Optical Innovations in Surgery

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Published in British Journal of Surgery, January 2015, 102(2):e56-72.
DOI: 10.1002/bjs.9713
ABSTRACT

BACKGROUND
In the past decade, there has been a major drive towards clinical translation of optical and, in particular, fluorescence imaging in surgery. In surgical oncology, radical surgery is characterized by the absence of positive resection margins, a critical factor in improving prognosis. Fluorescence imaging provides the surgeon with reliable and real-time intraoperative feedback to identify surgical targets, including positive tumour margins. It also may enable decisions on the possibility of intraoperative adjuvant treatment, such as brachytherapy, chemotherapy or emerging targeted photodynamic therapy (photoimmunotherapy).

METHODS
This article reviews the use of optical imaging for intraoperative guidance and decision-making.

RESULTS
Image-guided cancer surgery has the potential to be a powerful tool in guiding future surgical care. Photoimmunotherapy is a theranostic concept (simultaneous diagnosis and treatment) on the verge of clinical translation, and is highlighted as an effective combination of image-guided surgery and intraoperative treatment of residual disease. Multispectral opto-acoustic tomography, a technique complementary to optical image-guided surgery, is currently being tested in humans and is anticipated to have great potential for perioperative and postoperative application in surgery.

CONCLUSION
Significant advances have been achieved in real-time optical imaging strategies for intraoperative tumour detection and margin assessment. Optical imaging holds promise in achieving the highest percentage of negative surgical margins and in early detection of micrometastastic disease over the next decade.
INTRODUCTION

For decades, doctors have relied solely on their vision and tactile information for diagnosis and treatment monitoring during surgery. After World War II, medical doctors started to use lenses and microscopes in the operating theatre to zoom in on the field of interest during a procedure. \(^1\) At the same time fluorescein was used in combination with an ultraviolet lamp to enhance contrast in tumour tissue. \(^2\) Since then contrast agents have been used for several procedures during surgery, such as 5-aminolevulinic acid in neurosurgery \(^3\) and blue dye in detection of sentinel lymphnodes \(^4\) (Fig. 1). \(^2\)\(^–\)\(^5\) During the past decade there has been increased interest in the clinical application of optical imaging techniques in the operating theatre. The surgeon’s eyes and hands are useful instruments for detecting anatomical structures, but unfortunately cannot detect the precise molecular processes related to a particular disease stage. For example, in patients with peritoneal metastases it is often difficult to distinguish healthy scar tissue from malignant lesions. Moreover, there are no scientific data available on the sensitivity and specificity, nor diagnostic accuracy, of the detection of cancer by human inspection and palpation. The volume of publications in the field of intraoperative optical imaging has doubled in the scientific literature in the past 20 years, particularly in the last 10 years (Fig. 2). The standard for the direct result of a surgical resection is the pathology report. This usually takes 4–7 days to obtain, while the patient is recovering from surgery. In the event of incomplete resection, this is not an ideal time for reintervention. Ideally, a surgeon needs direct feedback during an operation, so there is still the possibility to adjust the procedure in order

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**Figure 1 Development of clinical optical imaging.** The first contrast agents were used by Moore \(^1\) between 1940 and 1950. Other milestones were the introduction of a blue dye for localizing the sentinel lymph node in breast cancer treatment \(^2\), the introduction of 5-aminolevulinic acid (5-ALA) in brain surgery \(^3\) and, more recently, targeted imaging using folate–fluorescein isothiocyanate (FITC) in a human ovarian cancer \(^4\). UV, ultraviolet. (Reproduced from Intraoperative Imaging and Image-Guided Therapy, Jolesz FA (ed.), 2014, with permission from Springer Science and Business Media)
to improve the outcome. Several clinical studies have used optical contrast agents (non-targeted and targeted) during surgical procedures. The non-targeted, clinically available dye indocyanine green (ICG) has been used for lymph node detection\(^6\), intraoperative angiography\(^7\), intraoperative cholangiography\(^8\) and visualization of liver metastasis\(^9\). As the efficiency of this agent is based on perfusion or the enhanced permeability and retention effect, it is not ideal for tumour-specific delineation. More recently, targeted optical probes have been introduced, which are based on existing targeted therapies.

**OPTICAL IMAGING IN SURGERY**

There are many imaging modalities available for preoperative staging. The introduction of ultrasonography, CT, MRI and PET has made a big impact on preoperative staging and therapeutic decision-making, which has significantly affected how patients with cancer are treated and monitored. Unfortunately, these modalities cannot easily be used in the operating theatre. For detection of small lesions, the resolution of CT (millimetres) is low compared with human vision (approximately 50 μm). Techniques like CT achieve high resolution in whole-body imaging but cannot detect small lesions in the surgical field during operation. Optical imaging methods can detect lesions smaller than 10 μm.\(^10\) Optical imaging can also provide direct feedback and is related to the natural surgical field of view. Together with relatively low costs and flexibility, optical imaging modalities fit well in the operating theatre (Fig. 3; Video S1, supporting information).

Ultrasonography, portable high-energy (ionizing) detectors, such as a handheld γ probe or handheld PET detector, and optical methods are more suitable in the operating theatre. Ultrasound imaging can achieve relatively high resolution (less than 30 μm), combined with a penetration depth of up to several centimetres. The disadvantages of ultrasonography are the relatively small field of view and the need for contact with the tissue. It is often used in liver surgery and recently has been applied successfully in breast-conserving therapy, reducing
the rate of positive margins.\textsuperscript{11} The γ probe can be used to detect a target with a much higher penetration depth, although the resolution cannot be compared with that of the human eye or optical methods. Moreover, the use of ionizing isotopes needs strict standard operating procedures and prevention of overexposure to irradiation, a potential hazard for the surgeons and scrub nurses working with these patients. In contrast, optical or fluorescence imaging has near-perfect features for intraoperative use; it has good correlation with human vision, is non-ionizing, and open-air systems can be built into laparoscopes with relatively low costs. A limitation of optical imaging is the penetration depth (1–2 cm) of the near-infrared (NIR) light into the tissue owing to scattering and absorption of photons.\textsuperscript{12} Superficial activity can be detected with high sensitivity, which is an advantage during an operation such as removal of peritoneal metastases from ovarian or colorectal cancer. The principles of the technique, together with its recent and future applications, are described below.

**PRINCIPLES OF INTRAOPERATIVE OPTICAL IMAGING**

Humans can detect light in the visual spectrum (± 400–750 nm) with a high resolution of approximately 50 μm. Humans can see depth and are therefore able to reconstruct shape and architectural features, although the human eye is not able to differentiate between spectra with a small separation in wavelengths. This implies that it is difficult to differentiate between two
different objects that are almost the same colour. For example, it is more difficult to count green apples in a tree with green leaves; if the apples are red it is easier to count them owing to enhanced contrast. This phenomenon to mislead the eye is employed in battle by the use of camouflage, or in nature as showcased by the octopus (Video S2, supporting information). The eye cannot detect molecular changes if the colour remains the same, which is often the case. During surgery, for instance, it is difficult to differentiate visually between tumour growth and benign scar tissue. Nor is it possible for the eye to detect microscopic residual disease in a resection plane. Light in the visible light range of 350–740 nm does not penetrate deep into tissue; NIR wavelengths ranging from 750 to 1000 nm penetrate up to 2 cm deep (Fig. 4). Red light penetrates deeper into tissue than green light, which is right in the middle of the visible spectrum (Fig. 5). Besides absorption and scattering properties of light, inherent autofluorescence of tissue is significantly higher in the visible spectrum, compared with the NIR region, clearly defining the optimal diagnostic window for optical imaging. Reduced tissue absorption, scattering and autofluorescence, combined with image reconstruction algorithms such as the Born normalized ratio, result in improved signal-to-noise ratios. The higher this ratio, the better the diagnostic performance of the imaging system combined with a NIR fluorescent probe. Fluorescence imaging has advantages that need to be considered. Fluorescence itself is a phenomenon that occurs when a molecule absorbs a photon (excitation) activated at a certain wavelength that triggers the release of photons at a longer wavelength (emission). These photons can be detected with a sensitive charged-coupled device camera. Such cameras can inspect the entire operating field in real time, with colour and fluorescence imaging, either separately or on an overlay pseudocolour image. Interpretation of the images is straightforward by the amount of fluorescence detected. Simple optical methods, such as use of magnifying glasses and microscopes, and more complex instrumentation, such as two-photon microscopy and optical coherence tomography, can be used in surgery, but these are beyond the scope of the review. The NIR imaging set-up that can be used during surgery is as follows. The field of view (operating field) is illuminated with two different light sources. First, a white light source is used for colour
Figure 5  a Light dependency on tissue scattering and absorption properties. The absorption coefficient of light in tissue is dependent on wavelength, and results from absorbers such as haemoglobin, lipids and water. The graph is calculated assuming normally oxygenated tissue (saturation 70 per cent), a haemoglobin concentration of 50 mmol/l, and a composition of 50 per cent water and 15 per cent lipids. It also shows the emission range of several common fluorochromes and luciferases used for imaging. GFP, green fluorescent protein; ICG, indocyanine green. b Mouse images show experimentally measured photon counts through the body of a nude mouse at 532 and 670 nm. The excitation source was a point illumination placed on the posterior chest wall. Signals in the near-infrared (NIR) range are about four orders of magnitude stronger compared with illumination with green light under otherwise identical conditions, illustrating the advantages of imaging with NIR photons. (Reproduced from reference 13, with permission from Macmillan Publishers, copyright 2003)

Figure 6  Clinical prototype of image-guided surgery system. a Multispectral fluorescence camera system in the operating theatre. b Schematic of a multispectral fluorescence camera system capable of capturing three imaging channels in real time simultaneously: colour reflectance, fluorescence and intrinsic excitation. A halogen light source is used for white light illumination and a diode laser for fluorescence excitation. CCD, charge-coupled device. (Adapted from reference 5)
registration of the tissue combined with a filtered white light source (light-emitting diode or laser) in the wavelength(s) needed for excitation of the fluorescent optical contrast agent to be used to detect the tumour. The light emitted from the field of view is guided through optics and divided to different detectors. Computer software can reconstruct the fluorescence signal, predominantly in a pseudocolour overlay on the colour image (Fig. 6). This set-up can be adapted to a NIR laparoscope or endoscope for evaluation of the oesophagus, stomach, distal small bowel and colon.\textsuperscript{15,16} Besides a sensitive camera system, the fluorescent dye, or contrast agent, often termed the probe, is of the utmost importance. Different types of optical contrast agent can be used. For example, fluorescein can be used as an angiography blood pool agent in the retina. ICG can be used for detection of lymph nodes\textsuperscript{6} or for perfusion of different organs such as the liver, or skin/muscle grafts in reconstructive surgery.\textsuperscript{17} ICG has advantages over fluorescein related to the absorption and scattering properties of NIR. The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) has approved ICG and fluorescein for clinical use. More recently, the development of tumour-specific optical contrast agents in oncology has changed the field dramatically. These probes enable visualization of biological processes by targeting important biomarkers of cancer (Fig. 7)\textsuperscript{18,19}, inflammation\textsuperscript{20}, neuro-degenerative diseases\textsuperscript{21}, infectious diseases\textsuperscript{22} and cardio-vascular disease.\textsuperscript{23} The imaging agents in surgical oncology can be selected by using the TArget Selection Criteria (TASC) (Fig. 8, Table 1).\textsuperscript{24} This is a helpful method for selecting the most suitable biomarker for different types of malignancy, based on their characteristics and score. A total

Figure 7 Hallmarks of cancer and their targets for optical imaging. EGF, epidermal growth factor; NIRF, near-infrared fluorescence; PEG, polyethylene glycol; cRGD, cyclic arginine–glycine–aspartate; VEGF, vascular endothelial growth factor. IRDye®800CW (LI-COR Biotechnology, Lincoln, Nebraska, USA); AngioSense™, IntegriSense™, MMP-Sense™ and ProSense™ (PerkinElmer, Waltham, Massachusetts, USA). (Adapted from reference 18, with permission from Keereweer and colleagues)
score of 18 or over indicates that the biomarker is potentially suitable for tumour-targeted imaging. The following biomarkers were selected for colorectal cancer based on the TASC system: vascular endothelial growth factor (VEGF) A, carcino embryonic antigen, epidermal growth factor receptor, matrix metalloproteinases (MMPs), epithelial cell adhesion molecule, mucin 1 and CXC chemokinereceptor 4. After selecting the biomarkers, preferably an existing drug needs to be conjugated with a fluorescent dye. This needs to achieve optimal pharmaco-kinetics and biodistribution without any toxicity, resulting in high signal-to-noise ratios and a homogeneous distribution within the tumour. Successful clinical examples are folate–fluorescein isothiocyanate (FITC) targeting the folate receptor α, bevacizumab–IRDye®800CW (LI-COR Biotechnology, Lincoln, Nebraska, USA) (NTR4632, http://www.trialregister.nl) and cetuximab–IRDye®800CW (NCT01987375, http://www.clinicaltrials.gov).

**IMAGING STUDIES FROM BENCH TO BEDSIDE**

Use of optical imaging in the clinic enhances surgical vision, but the extent depends on the site of application. Superficially located tumours are easily visualized, such as head and neck cancer, skin cancer, melanoma, bladder cancer and peritoneal metastases resulting from colorectal and ovarian cancer, whereas deeper-seated tumours, such as breast cancer and sarco-
Figure 9 Optical imaging for detection of residual disease in a xenograft mouse model of breast cancer. A human breast cancer tumour cell line (MDA-MB-231-luc-D3H2LN) was injected into the mammary fat pad of a nude mouse. 

a Colour image after removal of the skin. 
b Corresponding fluorescence image. 
c Haematoxylin and eosin histopathology after resection of 90 per cent of the tumour (original magnification ×10). 
d Colour image of the planned residual tumour tissue (approximately 10 per cent). 
e Corresponding fluorescence image showing an additional spot proximal to the 10 per cent that was intentionally left behind; this spot was not visible on the colour image and was sampled separately. 
f Haematoxylin and eosin histopathology revealed a small rim of tumour tissue on top of the pectoral muscle (original magnification ×5). Also see Video S3 (supporting information). (Reproduced from reference 25, with permission from Springer Science and Business Media)
mas, are more challenging owing to the significant overlay of fat, muscle or glandular tissue. However, progression to the clinic has been limited primarily because the development of clinical-grade optical contrast agents is expensive and time-intensive with regard to risk regulatory aspects, financial and administrative procedures. Despite these barriers, experimental data have provided evidence in preclinical animal models that detection, margin control and survival can be improved by use of fluorescently labelled tumour-targeting contrast agents in a variety of cancer types; there are also early clinical data in humans (Fig. 9).25 Videos S3 and S4 (supporting information) illustrate intraoperative use in an experimental set-up. In these experiments, a real-time resection of a breast cancer tumour and ovarian cancer was undertaken in a xenograft mouse model.25,26 Multiple strategies have been proposed to target tumour cells in vivo, using probes that can be considered in three broad categories: non-targeted fluorescent probes, targeted fluorescent probes, and targeted activatable probes (Fig. 10). Each of these has been studied extensively in vivo for potential clinical translation, and some probes have recently reached the operating theatre in the context of clinical trials, as mentioned above.

**NON-TARGETED FLUORESCENT PROBES**

Among the various non-targeted fluorescent probes currently available for research and clinical use are fluorescein, ICG, cresyl violet acetate, toluidine blue and Lugol’s iodine. ICG has...
optical properties (805 nm excitation, 835 nm emission) favourable for NIR imaging, because absorbance by haemoglobin and lipid, the predominant absorbers, occurs less in the NIR range. Promising results have been reported for imaging of angiogenesis, sentinel lymph node mapping (Fig. 11), and investigating blood perfusion in various organs.\textsuperscript{27–29} Recently, real-time demarcation of unidentifiable liver cancers has been described using ICG, as it is thought that cancer and non-cancerous liver tissue compressed by the tumour exhibit disordered biliary excretion of ICG.\textsuperscript{30} In general, however, these agents are not tumour-specific and therefore not optimally suited to enhance visual demarcation between normal and cancerous tissues.

**TARGETED FLUORESCENT PROBES**

Tumour cells differ significantly from normal adjacent cells owing to dysregulation of certain
genes, which ultimately contribute to increased expression rates of growth signalling receptors (Fig. 7). Numerous preclinical studies have demonstrated that targeting a tumour-specific receptor with fluorescence-labelled antibodies, nanobodies, affibodies or small peptides (such as arginine–glycine–aspartate, RGD) may have high potential for visualizing cancer in real time. Antibody-labelled fluorophores can be visualized either in the visible spectrum or in the NIR spectral range by using fluorophores such as FITC and IRDye*800CW respectively.

The first in-human proof of principle of the potential benefit of intraoperative optical imaging was provided in 2011. Here it was shown that the use of tumour-specific fluorescence imaging following systemic administration of the folate receptor α-targeted agent, folate–FITC, offered specific and sensitive real-time identification of tumour tissue during surgery in patients with peritoneal carcinomatosis resulting from ovarian cancer (Fig. 12; Video S5, supporting information). The authors showed that seven times more malignant lesions could be detected by fluorescence imaging compared with visual observation alone. Subsequent research, however, has focused on fluorescent dyes that emit in the NIR spectrum, which allows identification of deeper tumours, owing to the stronger penetration properties of NIR dyes compared with FITC (Fig. 12). Another approach uses the application of a therapeutic antibody such as bevacizumab, with a soluble ligand, VEGF-A, commonly involved in tumour-induced angiogenesis. For imaging purposes in preclinical and clinical experiments, bevacizumab has been conjugated with single-photon emission CT and PET isotopes. More recently, the conjugation of a NIR fluorescent dye, IRDye*800CW, with bevacizumab was achieved, and the conjugate demonstrated the ability to detect tumour lesions in vivo with high specificity and sensitivity. Several key preclinical findings in head and neck cancer (such as use of cetuximab–IRDye*800CW and panitumumab–IRDye*800CW to target epidermal growth factor receptor) and breast cancer (such as use of trastuzumab–IRDye*800CW to target Her2/neu, and bevacizumab–IRDye*800CW to target VEGF-A) have resulted in the swift progression from preclinical animal studies towards human applications. The first clinical studies are currently under way to assess feasibility in patients with breast cancer (NCT01508572), colorectal cancer (NCT01972373, NTR4632) and head and neck cancer (NCT01987375). The introduction of a new optical disease-specific contrast agent for clinical use has to overcome significant regulatory and safety concerns, but is feasible and safe. This process is challenging and often needs a long time path of Good Laboratory Practice, Good Manufacturing Practice (GMP) and Good Clinical Practice before approval by the responsible governing board (Investigational Research Board, FDA and/or EMA).
Figure 12 First human imaging of ovarian cancer, targeting folate receptor α by systemic injection of folate–fluorescein isothiocyanate. a Colour image showing peritoneum with small tumour deposits; some spots are hardly visible to the naked eye. b Fluorescence image of the same field; a multitude of tumour spots light up. c Overlay of fluorescence image on colour image. d Colour image of four excised specimens, containing tumour tissue and healthy tissue. e Fluorescence image of the four excised specimens. f Overlay of the fluorescence image on top of the colour image. g Quantitative representation of the number of spots detected by the naked eye compared with fluorescence imaging. Scoring by five independent surgeons was based on three different colour images and their corresponding fluorescence images (Graph reproduced with permission from reference 5). Error bars denote 2s.d. *P < 0.001. (Reproduced from Intraoperative Imaging and Image-Guided Therapy, Jolesz FA (ed.), 2014, with permission from Springer Science and Business Media)
TARGETED ACTIVATABLE PROBES
The advantages of antibody-based fluorescence optical imaging seem obvious; however, target finding itself is often complex. The chance of finding a universal generic receptor that targets all tumours equally is small, and probably unrealistic. Besides the increased expression rates of growth signalling receptors, the upregulation of certain proteolytic enzymes is another hallmark underlying the invasive character of cancer (Fig. 7). Using these proteolytic enzymes as targeting markers for more general phenomena in cancer invasive growth may be a promising way to circumvent the intratumour and, even more so, inter tumour heterogeneity. Detection of the homogeneous tumour margin is more important than homogeneous whole-tumour painting, including its core. Typically, these agents are injected in an inactivated state and become active upon cleavage by proteases such as cathepsins and MMPs, from which the resulting fluorescence can be measured. Mahmood and Weissleder and Wunderbaldinger et al. have developed a number of such activatable NIR probes. The mechanism involves macromolecules loaded with multiple fluorochromes that, owing to their close proximity to one another, exhibit self-quenching. However, after enzymatic cleavage by MMPs the fluorochromes become detached and are free to fluoresce. Jiang and colleagues recently introduced a different quenching mechanism based on activatable cell-penetrating peptides (CCPs). Activatable CCPs are small peptides that are able to facilitate cellular uptake of cargo, such as fluorochromes and therapeutic agents after linkage with the peptide. CCP uptake is effectively inhibited when an inhibitory domain made up of negatively charged residues is fused to the CCPs. Fusing the anionic residues to the CCPs (linked to the NIR probe) by means of a photolytic substrate allows visualization of proteolytic enzymes that are abundant in malignant tissue, thereby increasing the cancer-to-background contrast for sensitive intraoperative real-time tumour detection. A second generation of such activatable CPPs are known as ratiometric activatable CPPs, in which the tumour-to-background signals are significantly enhanced. No clinical prototype camera systems are currently available for clinical translation of this concept.

FUTURE DIRECTIONS
PHOTOIMMUNOTHERAPY
High rates of positive margins, and thus locoregional recurrence after curative surgical resection, occur in many cancer types, and highlight the importance of improving the ability to define tumour margins during surgery. Among the array of imaging modalities currently being investigated to localize microscopic disease, fluorescence optical imaging seems the most promising for real-time image-guided surgery. The efficacy of optical imaging is
based largely on the detection of photons.\textsuperscript{46} Owing to the complex geometry and density of tissues being imaged, fluorescence images display various intensities. Because light travelling through tissue is subject to various amounts of absorption and scattering, detecting fluorescence signals in an environment dominated by heterogeneous optical absorption is challenging.\textsuperscript{47} In these cases, photoimmunotherapy (PIT), comprising antibody-based photodynamic therapy (PDT), is a promising approach as photoimmunodetection in combination with PDT can be achieved.\textsuperscript{48} Conventional PDT has been a therapeutic modality for decades.\textsuperscript{49} In PDT, a photosensitive dye (photosensitizer), with both diagnostic and therapeutic applications, is administered intravenously, after which it accumulates selectively in metabolically active tissues. On exposure to light of the appropriate wavelength, the photosensitizer is excited to enable fluorescence imaging, but also generates cytotoxic singlet oxygen molecules that directly kill the surrounding cells.\textsuperscript{50} However, progress has been limited, mainly because the off-target uptake of photosensitizers in surrounding tissue results in significant damage to normal tissue. Furthermore, determining treatment depth and successful tumour removal can be very difficult. Therefore, this is not considered an appropriate primary treatment modality and is currently used only for palliative treatment of cancers that are not amenable to curative therapy. PIT uses photosensitizers coupled to monoclonal antibodies directed at tumour-specific antigens. Targeting tumour-specific antigens by using monoclonal antibodies as vehicles for delivery of photosensitizers has the potential to be much more efficient and cause less damage to surrounding normal tissue than conventional PDT.\textsuperscript{48} Although generally not considered ef-

\textbf{Figure 13 Intraoperative photoimmunotherapy.} a Photoimmunotherapy allows intraoperative photoimmunodetection of tumour. b Visually undetectable or microscopic tumour strands can be treated with phototherapy during surgery. NIR, near-infrared
fective for single-modality treatment because of the known limitations of conventional PDT, it may be highly effective as an adjuvant intraoperative wound bed treatment after resection for detection and treatment of positive margins. As such, PIT may be a very promising therapeutic approach for future NIR fluorescence-guided surgery. Integrating real-time imaging and selective destruction of tumour cells in the operating room can shift the standards of care in surgical oncology, offering the unique opportunity to visualize tumour borders and treat residual invasive disease with adjuvant PIT (Fig. 13).

**OPTOACOUSTIC IMAGING**

Ultimately, NIR optical imaging is limited by the strong scattering of light in biological tissue, which severely degrades the spatial resolution from targets deeper than first few hundred microns beneath the illuminated tissue surface. Optoacoustic imaging has been developed to allow high-resolution optical imaging at depths of up to several centimetres. Optoacoustic imaging detects optical absorption by means of the ultrasound emitted as a result of thermal expansion. In particular, the use of multiple optical wavelengths and spectral unmixing algorithms, referred to as multispectral optoacoustic tomography (MSOT), provides the ability to recognize specific absorbers, including endogenous tissue chromophores (such as haemoglobin, melanin, lipids), organic dyes also used in fluorescence imaging (ICG, IRDye®800CW), and a range of novel light-absorbing nanoparticles (gold nanorods, carbon nanotubes). Optoacoustic imaging is experiencing a surge of interest in clinical investigation following technological developments that have enabled imaging systems suitable for clinical use. The development of real-time hand held operation has allowed systems that are similar to clinical ultrasound technology in form and handling (Fig. 14a). Such systems allow rapid imaging at the bedside or in the operating theatre, and provide immediate feedback in the form of live images. Furthermore, multiple-wavelength light sources, capable of tuning rapidly to several distinct wavelengths, enable spectrally resolved detection of tissue absorbers or exogenous imaging agents, which is crucial for robust understanding of the source of optoacoustic image contrast. Miniaturized systems capable of endoscopic imaging have been described for use in the gastrointestinal tract in animal studies, and can be expected to reach human investigation in the near future. The diverse sources of optoacoustic image contrast promise a range of clinical applications. Haemoglobin contrast can be used to assess tumour vasculature (Fig. 14c), as well as tissue or organ perfusion. Melanin contrast can be used to detect melanoma metastasis in lymph nodes and to measure primary melanoma depth. The detection of lipids provides the potential for atherosclerotic plaque characterization and improved risk stratification. Breast cancer has long been considered a potential target, as breast tissue is relatively easily penetrated by NIR light, independent of
Tumours can be detected by means of haemoglobin contrast resulting from their increased vascular density.\(^{67-69}\) Optoacoustic imaging therefore has the potential to offer increased sensitivity compared with mammography in patients with dense breasts, and improved specificity compared with ultrasonography. Exogenous contrast agents for MSOT include those investigated for fluorescence imaging (Fig. 14d). ICG and methylene blue detection has been described in many different scenarios.\(^{54}\) MSOT-based molecular imaging using NIR dyes combined with targeting ligands has been demonstrated in animal models,\(^{58,59,70}\) and it is therefore likely that agents under investigation in clinical trials for fluorescence imaging will also be studied by optoacoustic imaging in the near future. In addition, a wide range of novel nanoparticles have been considered for optoacoustic imaging.\(^{71-76}\) However, because safety would have to be assessed for each form of nanoparticle agent under complex regul-
tory processes, it is unlikely that these agents will see human use in the short term. Overall, whereas fluorescence imaging provides a map of surface contrast over a wide field of view, emerging optoacoustic imaging enables high-resolution optical assessment of subsurface targets.

**DISCUSSION**

Imaging is an essential element in the daily practice of surgeons. Modalities such as ultrasonography, CT, MRI and PET all have their disadvantages. Their unsuitability for real-time feedback with regard to detection of (metastatic) tumour deposits is a major drawback for intraoperative surgical use. Achieving real-time intraoperative tumour visualization could improve patient outcome and survival by assisting the surgeon in a more complete resection of the tumour followed by an adjuvant (intraoperative) treatment. Therefore, alternative innovative optical imaging methods for intraoperative tumour assessment are being studied. Tumours can be detected by targeting various hallmarks of cancer. Non-targeted agents are used to visualize tumour by exploiting the enhanced permeability and retention effect. Overall, however, the majority of research is focused on exploiting the intrinsic properties inherent to tumours to enhance visual demarcation between normal and cancerous tissues. Invasive tumour borders can be imaged using NIR fluorescence agents that become activated by proteolytic enzymes involved in degradation of the extracellular matrix, a prerequisite for tumour invasion. Tumour cells differ from normal cells owing to different expression rates of their respective growth factor receptors. Targeting a tumour-specific receptor yields a high signal-to-noise ratio in diagnostic imaging. The first steps of translation of optical imaging to the clinic are being made in Europe as well as North America. Image-guided cancer surgery has the potential to be a very powerful tool in guiding patient care in surgical oncology. However, during the process of implementation, attention must be paid to the efficacy, accuracy and standardization of imaging systems and probes, in terms of clinical grade GMP production, quality control and standard operating procedures related to stability. Complex geometric and variable densities of tissues might compromise the detection of fluorescence signal. PIT and, in particular, optoacoustic imaging might be promising innovative imaging techniques to circumvent these issues. PIT allows both photoimmunodetection and phototherapy of undetectable fluorescence. Optoacoustic imaging is based on the absorbance of light inside tissue, which results in the generation of ultrasound waves that can be detected, enabling high-resolution optical assessment. It is anticipated that the next 5 years will deliver many clinical studies in the field of image-guided surgery in various cancer types, with increased use in certain routine procedures, such sentinel lymph node detection.
ACKNOWLEDGMENTS

E.d.B. and N.J.H. contributed equally to this work. In addition to institutional funding provided by the University Medical Centre Groningen and the University of Alabama at Birmingham, this work was supported by the Stichting Prof. Michaël-van Vloten Fonds.

Disclosure: The authors declare no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Video S1 Image-guided surgery in breast cancer (wmw file)
Video S2 Camouflaged octopus (mp4 file)
Video S3 Mouse breast cancer (mov file)
Video S4 Mouse ovarian cancer (m4v file)
Video S5 Human targeted fluorescence detection of ovarian cancer (mov file)
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