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Biofluorescence in surgery: Present and future

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A B S T R A C T

Biofluorescence is a physical phenomenon that has gained a multitude of clinical applications since its introduction to medicine in the 1940s. The utilisation of biofluorescence in colorectal surgery has grown from the development of the fluorophore indocyanine green (ICG) and its prior applications in assessing vascular beds in other fields of surgery. However, despite the increasing adoption of ICG in the assessment of colonic conduit perfusion, the evidence base for its utilisation remains controversial, although a range of other uses for this technology are emerging. Advances in semi-quantitative and artificial intelligence augmented platforms are providing greater objectivity in the application of biofluorescent techniques in colorectal surgery, although they are still in a largely developmental phase. Molecular-targeted biofluorescent technologies are also opening up new surgical paradigms for intraoperative real-time assessment of tumours and their locoregional spread and may in time facilitate surgeons to find equipoise in the radicality of oncologic resection.

Introduction

Biofluorescence (BF) is the physical event observed when electromagnetic radiation (light) is absorbed by a living organism and reemitted at a different wavelength in the visible spectrum. BF is a common phenomenon in the natural world and is observed in a huge variety of biological systems. BF has been unitised in a multitude of medical applications since the late 1940s, when fluorescein was first used to guide the removal of intra-cranial malignancies.[1] In 1959 indocyanine green (ICG) was approved for human use by the United States Food & Drug Administration and ICG is now utilised in a variety of clinical scenarios, including in colorectal surgery. Due to its pharmacokinetic properties and behaviour under near-infrared excitation, ICG predominates as the primary fluorophore in modern surgical practice, although considerable contention exists within the evidence base for its utilisation.

Despite the need for ongoing research, BF is finding an ever-more established place in routine colorectal practice and is at the forefront of advances in finding equipoise in strategies in surgical oncology. Real-time fluorescence imaging in intraoperative decision making for cancer surgery is advancing how we deliver surgical interventions to patients, in the expectation that both cancer-specific clinical outcomes and surgical morbidity may both be optimised.[2] Further developments in the dual technologies of ligand-specific fluorophores and artificial intelligence-led quantitative imaging provide an optimistic future for BF in molecular fluorescence guided surgery.[3]

Biofluorescence & biofluorescent agents in medicine

Biofluorescence (BF) is the physical event observed when electromagnetic radiation (EMR; light) is absorbed by a fluorophore in a living organism and reemitted at a different wavelength, commonly in the visible spectrum. When electromagnetic radiation excites a fluorophore the molecule temporarily enters a higher-energy state and then relaxes to its resting state (known as the Stokes shift), resulting in the emission of a photon which can be detected with the naked eye or through a variety of sensory apparatus.[4]

Although classically described in marine ecosystems, there is a growing appreciation of the role of biofluorescence in terrestrial animals including fireflies, salamanders, and other amphibians; where it has a role in communication, sexual selection, and visual acuity.[5,6] Excitation in the natural world is predominated by sunlight, although it may be stimulated by chemical bioluminescence or other sources of EMR. There is limited evidence that BF plays a role in mammalian biology, although its application in medicine has developed over the past 75 years.[1]

In 1948, Moore utilised the green-yellow fluorophore fluorescein under ultraviolet (UV) excitation to facilitate surgery for intracranial malignancy. This early trial utilised a wavelength of ~400 nm by means of a CH-4 Mercury Vapor Lamp with a Wood's filter but was limited to visualisation *ex vivo*, and is a methodology that continues to be utilised in dermatology and ophthalmology clinical practice today.[7] However, despite demonstrating the potential of fluorescence technologies for

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guiding real-time intra-operative decision making, the *ex vivo* methods employed in this early work were clearly limiting. Further neurosurgical innovation led to the development of second-generation fluorophore aminolevulinic acid (5-ALA), which breaks down into the compound PpIX to emit violet-red (~635 nm) fluorescence after excitation with blue light at UV wavelengths of 375–440 nm, but which can be used *in vivo* to facilitate in the visualisation of brain tumours.[8] Use of 5-ALA has demonstrated improved gross total resection over traditional neuronavigation on meta-analysis of almost 1000 cases over 17 years.[9,10] Although both of these techniques are used in today's clinical practice, the application of UV light is limited to relatively superficial or low-density tissues, as the penetration of light at this wavelength is limited, particularly in dense or pigmented tissues. An ideal fluorophore should be excitable and detectable at practical wavelengths, have predictable and safe pharmacokinetics and toxic profile, and be easily visualisable in target tissues. Methylene blue (MB), a thiazide dye with multiple medical applications, is also a fluorophore and can be excited at a peak of ~668 nm. However, unlike other fluorophores, MB is observable in the visual light spectrum (to the naked eye) and the Stokes shift is small, thus fluorescence is difficult to distinguish from background colour.[11] MB is also highly hydrophobic and demonstrates poor tissue penetration, further limiting its use as a medical fluorophore.

Indoscyanine green and colon prefusion assessment

Indocyanine green (ICG) is an amphiphilic, tricarbocyanine iodine dye suitable for intravenous or intratissue injection. Following administration ICG binds to plasma proteins, primarily albumin, and is transported to the liver where it is excreted into bile *via* glutathione S-transferase.[12] The rate of excretion, and therefore intravascular half-life of ICG is determined by the rate of hepatocyte uptake following the rules of first-order kinetics, but is typically between 3 and 4 min, although is extended in cirrhosis and other liver diseases.[13] In fluorescent applications *in vivo*, the concentration of ICG should be kept below 15 mg/L due to the molecule's tendency to aggregate at higher concentrations, leading to mitigation of effective fluorescence and reduced acuity.[14]

ICG has had US Food and Drug Administration approval sine 1956 for intravenous administration at a concentration of 2.5 mg ml^{-1}, with doses of up to 25 mg in adults, 12.5 mg in children and 6.25 mg in infants.[15] Although ICG is minimally toxic (it can generate a low concentration of oxygen free-radicles under certain conditions), ICG is not suitable for patients with iodine allergy. ICG is excited with near-infrared (NIR) stimulation at a wavelength of ~750–800 nm and fluoresces at a peak of 823 nm. A significant advantage of stimulation at these longer wavelengths is that NIR is able to penetrate more deeply (in excess of 10 mm) and thus visualise targets below the surface of tissues; although there is some variability depending on tissue density, pigmentation, and the angle of incidence of the applied EMR.[16] Furthermore, NIR stimulation of background molecules such as haemoglobin and oxyhaemoglobin results in an effect that leads to greater tissue transparency and less interference with measurement of ICG fluorescence, resulting in greater acuity.[1] Each of these features makes ICG an optimal fluorophore for *in vivo* biological applications in medicine and surgery and has thus led to its adoption in a number of fields.

Initial use of ICG in studies of hepatic and cardiac physiology, and later in retinal angiography, drove the growth of ICG through the 1960′s and '70′s and validated its utility as a tool in assessing circulatory physiology and vascular anatomy.[17,18] Refinement in techniques in the application of ICG angiography (ICGA) led to expansion in the 2000′s to assessment of skin-flap viability in general and plastic reconstructive surgery. Semi-quantitative real-time assessment of skin perfusion by ICGA during reconstructive breast surgery has been proven to augment the ability of surgeons to predict, and therefore mitigate, poor flap perfusion above the performance of clinical judgement alone, as well as aid in flap design and vascular anastomotic assessment.[19] Semi-quantitative methods in ICGA have been further developed to facilitate the assessment of blood flow in a range of scenarios, including assessment of peripheral vascular disease, wound necrosis, and visceral perfusion.[20–22] The potential for FB to be utilised in the assessment of perfusion in visceral anastomosis has not been overlooked and has been adopted in small bowel, colorectal, and oesophogastric surgery for over 10 years.[23–25]

The excitation-sensor technology employed in NIR fluorescence surgery has developed in conjunction with the evolving application of ICG, and most systems are now tailored specifically to ICG fluorescence.[26] Most major biotechnology companies now offer NIR-ICG platforms integrated into standard white-light visual-spectrum systems to provide hybrid image overlay in open, laparoscopic, robotic, and endoscopic platforms.

Current applications in colorectal surgery

ICG fluorescence has been utilised in colorectal surgery since the turn of the millennium when initial reports of its application as an alternative to India ink for colonic tattooing prior to surgery demonstrated that it was visualisable intraoperatively.[27] Ultimately, ICG's utility in tattooing is limited due to its relatively early washout from tissue, even after extravascular injection, although its potential for assessing colorectal perfusion has proved more fruitful.

Anastomotic leak (AL) following colorectal anastomosis is a significant and feared complication for both patients and surgeons, and has a significant clinical and economic burden.[28] Leak-rates vary across the literature but range from approximately 2 % to 20 % of cases depending on site of anastomosis and a number of other factors. Despite their frequency and a growing body of evidence to support our understanding the pathophysiology of a leak, surgeons have not proved at adept at predicting those cases which will go on to suffer an anastomotic leak.[29,30] However, poor blood flow to the anastomosis is one factor understood to be important in AL and is potentially amenable to intraoperative assessment and mitigation by the surgeon.[31] Following its applications in assessing other vascular beds, the potential role of ICG in assessing colonic vasculature in colorectal anastomosis has been investigated in a number of case series and randomised trials.

In an early multicentre non-randomised trial (PILLAR II; 2018), Jafari demonstrated that the leak rate following anterior resection in the group undergoing ICGA was 1.4 % compared to 12 % in the control group.[32] The use of ICGA in this study led to a change in the point of proximal colonic transection in 11 patients (8 %), of whom, none leaked. The cohort of patients in this study were, however, relatively heterogeneous in terms of the primary pathology (including elective resections for diverticular disease as well as cancers) and the rate of inferior mesenteric artery (IMA) high-ligation. Furthermore, the mean height of anastomosis in this study was 10 ± 4 cm from the anal verge, but no sub-group analysis was performed to indicate whether ICG had a potentially more impactful effect on leak rate in lower, more high-risk, joins. Regarding low joins, the FLAG trial (Russia; 2020) randomised patients to ICGA vs visual assessment of colonic perfusion and found that although the use if ICGA was associated with a reduced risk of leak, this was only observed in low anastomosis, defined as being within 8 cm of the anal verge.[33] Several studies have utilised ICG to examine the blood flow to the rectal stump rather than the colonic conduit to determine if this is a factor in anastomotic leak. Although some association was demonstrated between "delayed time to arterial [stump] perfusion" and anastomotic leak, the intraoperative assessment relied on semi-quantitative analysis of flow combined with vascular anatomy.[34] Other authors, who also examined leak following non-rectal anastomosis, suggest that leaks occurred most commonly in the subgroup whose anastomotic perfusion was *via* a marginal vessel rather than by the main native vessel (i.e. following high IMA ligation), but that a pragmatic course of action in such as case would be to prophylactically defunction such as patient and observe them closely postoperatively.[35] Despite the application of ICG and relatively complex perfusion analysis

in these studies, the authors did not arbitrate for a change in anastomotic strategy and seem to argue simply for the accepted wisdom of protecting subjectively high-risk anastomoses and good clinical care.

Subsequent studies have, however, broadly been in keeping with findings of earlier and smaller trial data in supporting ICGA as a tool for reducing the risk of AL in colorectal anastomosis. A systematic review and meta-analysis of 10 studies conducted between 1998 and 2014 and including approximately 1400 patients found that the use of ICGA was associated with a reduced risk of AL ($n = 23/693$; 3.3 % (95 % CI 1.97–4.63 %) compared with no ICGA assessment ($n = 19/223$; 8.5 %; 95 % CI 4.8–12.2 %); although the studies were heterogenous in the methods of ICGA assessment and protocols for intraoperative decision making in the case of suspected poor perfusion.[36] None of the studies had unbiased assessment of the endpoints, nor appropriate power calculation. In 2022, a broadly inclusive systematic review and meta-analysis of over 11,000 patients by Safiejko demonstrated that the colorectal anastomotic leak rate in ICGA and non-ICGA groups was 3.7 % vs. 7.6 % ($p < 0.001$) in all trials; 8.1 % vs. 12.1 % ($p = 0.04$) in randomized controlled trials (RCTs); and 3.1 % vs. 7.3 % ($p < 0.001$) in non-RCTs, respectively.[37] However, despite the large numbers, there remains a significant degree of heterogeneity in the included studies, some of which included only a small number of patients, some included non-rectal anastomosis, and some were singe-centre or even single-surgeon case series. Furthermore, the multivariate regression analysis reported in the recent multicentre Phase III PILLAR trial (USA; 2021) did not demonstrate any significant difference in leak rates between ICGA and standard assessment of perfusion (OR = 0.845 (95 % CI, 0.375–1.905); $p = 0.34$).[38]

Currently, a number of randomised controlled trials are recruiting with the objective of determining the true utility of ICGA in reducing the AL rate in colorectal anastomosis: IntAct (UK & Europe), AVOID (Netherlands), and FLUOCOL-1 (France).[39-41] Together, these trials aim to recruit almost 3000 patients, each has a clear protocol for utilisation of ICG and assessment of perfusion, intraoperative decision making, and have relevant clinical endpoints. Additionally, the IntAct trial will also include a sub-trial investigating the role of the gut microbiome in the pathophysiology of anastomotic leak as there is a growing appreciation that this a critical confounding factor.[42]

Indoscyanine green and ureteral identification

Beyond the assessment of colonic perfusion, ICG currently has a more straightforward role in aiding surgeons define at-risk anatomy during pelvic surgery; specifically, the ureters. Although only adopted sporadically and originally a technique borrowed from colleagues in gynaecology, the technique of injecting ICG into the ureters prior to pelvic colorectal resection has been reported to aid in the localisation of the ureters, particularly in challenging cases involving re-do surgery or sidewall dissection.[43,44] Although rates of ureteric injury in colorectal surgery are thankfully low, and therefore a reduction in risk by utilisation of ICG difficult to estimate, ICG may facilitate the identification of the approximately thirty percent of ureters that are "difficult to identify" under normal laparoscopic white-light illumination.[45] However, as ICG is metabolised in the liver and excreted in the bile, it must be introduced to the ureters *via* direct ureteral catheterisation prior to or during surgery. This is in contrast to the widespread utilisation of ICG in biliary surgery for guiding hepatic resection (including in surgery for colorectal metastasis), identifying bile leaks, and defining biliary anatomy at ductal surgery, where it can be conveniently injected intravenously.[46–48] Although there are no studies comparing the relative efficacy of ICG ureteric localisation versus prophylactic ureteric catheterisation/stenting in preventing ureteric injury, instillation of ICG may be performed quickly (mean time ~10 min) and ICG remains visualisable in the ureters for over 8 h.[49] There may also be an emerging role for ICG in surgery for endometriosis (both in localisation of nodules and perfusion assessment of treated organs); a surgical domain where colorectal surgeons not infrequently find themselves involved.[50,51]

Future developments

Indoscyanine green and lymph node identification

The resection of a colorectal cancer with clear margins (R0) remains the cornerstone of colorectal surgery and is associated with improved cancer-related outcomes.[52] Standardisation in surgical technique, such as total-mesorectal excision (TME) have led the way to improving clinical outcomes and have latterly been augmented by advances in neo-adjuvant and adjuvant therapies. Complete mesocolic excision (CME) may bring similar advances as the evidence base grows.[53] However, it is ultimately the skill of the surgeon in assessing the extent of a tumour, with the help of colleagues in radiology, that determines whether an R0 resection will be achieved and this is still predominantly dependent on intraoperative visual and tactile assessment.[54] One strategy for trying to ensure an R0 resection is a move towards ever-more radical resection, although this comes with significant associated morbidity and mortality, and thus techniques that facilitate clear margins without excessively high and wide resection are particularly valuable.[55] Biofluorescence is at the cutting edge of new surgical techniques for determining surgical resection margins in colorectal cancer surgery, and may facilitate in finding equipoise in surgical strtegy.

As previously discussed, techniques harnessing fluorescence are already utilised in real-time intraoperative decision making in neurosurgery for cancer, and are starting to be implemented in other surgical oncology domains.[2] In 2012, Hirche et al. reported the feasibility of sentinel lymph node (SLN) mapping in colonic cancer using a method of peri-tumoural injection of ICG and selective nodal sampling. Through this technique they were able to identify 82 % of involved sentinel lymph nodes, with an average SLN harvest of 1.7 nodes per-patient in 96 % of patients.[56] The authors of this small study discuss that although a standard oncologic resection of the lymph-node package would not be abandoned in the absence of positive SLNs, ICG could be utilised as a means of identifying aberrant lymphatic drainage and capture of nodes that would have otherwise fallen outside of resection margins. This is similar to the technique of "road mapping" and "cherry-picking" described by Cahill et.al. in their study on NIR ICG lymph node mapping in colonic cancer.[57] However, the authors in this study do admit that the so called *ultrastaging* of lymph nodes, whether SNL or non-SLN by ICG or by traditional staining at histopathology, did not significantly increase the number of nodes detected, nor did it upstage a significant number of patients (<1 %), as demonstrated by Wiese et al.[58] In other studies, Handgraaf and colleagues describe ICG as a method of tumour and nodal mapping in 5 cases of rectal cancer, but their method is limited to nodal groups within the TME.[59] Similarly, Kusano demonstrated that ICG was a reliable method of detecting colorectal lymph nodes (as well as gastric cancer lymph nodes) but within expected drainage basins.[60]

What is significant about these studies is their demonstration of the concept that SLN can be identified by ICG reliably, and may have some advantages over other methods of SLN detection such as radiocolloid or blue dye.[61] There are however only limited reports of the implementation of biofluorescence influencing the lymph node harvest or surgical strategy in colonic surgery, and specifically only in the context of CME. These studies, presented from the same unit in Italy, indicate that the implementation of ICG affected planned resection margins in approximately 35 % of cases, although no oncological outcomes are presented.[62,63]

When applied to rectal cancer, particularly low rectal cancer, the pertinence of bioflourescence to SLNs becomes more apparent. The differences in the management of low rectal cancer has historically been different in the global West when compared with the East, especially in the application of neoadjuvant therapies, and selective or default dissection of the internal iliac and obturator lymph node groups (lateral pelvic node dissection – LPND).[64] Rates of local recurrence in following

standard TME surgery for rectal cancer are in the order of 10–12 % and may be reduced by application of either neoadjuvant therapy or LPND to 6–7.5 %. However, the absolute rate of pelvic sidewall nodal involvement remains controversial, as does it impact on survival. Suspicious sidewall nodes were detected in approximately 12 % of cases in the MERCURY trial, and were found to be significant in relation to worse 5-year survival, although were not an independent factor in multivariate analysis and this prior association with poor survival was lost after application of neoadjuvant therapy.[65] This is in keeping with a Japanese study which found that lateral nodes were involved in 14 % of 930 patients undergoing prophylactic LPND, with T3 and T4 being the most significant risk factors.[66] Other studies of lateral pelvic nodes have indicated that rate of micrometastasis may be up to 20 % and be associated with an increased risk of local recurrence, but detection, particularly preoperatively and based on radiology, is challenging.[67] Each of these factors has led to a tendency towards variability in how a potentially involved sidewall is managed, with a propensity to either overtreat (thus risking morbidity associated with neoadjuvant treatment or LPND) or undertreat (leading to a higher risk of local recurrence).

Mapping and SLN biopsy (SLNB) is now the standard of care for most gynaecological cancers, including vulvar, cervical, and endometrial cancers, and is predominated by methods ulilising ICG across open, laparoscopic, and robotic platforms.[68] Open and minimally invasive techniques for PSW mapping utilising ICG have demonstrated high sensitivity, specificity, and negative predictive values for SNL metastasis across a range of different cancer types and excitation-detection platforms (typically approximately 95 %, 98 %, and 95 %, respectively).[69] SLNB in pelvic surgery for gynaecological cancers has three primary benefits that may also be considered as potential benefits if applied to low rectal and anal cancers: it (i) provides staging information with the potential to reduce intraoperative risks and patient morbidity associated with PLND; (ii) identifies unexpected lymphatic drainage patterns potentially identifying 'at-risk' nodes otherwise missed by standard dissection; and (iii) submission of fewer 'high-risk' nodes may be facilitate enhanced pathological examination which would otherwise be impracticable in a routine lymphadenectomy, thus reducing false negatives by oversight of single-cell or micro-metastasis.[70]

Unfortunately there is a paucity of evidence to support the use of biofluorescent techniques for pelvic sidewall mapping or SLNB in the context of rectal cancer, and none for anal cancer.[71] From a technical perspective, a number of small studies from the global East have demonstrated a small non-oncologic benefit to ICG guided surgery in minimally invasive routine PLND; specifically in reduction in blood loss and visualisation of critical structures.[72–74] Similarly, in a small series Noura et al. report that they readily identified nodal chains (both involved and uninvolved) in T1, T2, and T3 rectal cancers, but were not able to distinguish involved tumours on visual assessment alone.[75] In the West, one case series of five patients undergoing resections for low rectal cancer following neoadjuvant therapy received PSW mapping with ICG; also demonstrating that the technique was feasible and nodal chains demonstrable.[76] Interestingly, none of the identified PSW nodes which were cherry-picked as individual specimens in this study contained tumour. In the only randomised study, Wan randomised sixty-six (1:1) patients undergoing radical (D3) lymphadenectomy for rectal and sigmoid cancers to either white-light or ICG facilitated surgery; demonstrating that although rates of nodal positivity were no different between the two groups, the total number of nodes harvested in the ICG group was slightly higher than that in the standard surgery group.[77]

Clearly, there is a long way to go before BF has an established and evidence-based role in the management of the PSW in rectal and anal cancers. Despite the growing evidence for the utilisation of BF in gynaecology, neurosurgery, and increasingly in head-and-neck cancers; the limitations of the current techniques are frequently related to using ICG (or other fluorophores) in their freely circulating albumin-bound or hepatically metabolised form. Future advances in FB in surgery in colorectal cancer are currently being developed utilising technologies that specifically target the cell type and to improve imaging and special resolution.[3]

Alternative fluorophores

Antibody conjugated fluorophores have already been utilised in preclinical trials; the first of which reported in the early 2010′s and targeted established cancer-specific ligands such as VEGF, EGFR, and HER2 in head and neck cancers.[78,79] Key to the development of ligand-targeted fluorophores is a high sensitivity and specificity for target tissues relative to background binding; increasing spacial resolution.[80] In a small clinical trial, the anti-CEA ICG-conjugated monoclonal antibody SMG-101 was examined as a potential agent for the intraoperative identification of colonic tumours; finding that 19 (43 %) of 43 lesions were detected using fluorescence imaging and were not clinically suspected before fluorescent detection, which changed the treatment strategy in six (35 %) of 17 patients. Sensitivity was 98 %, specificity was 62 %, and accuracy of fluorescence intensity was 84 %.[81] Although this was a small study, it did demonstrate that techniques utilising ligand-targeted fluorescence in colorectal cancer surgery can influence intraoperative decision making; optimising planes of resection. In another study by Harlaar et al., the anti-VEGF-α fluorophore-conjugated monoclonal antibody bevacizumab-IRDye800CW demonstrated its utility in identifying occult serosal deposits in patients undergoing cytoreductive surgery for peritoneal metastasis of colorectal origin.[82] As increasingly tumour-specific antibodies are developed, cross-over from the field of medical oncology will facilitate delivery of fluorophores that can be utilised intraoperatively, optimising techniques in molecular fluorescence guided surgery (MFGS).[83] Due consideration must however be made of confounding factors in solid-tumour biology, most notably tumour heterogeneity and clonal evolution, which has made the reliable identification of a single agent that will target the entirety of a tumour as well as any metastatic deposits challenging.[84,85]

As well as monoclonal antibodies (>150 kDa), other protein-based binders such as antibody fragments, knottins (a type of sulphide-rich protein), and small peptides (5–15 kDa) are being investigated as potential vehicles for delivering fluorophores for MFGS. Some of these molecules have properties that facilitate in the specificity of tumoural targeting, whereas others are chosen for their broader pharmacokinetic properties such as circulating half-life, imaging contrast, or reduced administration-imaging interval.[3] Synthetic binders such as nanoparticles and other small molecules (such as folic-acid binding agents; <1000 Da) are also being assessed for targeted fluorophore delivery or inherent biofluorescence; some of which are undergoing Phase I trials (none colorectal).[86,87] Some of these small molecules interact with the tumour microenvironment, often by means of a pH mediated structural re-configuration or enzyme-cleavable link, optimising their performance in and around a tumour.[83]

As fluorophore delivery becomes more elegant, the demand for more sophisticated imaging and analytical systems to fulfil the potential of the molecular technology becomes ever-more pressing. Early *ex-vivo* imaging techniques with wide-spectrum filtered-lamps have developed into highly sophisticated laser or light-emitting-diode (LED) sources of EMR with a specific or narrow-spectrum excitation wavelength (<20 nm) often targeted to ICG use *in vivo*. Emitted photon detection has also advanced, with most systems designed for clinical use employing charged-coupled devices with high dynamic ranges and low image integration times.[15]

Advances in semi-quantitative, quantitative, and artificial intelligence-augmented imaging will be forthcoming, but effective clinical implementation will necessitate a dedicated framework for introducing technologies to the clinical workspace.[2] Future advances in machine learning and artificial intelligence may also offer deeper real-time and spatial tissue analysis, thus reducing or removing surgeon-subjectivity or other confounding factors; although the challenges of overcoming the relatively heterogeneous environment of the

human body and tumour environment mean this will not necessarily be an easily won battle.[88,89] Frameworks such as those set-out in the IDEAL recommendations are designed to facilitate this rapid technological advancement through thorough evaluation.[90–92]

Summary

Biofluorescence has evolved tremendously since its introduction to surgery over seventy-five years ago, and now has an established role colorectal surgery. Although the evidence base for some applications is still under scrutiny, the technology of biofluorescence will likely find new and exciting applications in colorectal surgery, and perhaps particularly so in molecular fluorescence guided surgery for oncology. Technological advancements will underly the development of the clinical role for biofluorescence; both at the level of the fluorophore as well as in the hardware and software used for detection and analysis.

Declaration of competing interest

The authors reported no conflicts of interest. (Author confirm complete and correct.)

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