

Fluorescence-guided surgery: comprehensive review

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Abstract

Background: Despite significant improvements in preoperative workup and surgical planning, surgeons often rely on their eyes and hands during surgery. Although this can be sufficient in some patients, intraoperative guidance is highly desirable. Near-infrared fluorescence has been advocated as a potential technique to guide surgeons during surgery.

Methods: A literature search was conducted to identify relevant articles for fluorescence-guided surgery. The literature search was performed using Medical Subject Headings on PubMed for articles in English until November 2022 and a narrative review undertaken.

Results: The use of invisible light, enabling real-time imaging, superior penetration depth, and the possibility to use targeted imaging agents, makes this optical imaging technique increasingly popular. Four main indications are described in this review: tissue perfusion, lymph node assessment, anatomy of vital structures, and tumour tissue imaging. Furthermore, this review provides an overview of future opportunities in the field of fluorescence-guided surgery.

Conclusion: Fluorescence-guided surgery has proven to be a widely innovative technique applicable in many fields of surgery. The potential indications for its use are diverse and can be combined. The big challenge for the future will be in bringing experimental fluorophores and conjugates through trials and into clinical practice, as well as validation of computer visualization with large data sets. This will require collaborative surgical groups focusing on utility, efficacy, and outcomes for these techniques.

Introduction

Advances in cross-sectional imaging have led to an improvement in diagnostic accuracy, patient selection, and surgical planning. Similarly, the evolution of minimally invasive platforms has led to advances in a number of surgical procedures, with demonstrated improvement in clinical outcomes. Despite these advances, operative procedures are mainly guided by the surgeon's perception of the tissue under ambient light. Fluorescence-guided surgery is an innovative and rapidly expanding field in modern surgical practice, which adds an additional dimension to the surgeon's understanding of the anatomy, physiology, and pathology.

A commonly used fluorophore in surgery is indocyanine green (ICG)^{1,2}. ICG is a water-soluble dye, which binds to albumin and distributes rapidly and uniformly in the blood. It is excreted by the liver and is therefore present in bile. The concept of ICG-guided surgery exploits the ability of ICG to absorb near-infrared (NIR) light at a wavelength around 800 nm and emit fluorescence around 830 nm^{1,2}. A fluorescent picture is captured by a dedicated camera and can be overlaid on to the white light image to provide additional information for the surgeon. Most major surgical imaging companies now provide such capabilities in their range of laparoscopic stacks.

A literature search on PubMed was performed for articles in English until November 2022 using Medical Subject Headings 'near-infrared fluorescence imaging' and 'optical imaging'. This emerging narrative review describes the role of ICG fluorescence and other less common fluorophores in improving intraoperative comprehension from visualization in blood to evaluate: tissue perfusion; anatomical structures, including bile ducts and ureters; the lymphatic system and its role in lymph node targeting; and uptake into peritumoral tissue.

Tissue perfusion

Using an NIR imaging system, ICG can be visualized in any tissue of interest^{3,4}. By visualizing fluorescence intensity over time, an understanding of dynamic perfusion can also be obtained. Most studies describe qualitative assessment of fluorescence (that is 'watching the tissue of interest becoming green'). Although the name ICG suggests otherwise, the green colour surgeons see on their screen is artificially chosen due to its contrast with other tissues, including skin, bowel, muscle, and fat. This qualitative assessment can be performed in real time to assist the surgeon in distinguishing between poorly and adequately perfused areas of interest^{5,6} (Fig. 1). This has already gained a lot of clinical traction in colorectal surgery for anastomotic perfusion

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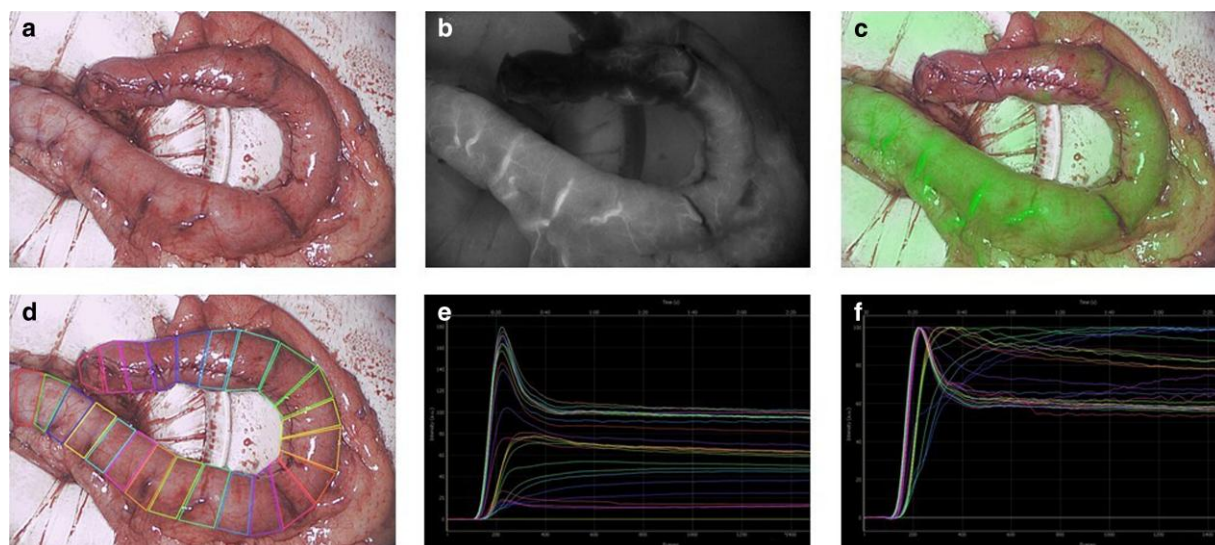


Fig. 1 Example of near-infrared fluorescence perfusion imaging using indocyanine green during preparation of a gastric conduit after oesophagectomy, demonstrating a tapering in the degree of perfusion

a White light image. **b** Near-infrared fluorescence image. **c** Colour-NIR merge image. **d** Subsequent regions of interest are drawn to plot time-intensity curves. **e** Time-intensity curves based on absolute fluorescent signal. **f** Time-intensity curves based on normalized curves.

assessment, with phase II⁷ and even small phase III^{8,9} studies now showing benefit. A large-scale randomized trial (IntAct) aimed at establishing the case for ICG fluorescence assessment of anastomotic perfusion is nearing completion, with results likely being published later this year¹⁰.

The interpretation of images, however, remains qualitative, and is prone to inter-observer variability¹¹. It is hoped that accurate quantification could allow even more data to be extracted from ICG perfusion curves, including venous outflow and better (perhaps even automatic) delineation between perfusion transition zones. Finally, the intensity of fluorescence is heavily dependent on the distance between the target and the camera, as well as camera angle, highlighting the need for quantification or at least standardization of viewing protocols. Using standardized camera settings and set up in a fixed position, the fluorescent signal can be plotted as arbitrary units over time (a time-intensity curve). This curve can be used to calculate various inflow- and outflow-based parameters. However, the maximum fluorescence intensity, which is dependent upon the distance between the camera and the target, has a significant influence on the shape and parameters of the curve, resulting in heterogeneous outcomes^{12,13}. A potential solution to this problem utilizes relative perfusion parameters, which are plotted as the proportion of the maximum intensity in a certain region of interest. Another technique that has been advocated is the use of curve normalization, plotting the maximum intensity as 100 per cent instead of using arbitrary units^{14,15}. In this manner, time-intensity curves can be better compared, without losing essential information. Unfortunately, despite efforts to improve software and hardware, the need to perform these analyses results in the inability to utilize quantification in real time. By comparing standardized quantification analyses with clinical outcomes, cut-off values differentiating the good, the bad, and the ugly will be created¹⁶. Large data sets, including NIR fluorescence parameters and clinical outcomes, are imperative to reaching this goal. The final step will be to prove the added value of NIR fluorescence in clinical decision-making through design and execution of RCTs.

Identification of anatomy

Biliary tract

Extrahepatic biliary tract anatomy has considerable variations. These variations combined with difficulties in identifying anatomy due to inflammation are an inherent contributory factor in bile duct injuries. Although bile duct injury is reported to be low (0.3–0.5 per cent), its occurrence is a serious complication that can have deleterious outcomes¹⁷. Since the introduction of laparoscopic cholecystectomy, the reported incidence of bile duct injury is higher than that for an open approach, despite introduction of the critical view of safety^{18,19}. Therefore, intraoperative cholangiography has been advocated to be utilized routinely²⁰. This technique can be difficult and time-consuming, with a significant learning curve and requirement for a patent cystic duct^{21,22}. Alternatively, NIR fluorescence using ICG has been shown to be capable of highlighting the extrahepatic biliary tract^{23,24}. Hepatic clearance after intravenous administration of ICG enables fluorescence imaging of the bile ducts 30 min after injection²⁵. Multiple studies have shown promising results, with two RCTs demonstrating improved detection of bile duct variations equal to that of intraoperative cholangiography, and a third study evaluating time to demonstrating the critical view of safety currently awaiting publication^{26–28}. In difficult operative conditions, for example inflammation from cholecystitis, ICG cholangiography has been demonstrated to be feasible and helpful in identification of the extrahepatic bile ducts²⁹.

Urinary tract

Iatrogenic ureteric injury is a rare, but serious, complication during surgery, with a reported incidence of 0.5–1 per cent and upwards of 10 per cent in advanced gynaecological oncology procedures^{30–32}. Preoperative ureteric stent placement can facilitate intraoperative identification; however, visualization during laparoscopic surgery can be challenging due to the retroperitoneal location and, particularly during robotic surgery, the lack of tactile feedback. The use of NIR fluorescence is a less invasive alternative to ureteric stenting for intraoperative ureter identification^{24,33–40}. The current clinically available dyes, ICG

and methylene blue (MB), are limited in their utility during laparoscopic surgery⁴¹. MB is preferred to ICG as it is renally excreted; however, it can only be visualized in the 700 nm range, with limited yield. MB is administered intravenously in doses ranging from 0.25 to 1.0 mg/kg 30 min before surgery^{33–35,42}; however, currently, there is no commercial system available to facilitate imaging. Identification of ureters is feasible in 50–95 per cent of patients, limited by high tissue autofluorescence and reduced penetration in the 700 nm imaging window⁴³. ICG can also be directly instilled into ureters^{36–38}. A volume of 10 ml (concentration of 2.5 mg/ml) is instilled into the ureter during cystoscopy. In patients with a nephrostomy in situ, ICG can be injected antegrade whilst clamping the urinary catheter to minimize drainage^{36,37}.

Of the currently experimental dyes, ZW800-1 and IS-001 are both renally cleared, with excitation in the 800 nm range, demonstrating promising results in the first clinical trials^{39,40}. ZW800-1 provides a large safety window in which a single low dose of ZW800-1 (1.0–2.5 mg) is sufficient for intraoperative ureteric visualization, with real-time assessment of function, even with overlying tissue, for an interval of at least 2 h after administration³⁹. An interesting possibility for ZW800-1 is conjugation to tumour-targeting moieties, for example the integrin-targeting small peptide cRGD in colorectal cancer, enabling simultaneous identification of both ureter and tumour^{44,45}. IS-001 has been demonstrated to be safe with the strongest fluorescent signal at a dose of 40 mg, 30 min after administration⁴⁰. Both dyes are the subject of further clinical evaluation.

Indications, technique, and interpretation

For bile duct imaging, ICG can be used in all patients undergoing open or minimally invasive surgery, with the greatest benefit likely to be in challenging cases, for example after biliary pancreatitis, drainage procedures, and biliary stenting. The technique is relatively straightforward: a 5–10 mg intravenous injection of ICG (concentration of 2.5 mg/ml) at least 30 min before the intended imaging time⁴⁶. The limitation of early ICG injection is the strong fluorescent signal arising from the liver; however, with modern camera systems, this signal does not hamper interpretation of extrahepatic bile ducts. In addition to bile duct imaging, an additional injection of 2.5 mg of ICG can be administered to allow for near instantaneous fluorescence of the hepatic arteries²⁹.

For ureteric imaging, ICG can be utilized as outlined above; however, the indications and advantages are less convincing. New experimental dyes are likely to prove useful in time, staining the urine fluorescently to facilitate visualization of peristaltic waves of urine flowing through the ureters. For this to work optimally, adequate diuresis is pivotal and therefore close communication with the anaesthetic team is required⁴⁷.

Lymph node targeting

Lymph node visualization and targeting with fluorescent dyes has been utilized in surgical oncology for many years. The first usage of ICG as a fluorophore was proposed by Kitai et al.⁴⁸ for sentinel node biopsy in breast cancer. Since then, new applications have been proposed for the identification and visualization of lymphatic channels.

In a meta-analysis of 22 studies by Mok et al.,⁴⁹ the detection rate of ICG for sentinel node biopsy in breast cancer was 97.9 per cent, with a false-negative rate of only 0.6 per cent. In their

analysis, ICG fluorescence outperformed all other analysed methods, including ^{99m}Tc, blue dye, dual, superparamagnetic iron oxide, and contrast enhanced ultrasonography techniques. Based on a more recent meta-analysis, ICG is equivalent to the radioisotope technique and superior to both blue dye and dual techniques⁵⁰. The clinical effectiveness of this technique is grounded in its real-time resolution tracing, visibility through the skin, good safety profile, cost-effectiveness, and no requirement for access to a nuclear medicine department. Limitations, however, include difficulties with detection of deeper sentinel nodes, a higher number of stained nodes, risk of anaphylaxis, and skin discolouration.

An ICG-^{99m}Tc-nanocolloid hybrid has been constructed, combining the advantages of both dyes used separately^{51,52}. Conjugation of ICG with rituximab produced a compound with 100 per cent sentinel lymph node mapping at 24 h, with no spread to secondary or tertiary lymph node stations⁵³. ICG may also be combined with an integrin-binding vector, which is overexpressed in some cancers⁵⁴. Finally, a tumour-targeted tracer of panitumumab-IRDye800CW injected intravenously showed very good results for the detection of sentinel nodes and metastatic sites in oral cancer⁵⁵. Use of MB and fluorescein for sentinel nodal biopsy in breast cancer and melanoma has also been described^{56–58}. Moreover, multispectral visualization of different lymphatic regions of interest using combinations of fluorophores has also been studied⁵⁹. Fluorescent visualization of sentinel nodes has not only been described for breast cancer and melanoma, but also other malignancies, including gynaecological, gastrointestinal, endocrine, head and neck, and urological malignancies^{60–65}.

In addition to sentinel lymph node identification, visualization of lymphatic channels can facilitate precision surgery in advanced lymphoedema⁶⁶. Fluorescent lymphography can also be used to classify lymphoedema, as well as for axillary reverse mapping and thoracic duct visualization during oesophageal surgery^{67–69}. Lymphography may also be used for tailored lymphadenectomy in gastric and colon cancer, facilitating the resection of additional lymph nodes outside the standard surgical field^{70,71}, as well as improving lymph node yield⁷². Metastatic lymph nodes can also be detected after intravenous administration in patients with peritoneal carcinomatosis⁷³, with an ongoing study evaluating the application of 'systemic sentinel node' visualization⁷⁴.

Imaging of tumour tissue

The cornerstone of treatment of most solid tumours, including colorectal, hepatopancreatobiliary (HPB), and endocrine tumours, remains radical surgical resection. The oncological benefit of this surgical approach relies on complete resection of all cancerous tissue, avoiding damage to vital structures and sparing healthy tissue⁴⁴. In this context, NIR fluorescence aims to enhance and optimize real-time surgical navigation for detection of tumour tissue, both at the primary site, as well as lymph nodes and metastatic sites, guiding surgeons with visual feedback during cancer surgery to improve safety, radicality, oncological outcomes, and survival⁴⁴.

The current clinically available fluorescent probes, such as ICG and MB (not currently licensed), have restricted utility for this purpose; an overview is given in Fig. 2. Although applicable for certain indications, visualization is hampered due to non-specific binding and lack of tumour specificity^{75,76}. ICG sentinel lymph node mapping in colorectal cancer is feasible

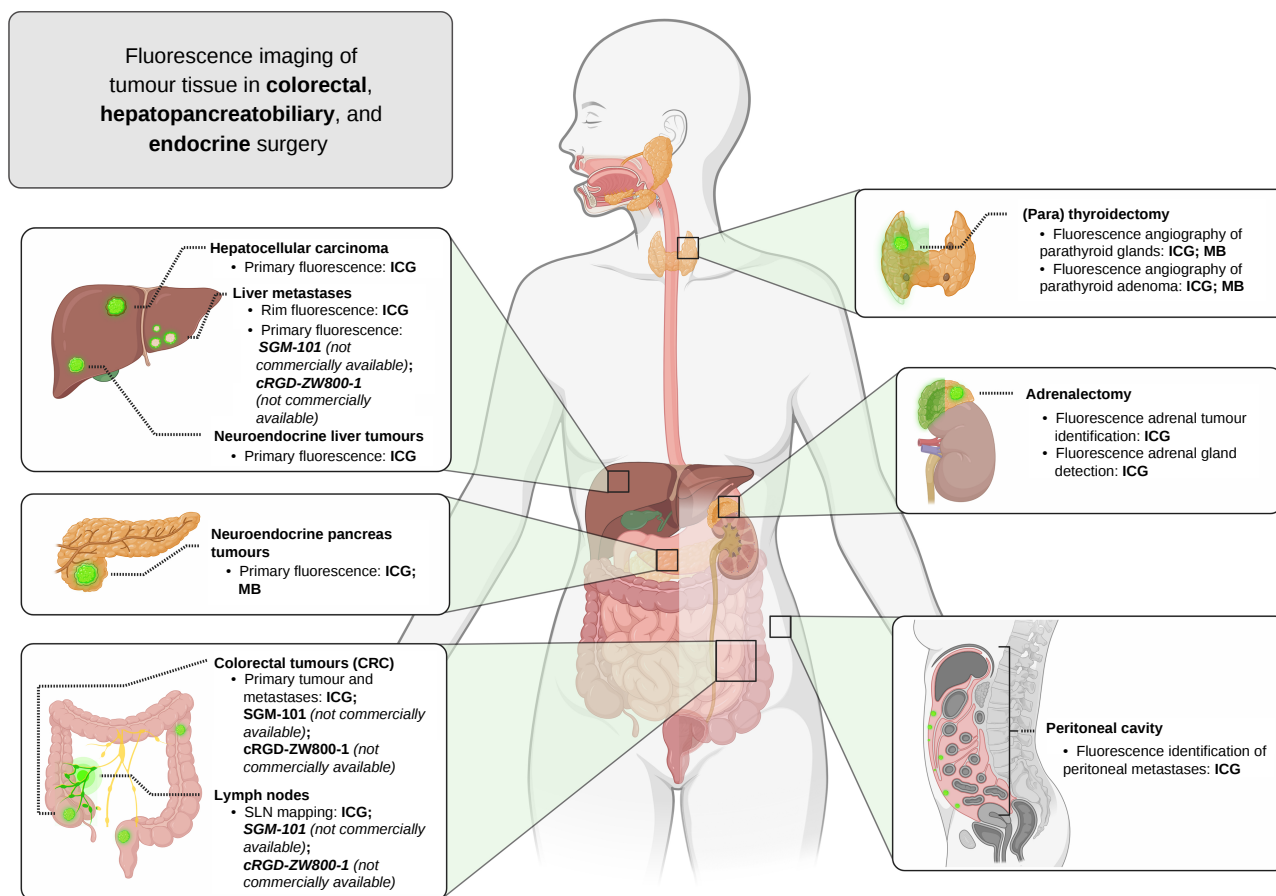


Fig. 2 Overview of clinical applications of fluorescence-guided surgery in colorectal, hepatopancreatobiliary, and endocrine surgery

Indocyanine green and methylene blue are commercially available fluorescent probes with applications in these patients. Specific dosing and dose timing for the indications above are described fully in the main text. Created using BioRender.com. ICG, indocyanine green; MB, methylene blue; SLN, sentinel lymph node.

with preoperative or intraoperative peritumoral injection of 0.5–1.0 ml ICG (2.5 mg/ml) in each tumour quadrant, although of limited clinical value at present^{77,78}. Accuracy decreases with advanced stage disease and a high false-negative rate remains a major disadvantage^{75,79}. While feasible, the clinical value of ICG-based visualization of peritoneal deposits in colorectal and HPB cancers is limited⁸⁰, with mucinous tumour type and neoadjuvant treatment adversely influencing the diagnostic sensitivity of this approach further⁷⁵. An increasingly utilized method for visualization of colorectal liver metastases using ICG takes advantage of ICG's hepatic clearance. It is hypothesized that ICG accumulates in immature hepatocytes surrounding the lesion, which are unable to rapidly excrete ICG in the bile, resulting in a typical rim-shaped enhancement pattern surrounding the lesion after preoperative intravenous administration (10 mg 24 h before surgery or 0.5 mg/kg 10–14 days before surgery)⁸¹. Dose and dosing interval need to be adapted in the case of liver cirrhosis or after chemotherapy-induced fibrosis, in order to decrease the false-positive rate (0.2 mg/kg 24–48 h before surgery)^{75,78,82}. Hepatocellular carcinomas can also be visualized using ICG; instead of a rim-shaped signal around metastases they demonstrate a fluorescent signal at the site of the primary lesion itself (0.5 mg/kg 2–7 days before surgery)^{82,83}.

Several methods for ICG- and MB-guided surgery have been explored and validated for (para)thyroidectomy and adrenalectomy⁸⁴. NIR fluorescence imaging can effectively be used for identification and preservation of the parathyroid

glands during thyroidectomy (intravenous injection of 0.2–1.0 ml ICG after thyroid gland dissection)^{85,86}. MB has been utilized in the same manner to visualize parathyroid adenomas, with a single dose (0.5 mg/kg) administered intravenously after induction of anaesthesia aiding the identification of parathyroid adenomas and normal glands⁸⁷. During adrenalectomy, ICG is effective for the identification of the gland and the adrenal tumour within the retroperitoneal fat using a (repeated) intravenous dose after exposure of the retroperitoneum and during dissection (5 mg, which could be repeated two to three times)⁸⁸. ICG- and MB-guided surgery of neuroendocrine tumours (NETs) has been evaluated in smaller studies, with clinical evaluation currently restricted to visualization of NETs in the pancreas and liver^{88–91}. Intraoperative administration of intravenous ICG (1 ml, 2.5 mg) aided the identification of pancreatic lesions and confirmation of their extent, particularly for NETs and cystic neoplasms⁹⁰. Similarly, intraoperative administration of MB (1.0 mg/kg) allowed successful identification of multiple metastatic lesions missed by preoperative imaging⁹¹. Metastatic NETs in the liver demonstrate the same enhancement as hepatocellular carcinomas with intravenous administration of ICG (0.5 mg/kg 24 h before surgery).

Target-specific probes

NIR fluorescence using target-specific imaging probes has gained interest over the past decade as a promising and effective method for visualization of malignant targets. To date, multiple fluorescently labelled antibodies, peptides, particles, and other

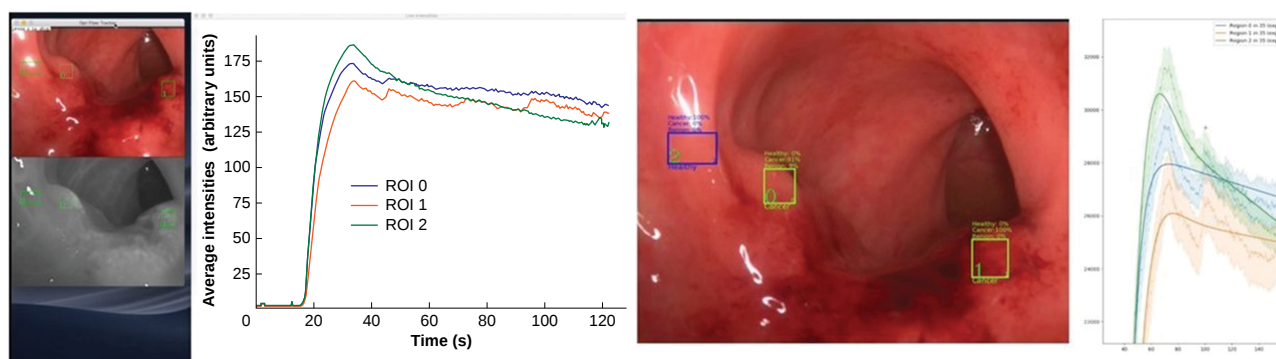


Fig. 3 Illustrative example of the application of computer vision and artificial intelligence methods to indocyanine green fluorescence angiography for the classification of rectal cancer

The images to the left show the white light endoscopic appearance of a rectal tumour above a still of the dynamic indocyanine green perfusion phase of the same lesion. Indocyanine green intensity time series from the boxed areas in these images allow prediction (including mathematical probability) of whether the lesion is cancerous or not based on rapid statistical inference of such time-series analyses. ROI, region of interest.

small molecules have been developed and are currently being explored and validated^{44,92–94}. These conjugates of a target molecule coupled to a fluorescent dye bind or interact with the target cell-surface proteins or tumour microenvironment of the cancer cells⁹⁵. Potent targets should have a strong specific expression on target tissue relative to (healthy) surrounding tissue, with their expression minimally influenced by neoadjuvant treatments⁹⁶. Well known cancer cell-surface targets, such as carcinoembryonic antigen (CEA), epidermal growth factor receptor, vascular endothelial growth factor, epithelial cell adhesion molecule, and integrins, as well as others, form the basis of these target-specific probes^{97–101}. The main challenge in the field is the evolution from early clinical trials to phase III trials aiming to evaluate the clinical benefit of target-specific NIR fluorescence¹⁰². An example is the CEA-targeting agent SGM-101 (Surgimab S.A.S., Montpellier, France). A phase II safety and feasibility trial using a single intravenous dose of SGM-101 (10 mg) 3–4 days before surgery in patients with colorectal cancer showed a sensitivity of 96 per cent, a specificity of 63 per cent, and a negative predictive value of 94 per cent for detection of the primary tumour. In more than 20 per cent of patients, the surgical plan was altered based on fluorescence, including the detection of additional malignant lesions¹⁰³. Additionally, SGM-101 was shown to detect colorectal and pancreatic liver metastases¹⁰⁴. An international multicentre phase III trial is currently recruiting, aiming to evaluate the clinical benefit of CEA-targeted NIR fluorescence in primary and recurrent colorectal cancer (NCT03659448). The focus for coming years should be selective validation of the most effective target-specific probes identified in early-phase clinical research to accelerate future clinical availability.

Future opportunities and horizon scanning

For tissue perfusion characterization, the dynamic appearance of the agent ('how it gets into the tissue') is an important variable to judge in addition to dye presence/absence. To date, for most proposed tumour identification modalities discussed above, the dye is given some considerable time before surgery in the expectation that it will then be viewed at whatever time its concentration is maximal in any cancerous areas and minimal in any adjacent non-cancerous regions. Therefore, although the dye's uptake into a tumour has been dynamic, its time of viewing is instead at a stable, rather static interval in its pharmacodynamic tissue phase. The human observer (the surgeon) must then

visualize the fluorescent tissue and make a judgement about what is being seen before proceeding with the operation with this additional information. This means that considerable preoperative planning is needed, including agent administration long before the intended operation, with a limited window for optimum visual conditions. Potentially useful information from the early dynamic phase is also lost with this approach.

Given that the fluorophore creates specific contrast in tissue that is easier to distinguish against a normal tissue background, the field of ICG fluorescence angiography is ripe for the application of computer visualization to more precisely and objectively distinguish the dye's presence and course¹⁰⁵. Important differences in dye concentration through the application of thresholding may better distinguish truly important dye concentrations over unimportant (false-positive) areas of dye leakage/persistence. For dynamic influx/egress, generation of fluorescence intensity–time curves can both record the time-series data and allow statistical comparison of dynamic dye tissue behaviours by the additional application of artificial intelligence (AI) methods, which can also then classify the tissue (that is whether it is healthy or unhealthy, or consisting of cancer or not cancer) with sufficient training data sets¹⁰⁶. Inclusion of information from the standard white light appearance of the tissue alongside the evolving fluorescence imagery against a black background on an NIR spectral view provides further information, enabling tissue classification (Fig. 3). Such an application of AI is appealing, because the predictions being made are based entirely on explainable, interpretable, and understandable underlying biological, physical, and pathophysiological processes, and so stronger confidence can be afforded when compared with other AI methods, such as deep learning¹⁰⁷. For these reasons, AI systems based on fluorescent dyes may be more likely to be viewed favourably by regulatory agencies¹⁰⁸. As even the most specific new fluorophores will still need interpretation (as quickly and precisely as possible), such adjunctive identification methods will still be applicable irrespective of their specific indication. Clinical proof of this fundamental concept has already been established with regard to distinguishing rectal cancer with high degrees of accuracy, with work ongoing to prove validation of the concept and generalizability at other sites and with other cancers¹⁰⁹.

Surgical contrast agents, especially most MB derivatives, are a significant cause of healthcare-associated morbidity¹¹⁰. Whilst ICG has a very good safety record, it still requires the systemic

administration of a compound and therefore carries some risk of anaphylaxis. There is also an added clinical step in administering the agent whether before or during a surgical procedure. Given the potential clinical impact of enhanced intraoperative visualization, there has been increasing interest in developing 'dye-free' ways of analysing tissue texture and character via multispectral analysis¹¹¹. Such hyperspectral imaging is technically possible and generates huge amounts of information, which again may benefit from computational interpretation¹¹². Early clinical use is proving encouraging, but it remains unclear as to how useful it will prove and whether it is best as an adjunct rather than alternative to NIR fluorescence.

Undoubtedly, all these innovations are technologically possible, but a key component of their clinical utility is how useable they are and indeed how to get the most out of them in clinical practice. Given that these are screen-based technologies that require additional understanding to visualization of the surgical field, there is a great need to share and build experiences, for example with surgical video recordings. In this way, innovations can be validated, as well as combined with emerging new computational strategies, such as AI, to achieve greater levels of accuracy in interpretation. There is a need for collaborative surgical groups to build independent, representative, and accessible data stores, and work closely with industry and academic partners to better help realize these technologies¹¹³.

Conclusion

Fluorescence-guided surgery has proven to be a widely innovative technique applicable in many fields of surgery. An important step has been widespread implementation, with increasing evidence of patient safety and surgical efficiency. The potential indications for its use are diverse and can even be combined in selected cases. The big challenge for the future will be in bringing experimental fluorophores and conjugates through trials and into clinical practice, as well as validation of computer visualization with large data sets. This will require collaborative surgical groups focusing on utility, efficacy, and outcomes for these techniques. Given the present experience, published literature, and future opportunities, fluorescence-guided surgery is here to stay.

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Author contributions

Paul A. Sutton (Conceptualization, Project administration, Supervision, Writing—original draft, Writing—review & editing), Martijn A. van Dam (Conceptualization, Project administration, Writing—original draft, Writing—review & editing), Ronan A. Cahill (Conceptualization, Project administration, Writing—original draft, Writing—review & editing), Sven Mieog (Conceptualization, Project administration, Writing—original draft, Writing—review & editing), Karol Polom (Conceptualization, Project administration, Writing—original draft, Writing—review & editing), Alexander L. Vahrmeijer (Conceptualization, Project administration, Writing—original draft, Writing—review & editing), and Joost van der Vorst (Conceptualization, Project administration, Writing—original draft, Writing—review & editing).

Disclosure

The authors declare no conflict of interest.

Data availability

No novel data are included in this comprehensive review.

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