mg/mL or 0.25 mg/mL). Fluorescence imaging assessments should begin immediately after ICG injection. Monitoring the subsequent perfusion in real time is important because marginal areas may demonstrate lagged ICG enhancement. This technique can similarly be used to visualize the actual arterial vessels when viewed in the very early phase of ICG distribution.

Assessing perfusion during flap reconstruction is a commonly used reconstructive application of ICG. IGC–NIR video angiography was used for intraoperatively assessing 88 consecutive free flaps and found to have excellent sensitivity and specificity for predicting tissue necrosis [26]. ICG angiography can likewise be useful for ensuring adequate anastomotic perfusion after bowel resection procedures. Although primary colon cancer is rare in children, bowel resection and anastomosis may also be required for other tumors, such as desmoplastic small round cell tumors. In a randomized trial of 252 adult patients undergoing laparoscopic left colon or rectal resection procedures, 11% of patients received additional resection of the colonic stump because of inadequate perfusion observed with ICG imaging. As a result, the rate of anastomotic leak was 5% in the ICG group versus 9% in the control group (p not significant) [27].

5. Limitations and false positives

As with all new technologies, FGS is neither a panacea nor a substitute for sound clinical judgment. False-positive and false-negative results do occur. Fig. 7 shows an example of a patient with multifocal

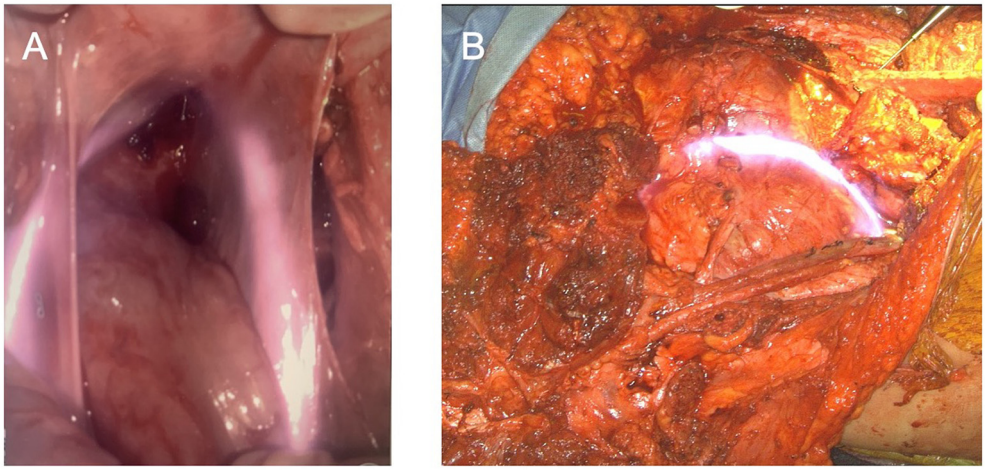

Fig. 6. Near-infrared–emitting ureteral stents allow for safe identification and protection of the ureters (A) during pelvic surgery and (B) in a retroperitoneal exposure for hemipelvectomy.

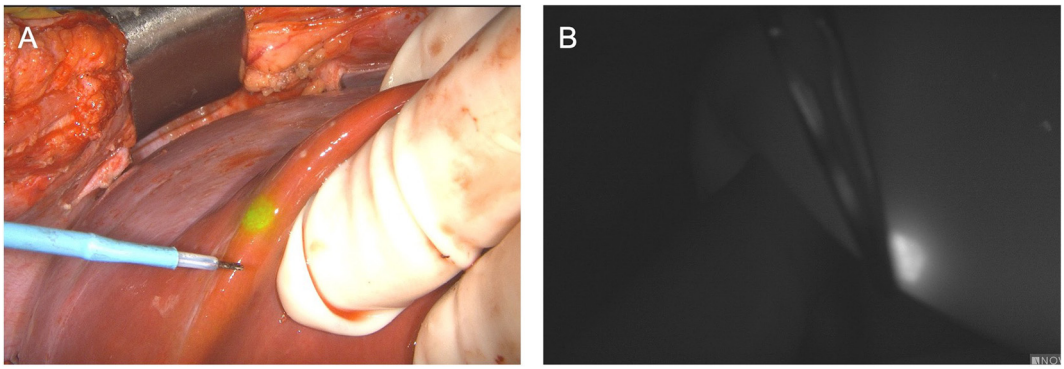

Fig. 7. An ICG false-positive nodule was (A) identified in overlay mode and (B) confirmed in contrast mode during partial hepatectomy for hepatoblastoma, despite no evidence of disease in this location during preoperative imaging. Histologic examination of the wedge biopsy showed no evidence of tumor or other abnormalities in the specimen.

hepatoblastoma who had an additional brightly fluorescent nodule in the right lobe. Although no mass was observed in this location during preoperative imaging or upon visual inspection by the surgeon, the FGS findings were sufficiently suspicious to warrant wedge resection. Histologic evaluation demonstrated only normal hepatic parenchyma. Further work is also required to better understand whether FGS is equally effective for identifying metastases in patients pretreated with chemotherapy and/or radiation therapy, or in those with new lesions. The percent viability of the tumor nodules may affect the degree of fluorescence intensity.

Adherence to the pearls and pitfalls outlined in section 2.2 can help minimize the occurrence of false positive and negative results. It is important to always bear in mind that the penetrance of the ICG infrared fluorescence through soft tissue is limited to about 1 cm. False negatives (i.e., nonvisualization of the intended target) will occur if attempting to identify deeper structures. This penetration depth can be decreased even further through multiple air/fluid interfaces, thus emphasizing the importance of meticulous lung isolation for thoracic cases and realistic imaging strategy for other planned FGS cases.

6. Conclusions and future directions

Modern FGS modalities are progressively becoming more routine in surgical practice. In adults, ICG was found to beneficial in randomized clinical trials for bile duct cholangiography [2,3], colorectal anastomosis [27], and cervical cancer SLNB [18]. Sentinel lymph node biopsy in other diseases such as breast cancer [28] and lung cancer [29] are also the subjects of intense interest for the prospect of FGS utility. Pediatric surgical trials have been less robust but have suggested potential promise [11].

Future efforts will most likely refine the utility of ICG by establishing an evidence base, developing targeted fluorophores, and discovering endogenous fluorescence patterns. Several pediatric clinical trials are underway to better determine the benefits of FGS in applications ranging from cardiac angiography and intestinal perfusion, to tumor resection margin delineation and SLNB. As NIR detection technology becomes increasingly integrated into modern surgical platforms, assessments of patient benefit, surgeon utility, and cost will ensue. While the cost of this technology currently restricts its general availability, it is hoped that the cost will be reduced with broader use.

Targeted fluorophores are promising and may improve the specificity and precision of FGS. Although ICG is generally nonspecific beyond its perfusion and clearance kinetics, some fluorophores can be activated only in tumor-specific environments. For example, the activated fluorophore 5-aminolevulinic acid is administered orally and is then metabolized within glioma cells to a fluorescent molecule but remains nonfluorescent in surrounding tissues [30]. Alternatively, fluorophores may be conjugated to monoclonal antibodies for targeting tumor-specific antigens [31]. Indeed, a first-in-human, proof-of-concept study recently demonstrated the utility of a fluorophore conjugated to an EGFR antibody for localizing glioblastoma tumors during resection [32]. The opportunities to expand the field of theranostics (i.e., using therapeutic agents for diagnostic purposes) are nearly limitless.

A final future direction is detection of autofluorescence. In contrast to using exogenous contrast agents such as ICG, this modality tunes the camera detection range to frequencies specifically associated with an organ or disease. For example, parathyroid glands autofluoresce at wavelengths distinct from their surrounding tissues, permitting precise intraoperative localization [33]. In other instances, pathologic states caused by a tumor or inflammation change the emitted or reflected NIR signature, which can be leveraged to facilitate diagnoses or discriminate between healthy and diseased tissues [34,35].

In summary, FGS with ICG has many current and evolving applications in pediatric surgical oncology. These adjunctive tools are still experimental but have demonstrated promise for identifying tumor margins, locating metastases, facilitating SLNBs, protecting peritumoral structures of interest, and facilitating reconstruction. The impact of specific tumor histology, tumor viability and prior treatment with adjuvant therapies also remain to be determined.

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