# Real-time fluorescence imaging in intraoperative decision making for cancer surgery

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**Summary**

Fluorescence-guided surgery is an intraoperative optical imaging method that provides surgeons with real-time guidance for the delineation of tumours. Currently, in phase 1 and 2 clinical trials, evaluation of fluorescence-guided surgery is primarily focused on its diagnostic performance, although the corresponding outcome variables do not inform about the added clinical benefit of fluorescence-guided surgery and are challenging to assess objectively. Nonetheless, the effect of fluorescence-guided surgery on intraoperative decision making is the most objective outcome measurement to assess the clinical value of this imaging method. In this Review, we explore the study designs of existing trials of fluorescence-guided surgery that allow us to extract information on potential changes in intraoperative decision making, such as additional or more conservative resections. On the basis of this analysis, we offer recommendations on how to report changes in intraoperative decision making that result from fluorescence imaging, which is of utmost importance for the widespread clinical implementation of fluorescence-guided surgery.

## Introduction

Surgeons often rely on experience, training, visual, and tactile information when delineating and resecting most solid tumours. However, identifying tumour margins based on these subjective parameters is challenging and can result in residual disease, which is a prevalent cause of local recurrence and ultimately affects patient prognosis. Complete resection of malignant tissue with adequate tumour-free margins must also be balanced with preservation of the surrounding structures to minimise functional impairment. Histopathological examination of the resected specimen is the current gold standard for margin assessment, but, for many oncological surgeries, it is technically and logistically unrealistic to intraoperatively assess the complete surgical margin by histopathology [1]. Although selective frozen section analysis of suspected regions during surgery is feasible, this technique is time-consuming, can only examine a small part of the specimen, and is subject to sampling errors [2]. Therefore, frozen section analysis is associated with a high false-negative rate [3]. A labour-intensive and still subjective improvement would be extensive intraoperative assessment of the resection margins on the specimen by the surgeon and a pathologist [4], with efforts to base such an assessment on objective optical methods [5]. There is a clear need for highly accurate, intraoperative, real-time methods for tumour margin identification. The increasing interest and preclinical and clinical findings in the field of fluorescence imaging emphasise its potential for intraoperative management.

Fluorescence-guided surgery is an optical imaging method that provides real-time guidance for the delineation of tumours during surgery, with higher sensitivity than direct visual inspection and palpation[6]. An additional benefit of this imaging technique is that it allows for scanning of large surfaces, which might result in the detection of clinically occult tumour lesions.

5-aminolevulinic acid has been well established for its use in fluorescence-guided neurosurgery and has been studied since 1998, leading to a landmark multicentre, randomised controlled trial of fluorescence-guided surgery in 2006 [7-9]. This study showed higher rates of gross total resection for patients in the 5-aminolevulinic acid group, and significantly improved progression-free survival after 6 months. In the past decade, several groups have started to report on the clinical impact of using 5-aminolevulinic acid to adapt the surgical plan, improving the intraoperative extent of resection [10-12]. Fluorescence imaging with indocyanine green, which is analogous to 5-aminolevulinic acid, was introduced in 2007 for the realtime identification of liver cancers, and remains a commonly used technique in various settings, including hepatic resection and

vascular perfusion assessment [13, 14]. Clinical implementation of 5-aminolevulinic acid and indocyanine green was facilitated by the fact that these compounds were already approved for clinical use by the US Food and Drug Administration. Considering the enormous costs associated with drug development, offlabel use of already approved agents was the logical next step [15].

These conventional fluorescence imaging agents led to the development of many near-infrared fluorescent agents with improved molecular specificity and image contrast. Indeed, imaging in the near-infrared region of the electromagnetic spectrum has optimal characteristics that limit the effects of autofluorescence and light scattering and increase tissue penetration [16]. These tumour-specific fluorescent agents consist of fluorophores attached to various targeted ligands, including peptides, antibodies, affibodies, activatable tracers, and small molecules [17, 18]. So far, clinical studies on intraoperative fluorescence imaging with several fluorescent targeting agents show promising results for various cancer types [18].

To date, most studies on intraoperative fluorescence imaging have been proof-of-principle studies [1, 19-54]. In addition to safety considerations, the technical and diagnostic performances of combinations of fluorescent targeting agents and imaging devices have been evaluated for tumour-to-background ratio and for the sensitivity and specificity of the fluorescent signal. These factors provide essential information about the quality of the imaging strategy, and these data are indispensable for further development of this new technology. However, the purpose of fluorescence-guided surgery is to improve oncological and functional outcomes. Therefore, when evaluating the clinical value of fluorescence-guided surgery, this technique could be considered not only as an imaging method, but also as a tool for decision making. In other words, how does surgical management change when fluorescence-guided surgery is added to the surgical procedure? Additionally, in how many cases does this change in management result in an improved oncological or functional outcome?

Providing insight into how intraoperative fluorescence imaging affects patient care is essential for funding agencies and regulatory bodies, which weigh costs and safety risks against clinical benefit—a crucial step towards approval and widespread clinical implementation[55]. Although US Food and Drug Administration guidance documents for approval of imaging products state that an agent’s ability to locate and outline normal structures, or distinguish between normal and abnormal anatomy, are sufficient evidence of its clinical usefulness, some health-care funders frequently enforce strict requirements regarding

efficacy [55]. Thus, to evaluate the added clinical value of fluorescence-guided surgery, its effect on clinical decision making needs to be identified. Most early-stage studies report on diagnostic performance and do not aim to assess the effect of fluorescence-guided surgery on surgical decision making. However, they can still provide an indication of the number of cases in which surgical management would potentially have changed if clinical follow through had been done. In this Review, we present an overview of the current reporting methods of clinical studies that mainly focus on diagnostic performance. We then explain why change in surgical management is an essential outcome measurement to evaluate the effect of fluorescence imaging on cancer surgery. Finally, we establish metrics to define changes in intraoperative decision making that result from intraoperative fluorescence imaging.

## Limitations of current reports on intraoperative fluorescence imaging

The tumour-to-background ratio shows the ability of an imaging strategy to provide contrast between tumour and surrounding tissue for accurate tumour delineation or identification of clinically occult lesions. It is a routinely reported endpoint in preclinical and clinical studies to establish the ideal dose of an imaging agent. Sensitivity and specificity, requiring large sample sizes for powered calculation, are generally evaluated in phase 2 and 3 clinical trials[56]. These test characteristics provide insight into the fluorescent agent's targeting efficacy: does the fluorescence colocalise with the tumour, and is a non-fluorescent area free of tumour[57]?

Direct comparison between the results of such clinical studies of fluorescence-guided surgery is difficult [18], partly because there is no consensus on standardised protocols for the clinical evaluation of fluorescent targets. Equally important is the nature of the outcome parameters. For example, calculations of the tumour-to-background ratio, an important determinant for the evaluation of the efficacy of a fluorescent tracer, can be influenced by selection bias for relevant regions of interest, tissue optical properties that are generally variable and unknown in each individual patient, and the technical specifications and performance of the chosen imaging instrument[16]. Interobserver variation, learning curves for technology use, tumour size, and antigen expression are just a few of many other variables that are challenging to quantify. Moreover, whereas the ex-vivo tumour-to-background ratio is often not reported, the more clinically relevant in-vivo tumour-to-background ratio is even less so.

We advocate for the reporting of all changes to intraoperative decision making precisely because of the challenge of objectively evaluating all possible influencing factors. Overall, the impact of fluorescence-guided surgery on intraoperative decision making is the most effective and objective outcome measurement that is independent of interinstitution and intrainstitution variance and can be compared across all cancer types, imaging systems, and fluorescent tracers.

Although tumour-to-background ratio, sensitivity, and specificity are important markers of the diagnostic performance of fluorescence-guided surgery, they do not provide information on how frequently fluorescence imaging information leads to a change in conventional surgical practice. Additionally, such changes brought on by fluorescence-guided surgery must be evaluated for their efficacy so that an improved clinical outcome can be attributed to it; disclosing the frequency of these changes is necessary to gain insight into how fluorescence-

guided surgery contributes to clinical outcome. Data obtained from consistent recording and quantification of adjustments made to the standard workflow in current and future clinical trials of fluorescence-guided surgery will exemplify its effect on clinical decision making, and are essential to establish an eventual correlation with patient outcomes in the long term.

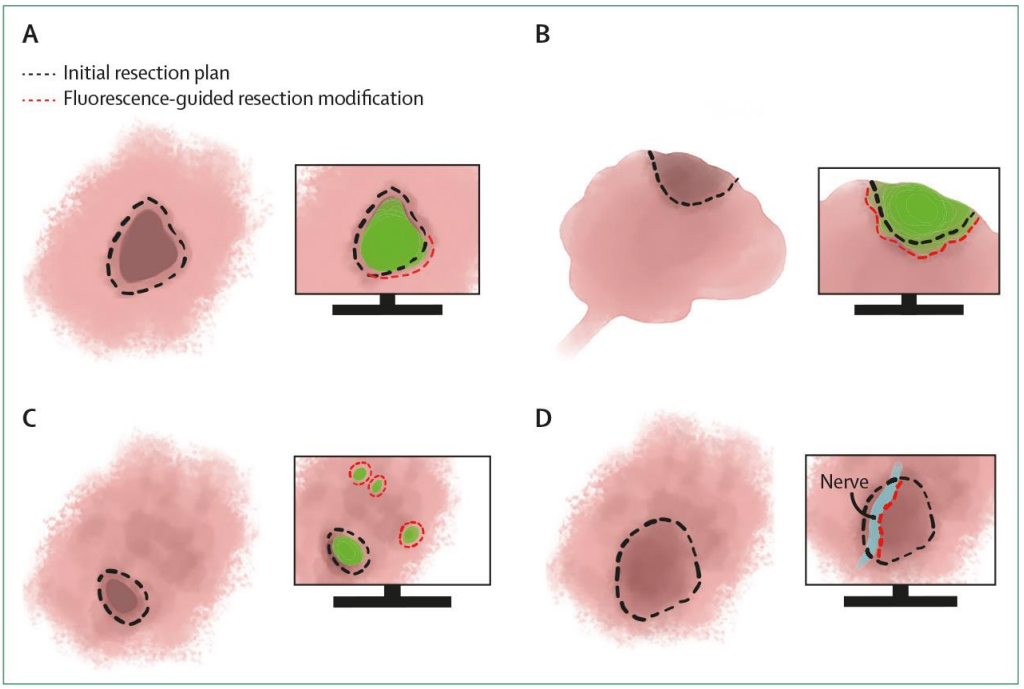


***Figure 1:*** **Schematic representations of the four basic applications of fluorescence-guided surgery**
Green shaded areas represent fluorescence emitted by tumour cells. (A) Excision with tumour-free margins. (B) Debulking procedures. (C) Identification of clinically occult lesions. (D) Identification of vital structures and vascular perfusion (blue shaded area represents fluorescence emitted by nerve). STRAKS VERDEROP SCHUIVEN

## Four basic applications of fluorescence imaging during cancer surgery

To describe a change in surgical management, the four basic applications of intraoperative fluorescence imaging need to be acknowledged. Each application brings a different benefit, having a slightly distinct standard surgical management (figure 1), and is therefore evaluated by different clinical trial endpoints [58]. Clinical examples of each application are illustrated in figure 2.

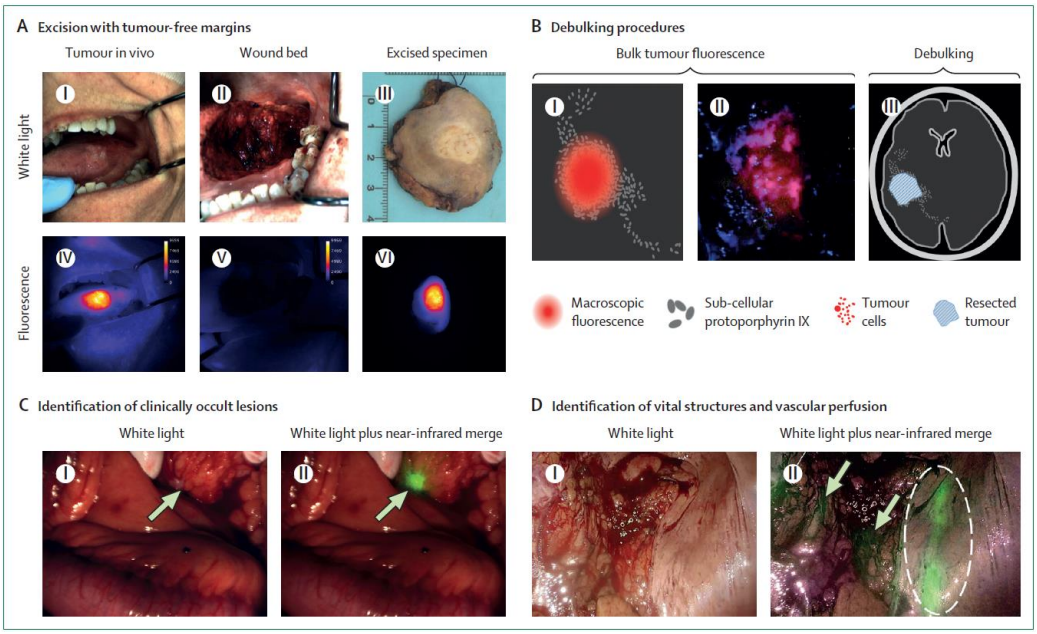


***Figure 2:*** **Clinical examples of the four basic applications of fluorescence-guided surgery**
(A) Fluorescence imaging using pH-activatable nanoprobe ONM-100 in head and neck squamous cell carcinoma of the tongue. In-vivo (I, II, IV, and V) and ex-vivo (III and VI) visualisation of high-fluorescence signal in the tumour and low-fluorescence signal in the tumour-free margin and wound bed. Reproduced from Voskuil and colleagues[39], by permission of Nature. (B) 5-aminolevulinic acid-based fluorescence imaging in neurosurgery. (I) Macroscopic protoporphyrin IX fluorescence is visible at the central portion of a high-grade glioma, but not at the infiltrative margins. (II) Example fluorescence microscopy image of protoporphyrin IX expression in the tumour. (III) Debulking guided by macroscopic protoporphyrin IX fluorescence resulting in residual tumour burden. Reproduced from Wei and colleagues[59], by permission of Frontiers. (C) Intraoperative detection with fluorescent tracer bevacizumab-IRDye800CW of a peritoneal metastasis (arrow) detected by white light alone (I) and fluorescence-guided surgery using the near-infrared signal merged with white light (II). Reproduced from Harlaar and colleagues[60], by permission of Elsevier. (D) Visualisation of ureter residing under peritoneum with fluorophore ZW800-1 using white light alone (I) and the near-infrared fluorescence signal merged with white light (II). The ureter (dashed circle) is captured during a pulse of urine flow during surgery. The darker green areas (arrows) show background fluorescence from vessels in the surrounding tissue. Reproduced from de Valk and colleagues[61], by permission of Nature. STRAKS VERDEROP SCHUIVEN. VOOR NU HIER IVM REFS

The first application for which fluorescence-guided surgery is suitable is excision aimed at achieving adequate tumour-free margins, which is the mainstay of surgical treatment for many solid tumours, including breast, colon, skin, prostate, lung, head and neck, and many others (figures 1A, 2A). In most cases, an adequate surgical margin is an important surrogate marker for overall survival [62]. Fluorescence imaging can be used in vivo during surgery or ex vivo on the specimen, to decide where to do a frozen section assessment, additional resection, or both [24, 36, 63]. The main oncological outcome parameter for fluorescence-guided surgery, compared with the standard of care, is an increase in the percentage of patients with adequate surgical margins. Additionally, precise margin delineation is imperative in delicate regions, where wider resection inevitably leads to increased morbidity and loss of functionality (eg, in head and neck cancer). For these cases, the extent of the resection might decrease when guided by the fluorescence signal. These parameters should be reported as a percentage of cases in which the conventional surgical management was changed, on the basis of intraoperative fluorescence imaging findings on the resected specimen (ie, ex vivo) or wound bed (ie, in situ). Importantly, efficacy should then be objectified by reporting the percentage of additional resections that are histopathologically confirmed true positives or false positives for tumour cells.

The second application of fluorescence-guided surgery is as a debulking procedure aimed at decreasing the tumour load as much as possible without compromising vital structures (figures 1B, 2B). In ovarian cancer and some brain tumours, debulking or cytoreductive surgery is done to increase the efficacy of adjuvant radiotherapy or chemotherapy [64]. For incurable disease, debulking can be done to improve quality of life. In these procedures, the main oncological outcome benefit of fluorescence-guided surgery is its ability to identify the perimeter of the tumour or additional clinically occult lesions [8, 49, 65]. This outcome should be reported as the percentage of cases in which additional resection is done to remove more tumour tissue based on the fluorescence signal. Again, the percentage of additional resections that are histopathologically confirmed true positives or false positives should be reported.

The third application in which fluorescence-guided surgery can be beneficial is in the identification of clinically occult lesions (figures 1C, 2C). There are many examples of the utility of fluorescence-guided surgery in this context, including identification of occult pulmonary [66] or abdominal [67] lesions, sentinel lymph nodes [68], and use in multifocal or multicentric tumours where multiple tumours might be present within a single organ (eg, breast, liver, kidney, and thyroid), separate from the lesion that is clinically evident. In these cases, fluorescence-

guided surgery might aid in identifying secondary, separately located, clinically occult tumours [58]. Reporting should be consistent with that of debulking procedures, whereby the percentage of additional resected lesions and histopathologically confirmed true-positive or false-positive rates are included. An additional element needs to be considered when fluorescence-guided surgery is evaluated as a diagnostic tool for sentinel lymph node detection, in that a false-negative sentinel lymph node can translate to more conservative treatment; hence, the incidence of false negatives should also be reported.

The fourth application of fluorescence-guided surgery considers the non-oncological aspects of cancer surgery (figures 1D, 2D). Fluorescence imaging can be applied to aid in the identification of relevant vital structures that need to be spared during oncological surgery. These structures can be identified and imaged during the operation, and the trial endpoints in these studies focus on reducing the rate of iatrogenic injury and related morbidity. Examples include imaging of the biliary tract during hepatobiliary surgery [69], and of nerves [70] or ureters during abdominopelvic surgery [60]. However, in these studies, objectively establishing the change in surgical management can be challenging because the surgeon is not masked to the fluorescence signal and is very likely to follow the fluorescent signal to prevent iatrogenic injury.

Another non-oncological application of fluorescence-guided surgery in cancer surgery is the assessment of vascular perfusion. Examples include the use of fluorescence-guided surgery to evaluate the perfusion of free-flap reconstructions [71], peritoneum [72], or skin [73]. Similarly, indocyanine green has been used to identify segmental and subsegmental boundaries to facilitate anatomical resection during liver and lung surgery [74, 75]. Moreover, anastomotic leakage, the complication in colorectal surgery that is most associated with increased morbidity and mortality, has been shown to be reduced with the use of fluorescence-guided surgery in randomised controlled trials [76]. The clinical benefit of fluorescence-guided surgery for such applications needs to be concluded from improved surgical outcome (eg, reduction of iatrogenic injury, tissue sparing, flap failure, or anastomotic leakage) [58].

## Current clinical trial designs for fluorescence-guided surgery

Several completed and ongoing clinical trials of fluorescent agents were analysed to identify studies that report a change in surgical management as an outcome parameter. Studies were categorised according to their design, focusing on how and when fluorescence-guided surgery was introduced into the standard of care (table, showing the lowest [type A] to the highest [types E and F] potential for reporting the effect of fluorescence-guided surgery on intraoperative decision making). This categorisation allowed for the identification of study designs that can provide information on (potential) changes in intraoperative decision making. The following questions regarding steps taken as a result of fluorescence imaging were assessed: did fluorescence imaging influence intraoperative decision making and, if so, to what degree? Were biopsies or additional resections done as a result of residual fluorescence in the wound bed, the resected specimen, or both, or was resection (also) guided by fluorescence imaging beforehand?

In type A studies, fluorescence and white light images or videos are collected before resection, after which fluorescence intensity is compared with the surrounding background tissue, fluorescence is correlated with tumour presence, and results are analysed offline. The primary motivation for these studies is to assess the sensitivity and specificity of the fluorescent agent. This study type could be thus adapted to evaluate potential changes in intraoperative decision making (not yet been done in published reports): first, delineate the conventional tumour resection, then use fluorescence imaging, and report whether the tumour outline changed. This procedure would thus still provide insight into how fluorescence imaging might have altered the surgical plan.

Studies of type B, C, and D describe cases in which observations by fluorescence imaging indicate potential for a change in intraoperative decision making. Unfortunately, the percentage of cases in which this potential change happens is seldom reported. In type B studies, fluorescence imaging of the resected specimen is done. Fluorescence on the specimen surface might indicate a close or positive resection margin. Although the surgical management is not altered, this finding clearly highlights the clinical impact that fluorescence imaging would have had if acted on.

In type C studies, fluorescence imaging is done before resection, followed by fluorescence imaging of the resected specimen, wound bed, or both. Regardless of the findings of fluorescent signals, no additional resections are done during the operation. Because no biopsies are taken from the fluorescent lesions in the

wound bed in these studies, whether fluorescence correlates with tumour presence (ie, whether these lesions are true or false positives) is unclear. However, the finding of residual fluorescent lesions still suggests that if the resection is directed by fluorescence imaging, the surgical management might be different from standard practice.

| | Timing of fluorescence imaging | Design | Reported changes in intraoperative decision making |
|---|---|---|---|
| Type A | Preresection [24–27] | Fluorescence imaging and white light imaging of lesions; standard removal of lesions | None (data might be available)* |
| Type B | On the resected specimen [28–32] | Standard identification and removal of lesions; fluorescence imaging of specimen | None (data available)† |
| Type C | Preresection; on the resected specimen, wound bed, or both [1,33–36] | Fluorescence imaging and white light imaging of lesions; standard removal of lesions; fluorescence imaging of wound bed, specimen, or both | None (data available)† |
| Type D | Type C with biopsies of fluorescent lesions [37–43] | Fluorescence imaging and white light imaging of lesions; standard removal of lesions; fluorescence imaging of wound bed, specimen, or both; biopsies of fluorescent lesions | None (data available)† |
| Type E | Preresection; on the resected specimen, wound bed, or both [44–53] | Fluorescence imaging and white light imaging of lesions; standard removal of lesions; fluorescence imaging of wound bed, specimen, or both; additional resection of residual fluorescent lesions, or other change in surgical plan | Yes; reporting not always comprehensive |
| Type F | Throughout the entire procedure [10,35,54–58] | Removal of lesions guided by fluorescence imaging and visual inspection or palpation | Yes; reporting not always comprehensive |

**Study design that could be adapted to provide data to inform on how often fluorescence imaging would have led to a change in intraoperative decision making. †Study design that provides data to inform on how often fluorescence imaging would have led to a change in intraoperative decision making.*

***Table:*** **Types of clinical trial designs for fluorescence-guided surgery**

Type D studies are similar to type C studies, except that biopsies of residual fluorescent lesions are taken, providing additional essential information on true-positive and false-positive detection rates. Despite the outcome of these biopsies, no additional resection is done, and surgical management is therefore not changed. This approach does, however, allow the researcher to report the

percentage of cases in which a change in surgical management is occasioned by the fluorescence imaging results. In some surgical cases in which areas with residual fluorescence are not also clinically suspect, and specificity of the targeting agent is not yet well established, limiting disruption of the standard of care by only taking biopsies for retrospective correlation with histopathology is ethically justified.

Type E includes studies in which resection is primarily based on standard-of-care palpation and visual inspection, with further resection of fluorescent lesions. This design is ideal for reporting the altered management of fluorescence-guided surgery compared with standard surgical practice. The resected lesions undergo pathological examination, so true and false positives can be reported. Although these studies suggest changes in intraoperative decision making, they do not always report in how many cases the standard of care was altered.

A type F study design implies true fluorescence-guided surgery, wherein the initial resection is based on the combination of fluorescence imaging and standard-of-care visual inspection and palpation. Fluorescence imaging and traditional cues are complementary and assessed simultaneously: fluorescence signals provide additional information to aid the surgeon in intraoperative decision making, without the intention to overrule a surgeon's judgment. Different from a type E study, where fluorescence-guided surgery might guide the resection of additional lesions that would have been missed otherwise, a type F design can also result in more restricted resection than would current standard practice (ie, the surgeon can decide to more conservatively resect lesions based on how they fluoresce).

**Insufficient reporting of the potential clinical effect of fluorescence-guided surgery**

As can be concluded from the table, only a few clinical trials currently report changes in surgical management as a separate outcome parameter. However, many of the type E and F trials suggest that fluorescence imaging information might (or did) alter the standard surgical procedure, although in how many surgical cases a change actually occurred is not generally reported. Several studies mention malignant lesions that were only identified with fluorescence imaging [31, 39, 40, 43-46, 48, 51, 53, 54], but the number of cases in which the management would have been changed is often not specified. This underreporting represents missed opportunities because, on the basis of their study design, it can be deduced that these data should be available to the researcher. If changes to surgical management were clearly reported, much information about the potential clinical impact of fluorescence-guided surgery would become available to the field.

The few trials that do report this specific parameter illustrate its clinical usefulness. One type F study reported that this parameter “improved surgical decision making”, showing that fluorescence imaging led to an improvement in surgical outcome in 21% of cases [50]. Several other studies describe additional action as a result of fluorescence imaging[31, 43-47, 54].

In the only completed randomised controlled trial of fluorescence imaging for primary tumour surgery, true fluorescence-guided resection with 5-aminolevulinic acid was done in patients with malignant gliomas[8]. In this phase 3 study, in which 322 patients were randomly assigned to either fluorescence-guided surgery or conventional microsurgery with white light, endpoints were solely concerned with differences in outcome (eg, amount of residual contrast-enhancing tumour and progression-free survival); the results showed higher rates of gross total resection for patients in the 5-aminolevulinic acid group and significantly improved progression-free survival after 6 months. There is a need to quantitatively assess also overall survival and quality of life attributable to fluorescence-guided surgery, but such endpoints require costly, time-consuming, and logistically cumbersome randomised controlled trials, and require long follow-up periods. The ability to draw conclusions on the potential clinical impact of fluorescence-guided surgery from earlier, smaller scale studies would facilitate the decision to proceed from phase 1 and phase 2 studies to new randomised controlled trials.

## Reporting changes in surgical management guided by fluorescence imaging

To show (potential) deviations from the standard of care as a result of fluorescence-guided surgery, it is important that comparisons can be made between standard-of-care surgery with and without fluorescence. For this comparison to be possible, it is necessary that more attention is given to the description of the method, as the study type (table) determines how and to what degree this effect can be analysed. The key factor in reporting the effect of fluorescence-guided surgery on intraoperative decision making is to describe clearly whether additional steps (eg, biopsy or resection) are taken as a result of fluorescence-guided surgery and in how many cases this decision was justified (true or false positive). Besides prompting additional resections, fluorescence imaging findings could also lead to abortion of the procedure in cases in which clinically occult, distant, and inoperable metastases are discovered [43]. Importantly, any complications or morbidity related to additional resection need to be reported. The usefulness of this parameter as a primary outcome measurement is underlined by the phase 3 trial on ovarian cancer (NCT03180307) assessing the efficacy of OTL38 (a folate-indole-cyanine green-like conjugate to folate receptor alpha). In this trial, efficacy was defined as the proportion of patients in which fluorescence imaging yields at least one additional tumour-positive lesion compared with normal conditions. Change in surgical management is also a secondary outcome in an ongoing randomised controlled trial of SGM-101, a fluorochrome-labelled anti-carcinoembryonic antigen monoclonal antibody, in colorectal cancer patients (NCT03659448).

Another important implication of fluorescence-guided surgery is its potential for more conservative surgical excision: to reduce unnecessary resection of non-malignant tissue (ie, retention of normal tissue). This objective is particularly important for tumours located in functionally or aesthetically delicate areas. When evaluating the added value of fluorescence-guided surgery, it is important to also provide insight into these changes in intraoperative decision making that lead to reduced unnecessary resection.

We propose a checklist of recommendations that are relevant for the evaluation of changes in intraoperative clinical decision making (panel; see table for study type details).

***Panel:*** **Recommendations for the evaluation of changes in intraoperative decision making**

**Fluorescence imaging preresection**
- Report whether the intended resection was, or could have been, altered on the basis of fluorescence imaging
- Include a fluorescence imaging step between delineation and resection of the tumour

**Fluorescence imaging sequence during resection**
- Report whether resection is done as per standard of care (without fluorescence imaging)
- Report whether and how surgical management changes when fluorescence imaging is turned on and off during resection

**Fluorescence imaging of specimen**
- Report the percentage of cases in which a fluorescent lesion on the specimen surface, corresponding to a close or positive surgical margin, would have caused a change in surgical management (types B or C study design), results in an additional biopsy (type D study design), or results in additional resection (type E study design)
- Compare standard-of-care sampling to fluorescence imaging-based sampling

**Fluorescence imaging of wound bed**
- Report the percentage of cases where a fluorescent lesion in the wound bed would have caused a change in surgical management (types B or C study design), results in an additional biopsy (type D study design), or results in additional resection (type E study design)

**Fluorescence imaging of other scanned surfaces**
Report the percentage of cases in which clinically occult lesions are detected and whether this detection changed intraoperative decision making, postoperative clinical management, outcome, or all (types E and F study design)

**In general, report:**
- Detection rate of true positives (fluorescent malignant lesions)
- Detection rate of false positives (fluorescent lesions without malignancy)
- Detection rate of false negatives* (non-fluorescent malignant lesions)
- Any complications or morbidity related to fluorescence-guided additional resections
- Change in duration of the surgical procedure as a result of fluorescence-guided surgery
- Changes in postoperative clinical management and outcome related to fluorescence-guided surgery

*The true negative rate is omitted because it would require histopathological evaluation of 100% of the imaged surface, which is unfeasible in the clinical setting. Conclusions on the true negative rate of fluorescence imaging can most likely be drawn only on the basis of patient outcomes, although there are many possible confounders, such as postsurgical therapy (eg, radiation).

## Discussion

The need for more uniformity in reporting results of fluorescence-guided surgery has been extensively discussed [55, 57, 77-79]. We believe that the effect of fluorescence-guided surgery on intraoperative decision making should be added as a standardised part of reporting. Our recommendations for reporting the change in intraoperative management aim to make this effect quantifiable and easy to evaluate and compare.

Phase 1 and phase 1/2 studies are frequently designed not to interfere with the standard of clinical care, but fluorescence imaging-related observations can still indicate the clinical potential of fluorescence imaging. Study types A and B are typical for phase 1 and 1/2 trials, wherein safety, feasibility, and efficacy of the imaging strategy (fluorescent agent, camera, or both) are measured simultaneously. These studies are generally required for medical and ethical reasons before alteration of the standard of care (ie, types E and F studies) can be approved. Type D studies provide a good alternative by incorporating the possibility of taking biopsies from fluorescent areas in the wound bed, specimen, or both for correlation with histopathology. Such biopsies add essential information about the potential impact of fluorescence-guided surgery on intraoperative decision making and whether additional resection would have been justified. In cases where areas with residual fluorescence are also clinically suspect, intraoperative frozen sections would be preferable to biopsies because these lesions can then readily be resected, which increases the likelihood of clinical benefit.

Introducing a new technique into the operating room might be initially associated with incremental operating time, more biopsies, and potentially higher morbidity in cases with additional resections. These changes can be associated with higher costs. However, there have been studies reporting a reduction in operation time because fluorescence-guided surgery allowed a clearer delineation of vascular structures, expediting dissection and preventing injury [80]. Moreover, fluorescence-guided surgery is anticipated to eventually replace the role of fresh frozen analysis, which would substantially reduce operating time. Finally, the use of fluorescence-guided surgery could increase the rate of clear resection margins or result in more personalised adjuvant treatment regimens. When resulting in improved oncological outcome, fluorescence-guided surgery will also lead to a reduction in costs related to diagnosis and salvage treatment of recurrent disease. Analyses have shown that fluorescence-guided surgery appears to be cost-effective in surgery for high-grade gliomas [81] and breast cancer [82]. Future cost-effectiveness studies are required to further elucidate the weight of all these variables. Therefore, it is important that these factors are reported.

The clinical impact of fluorescence-guided surgery encompasses more than changes to surgical decision making. Unanticipated intraoperative findings can also have an effect on postoperative clinical management and outcome. For example, fluorescence imaging might show occult tumour lesions, which can result in changed adjuvant therapy, potentially affecting tumour recurrence and patient survival. Another advantage of fluorescence-guided surgery is its ability

to reflect biological characteristics of viable cancer cells. Fluorescence signal intensity can visualise molecular expression patterns, which might have prognostic value [83]. Both surgical changes and additional intraoperative findings (that do not necessarily lead to immediate action) gained from intraoperative fluorescence imaging should be reported. Evidence supporting the clinical impact of fluorescence-guided surgery in a broader sense can be obtained much more effectively when early, smaller scale studies also report on potential clinical consequences: it is the first step towards correlation of intraoperative decision making and findings with patient outcomes.

The most scientifically robust study design to assess the clinical impact of fluorescence-guided surgery is a multicentre, randomised controlled trial with functional outcome and disease-specific survival as primary endpoints. To minimise surgeon bias, initial inspection and delineation of the tumour by the surgeon should be done while the surgeon is masked to the fluorescence signal—that is, before the first intraoperative imaging sequence. Under these semiblinded circumstances, the standard operating procedure is the control situation, and each patient is his or her own control [78]. Depending on the application and cancer type, numerous secondary endpoints could be considered. Examples include the change in rate of positive margins; retention of normal tissue; preservation of tissue function; occurrence of complications; prediction of postoperative outcomes (eg, risk of recurrence and sensitivity to chemotherapy); and change in the need for adjuvant therapies or salvage surgery [55].

## Conclusion

Current evaluations of fluorescence-guided surgery mainly focus on diagnostic performance through outcome parameters that are notoriously difficult to objectively evaluate and quantify, and that do not inform about the clinical implications of the technique. Without adequate reporting of the impact of fluorescent findings on intraoperative decision making, there is a serious risk that the field of fluorescence-guided surgery will not progress towards widespread clinical implementation. These relevant insights can be obtained by reporting how (and how frequently) fluorescence-guided surgery leads to changes in intraoperative decision making (eg, additional or more limited resections) compared with current practice. In addition, these changes need to be evaluated for the efficacy and safety attributed to fluorescence-guided surgery to be correlated with patient outcomes in the long term. The impact of fluorescence-guided surgery on intraoperative decision making is the most effective and objective outcome measurement to evaluate the clinical value of

fluorescence-guided surgery and to compare study results across all cancer types, imaging systems, and fluorescent tracers.

## Search strategy and selection criteria

Relevant studies were collated by searching clinicaltrials.gov, cinicaltrialsregister.eu, the Dutch Trial Register, and the International Standard Randomised Controlled Trial Number Registry with the search terms “cancer” or “tumo(u)r”, combined with “fluorescence”, “fluorescence-guided surgery”, “image guided surgery”, “molecular imaging”, and “optical imaging”. References for this review were then identified by searching PubMed using the same terms as mentioned above, as well as National Clinical Trial numbers. Only papers published in English between Jan 1, 2006, and July 1, 2020, were considered. The final reference list was generated on the basis of originality and was limited to papers presenting results from clinical trials of fluorescence-guided surgery.

## Contributors

SKe conceived this Review. LJL and SKe did the literature search and produced the figures. LJL, PBAAvD, and SKe wrote this Review. All authors critically revised, had full access to the data, and approved the manuscript for publication.

## Declaration of interests

We declare no competing interests.

## Acknowledgments

SKe is supported by the Bas Mulder Award from the Dutch Cancer Society (grant number 12175) and by the Daniel den Hoed Award from the Erasmus University Medical Center Foundation. This manuscript was edited for English language by Springer Nature Author Services, with funding from the European Commission under the H2020-MSCA-RISE Award (grant 872860).

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