Review

Modalities for image- and molecular-guided cancer surgery

M. A. Stammes[1,3], S. L. Bugby[7], T. Porta[6], K. Pierzchalski[6], T. Devling[8], C. Otto[4], J. Dijkstra[1], A. L. Vahrmeijer[2], L.-F. de Geus-Oei[1,5] and J. S. D. Mieog[2]

Departments of [1]Radiology and [2]Surgery, Leiden University Medical Centre, Leiden, [3]Percuros, [4]Medical Cell Bio Physics, University of Twente, and [5]Biomedical Photonic Imaging Group, MIRA Institute, University of Twente, Enschede, and [6]Maastricht MultiModal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, The Netherlands, [7]Space Research Centre, Department of Physics and Astronomy, University of Leicester, Leicester, UK, and [8]iThera Medical, Munich, Germany

Correspondence to: Dr J. S. D. Mieog, Department of Surgery, Leiden University Medical Centre, PO Box 9600, 2300 RC Leiden, The Netherlands (e-mail: j.s.d.mieog@lumc.nl)

Background: **Surgery is the cornerstone of treatment for many solid tumours. A wide variety of imaging modalities are available before surgery for staging, although surgeons still rely primarily on visual and haptic cues in the operating environment. Image and molecular guidance might improve the adequacy of resection through enhanced tumour definition and detection of aberrant deposits. Intraoperative modalities available for image- and molecular-guided cancer surgery are reviewed here.**

Methods: **Intraoperative cancer detection techniques were identified through a systematic literature search, with selection of peer-reviewed publications from January 2012 to January 2017. Modalities were reviewed, described and compared according to 25 predefined characteristics. To summarize the data in a comparable way, a three-point rating scale was applied to quantitative characteristics.**

Results: **The search identified ten image- and molecular-guided surgery techniques, which can be divided into four groups: conventional, optical, nuclear and endogenous reflectance modalities. Conventional techniques are the most well known imaging modalities, but unfortunately have the drawback of a defined resolution and long acquisition time. Optical imaging is a real-time modality; however, the penetration depth is limited. Nuclear modalities have excellent penetration depth, but their intraoperative use is limited by the use of radioactivity. Endogenous reflectance modalities provide high resolution, although with a narrow field of view.**

Conclusion: **Each modality has its strengths and weaknesses; no single technique will be suitable for all surgical procedures. Strict selection of modalities per cancer type and surgical requirements is required as well as combining techniques to find the optimal balance.**

Paper accepted 5 November 2017

Published online in Wiley Online Library (www.bjs.co.uk). **DOI:** 10.1002/bjs.10789

Introduction

In recent decades, multiple imaging modalities have emerged as essential tools in cancer diagnostics, providing information about the molecular and functional processes in normal and diseased tissues[1]. New technologies have been developed to enhance understanding of the diversity and behaviour of cancer *in vivo*[2]. Despite these resources, surgeons still primarily rely on their eyes and hands as tools during surgery[3–5]. In oncological surgery, clean and clear demarcation of the tumour boundaries is pivotal to determining the balance between excising too little or too much tissue. Therefore, careful examination of the tumour borders is essential[6,7]. Preoperative imaging does not always correlate well with intraoperative images owing to tumour growth, deformation of soft tissue, shifting of organs, or misalignment of the image display compared with the surgical field[8].

As Rosenthal and colleagues[9] discussed for patients with breast, melanoma or head–neck cancer, surgical excision requires three detection steps: initial assessment before resection; initial assessment during incision, including detection of regional metastasis as well as lymph nodes; and postresection margin analysis by the pathologist. Eyes and hands cannot detect the exact boundaries of a tumour, or create a clear three-dimensional (3D) morphological or functional overview of the operative site[5]. As a result, histological tumour involvement of the resection margins may be observed at least 20 per cent of the time in patients with breast cancer[3,4,9,10]. To improve cure and complication rates, the use of intraoperative *in vivo* and

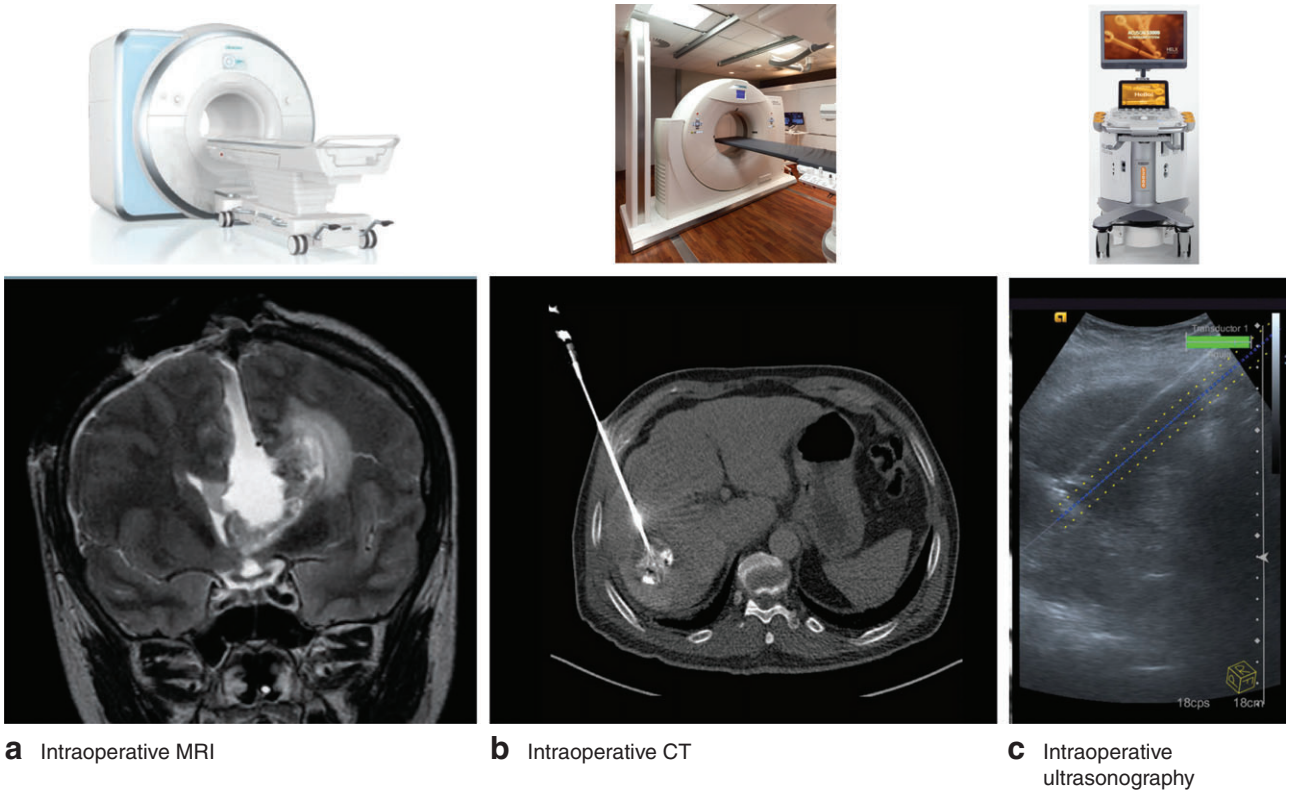

Fig. 1 Conventional image-guided surgery systems and examples of image output: **a** intraoperative MRI, **b** intraoperative CT and **c** intraoperative ultrasonography

real-time tools would be useful. To achieve this requires better spatial resolution than can be achieved by the human eye, minimal interference with daily practice, and operator-friendly instrumentation that is time efficient[11]. To go beyond visualization of anatomical boundaries, real-time molecular information would provide additional information to optimize surgical resection. The focus of this review was intraoperative modalities for image- and molecular-guided cancer surgery.

Methods

Twenty-five characteristics were selected to evaluate and compare the ten different image-guided surgery modalities reviewed here. As stated by Weissleder and Pittet[2]: 'for imaging technologies to be adapted more widely and to be complementary to other types of imaging the read-outs need to meet certain criteria; they need to be quantitative, high resolution, longitudinal, comprehensive, standardized, digital and sensitive'. This statement refers to cancer imaging in general, but the requirements apply equally well to image- and molecular-guided surgery in patients with cancer[2].

The chosen characteristics are based on relevant articles, which were found through PubMed searches (January 2012–January 2017) using one or more of the following keywords: 'surgery', 'cancer', 'oncology' and the specific names of the (imaging) modalities. Further searches were carried out for specific performance characteristics, such as resolution. Abstracts were reviewed and full-text articles obtained where possible. References and linked articles from included papers were studied to identify further relevant information.

To summarize the data in a comparable way, a three-point rating was applied to quantitate image-guided surgery characteristics. User friendliness was determined from discussions with end-users but differed from user to user; these were scored as easy (+), intermediate (–/+) or challenging (–).

Results

The study identified ten modalities that could be used for image guidance during surgery. Examples of imaging systems for each modality, along with a representative clinical image, are shown in *Figs 1–3*. In general, the modalities

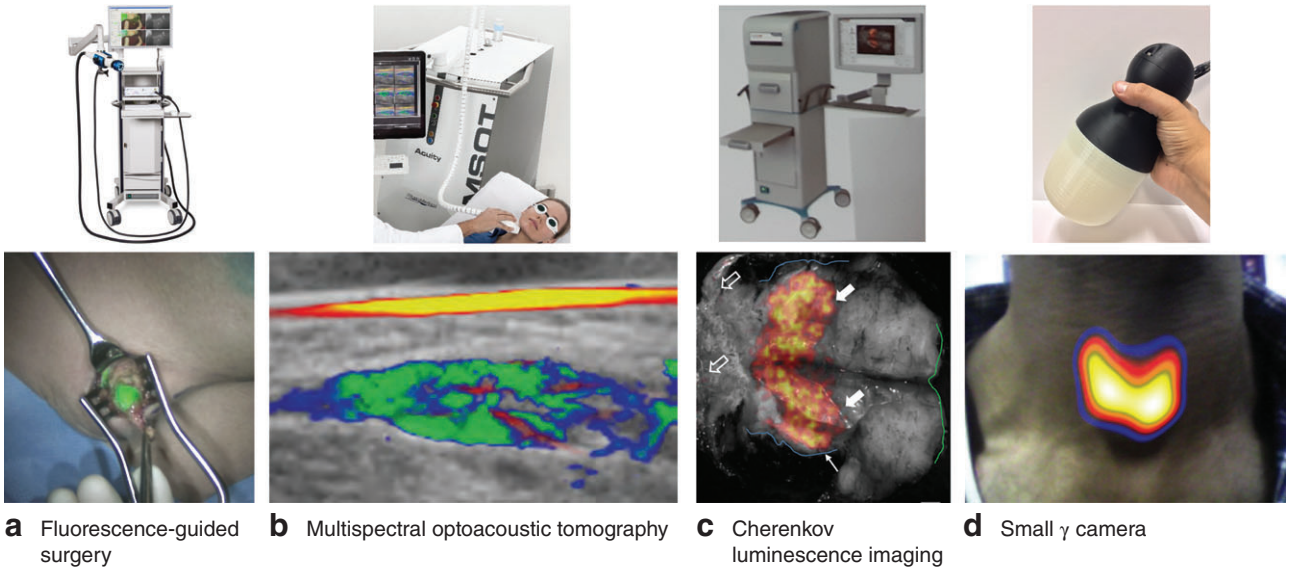

Fig. 2 Optical and nuclear image-guided surgery systems and examples of image output: **a** fluorescence-guided surgery; **b** MSOT, multispectral optoacoustic tomography; **c** Cherenkov luminescence imaging[12] and **d** small γ camera[13]

can be classified into four groups: conventional, optical, nuclear and endogenous reflectance. *Table 1* provides information on conventional modalities already familiar to many practitioners; the imaging modalities are described along with the type of information obtained together with the surgical interference and associated risks. *Tables 2* and *3* provide the same information for optical and nuclear, and endogenous reflectance techniques respectively. The same groupings are used for *Tables 4–6*, which compare the performance of each modality during surgery, including the criteria that Weissleder and Pittet[2] mention as being essential. *Tables 4–6* additionally provide information about the clinical potential and major challenges for clinical implementation of each of the ten modalities.

Comparison between modalities

Modalities within each group are compared in the tables, and it is also possible to compare techniques between groups across tables. *Fig. 4* provides a comparison of all ten modalities based on the characteristics most interesting in clinical practice: penetration depth, resolution and acquisition. This clearly demonstrates a common trade-off in image-guided surgery – greater penetration depth often coincides with degradation of resolution.

Conventional modalities

The use of non-invasive imaging for disease diagnosis has become a standard operating procedure, and these conventional modalities are widely available. The current standard consists of conventional imaging modalities that yield anatomical and macroscopic structural information. The images and information obtained with any new technologies must be compared with those from these established techniques[64].

Intraoperative MRI

To be able to use MRI during surgery, the magnetic resonance compatibility of surgical equipment needs to be guaranteed, together with special policies for safety and staff training. The implementation of such policies can be prohibitively expensive, although the costs are dependent on the field strength of the system. High-field systems (more than 1·0 T) require far more investment as shielding of the operating room is essential, but provide high-resolution images within a shorter acquisition time. Low-field systems (below 0·3 T) are cheaper as no additional requirements for the operating theatre are necessary, and they can be integrated into existing operating rooms[22]. Another advantage of using a low-field system is the availability of open systems, which are more useful during surgery. Nevertheless, the lower the field strength, the lower the image quality or the longer the acquisition time[19,25].

Despite these limitations, the main reason for still making use of MRI in neurosurgery is that the maximum amount of tumour can be removed in a safe manner[25].

Intraoperative CT

In general, CT offers high throughput with high-resolution imaging; however, this is not the case when

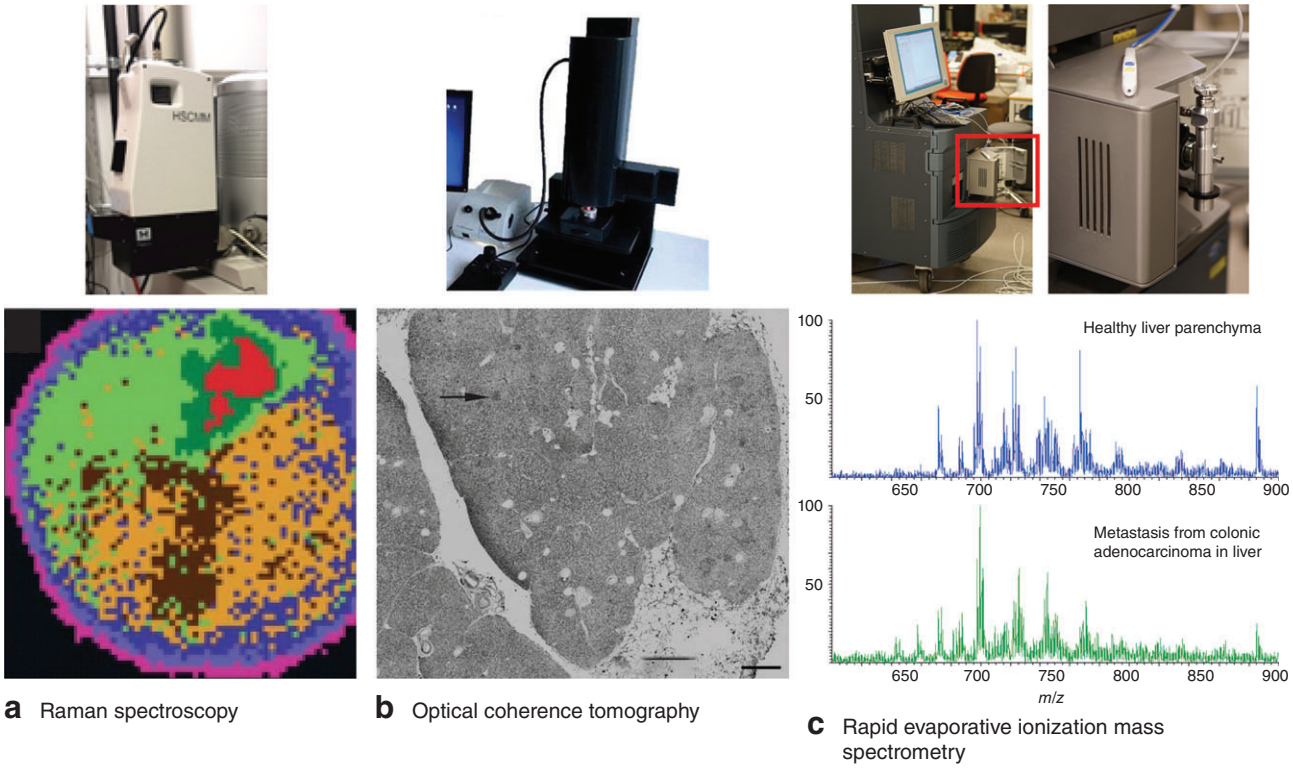

Fig. 3 Endogenous reflectance image-guided surgery systems and examples of image output: **a** Raman spectroscopy[14], **b** optical coherence tomography and **c** rapid evaporative ionization mass spectrometry[15]

it is used for intraoperative imaging. Acquiring CT images during surgery takes 10–15 min, partly owing to the interference caused by the shape of the gantry, as using a bore will cause more interference than use of a C-arm. For assessing surgical specimens instead of the cavity, micro-CT can be used as there is less interference with the procedure and a high spatial resolution of less than 1 μm. Nevertheless, the accuracy of margin assessment is variable owing to specimen orientation, and there can be a high rate of non-specific findings due to dense parenchyma and architectural distortion resulting from the surgery[20].

Intraoperative ultrasonography

Of the conventional imaging modalities, ultrasonography is the easiest to incorporate during surgery as it does not interfere with the procedure or present logistical challenges, gives real-time information and surgeons are already used to interpreting the images obtained. In addition, intraoperative ultrasonography is one of the most sensitive imaging modalities for assessing small lesions as a high-frequency transducer can be used. In addition to sensitivity, the specificity of discrimination between healthy tissue and residual disease is a benefit of this technique[27]. Because intraoperative ultrasonography can be used in an iterative mode, one should be aware of an essential drawback, that surgical manipulation can cause artefacts so the image quality will decrease as the operation proceeds[23].

Optical imaging

Optical imaging techniques such as fluorescence-guided surgery (FGS) and multispectral optoacoustic tomography (MSOT) can provide real-time feedback with limited workflow disruption. They require a targeted probe that consists of a fluorophore in the near-infrared window (approximately 700–900 nm) which has the largest penetration depth of optical light in tissue. In this window, penetration is 1 cm for FGS or a few centimetres with MSOT, compared with only a couple of millimetres for wavelengths below 700 nm[3,4,6,38]. There is also a window above 900 nm, the so-called second-window near-infrared light (NIR2), ranging from 900 to 1450 nm. This window has the advantages of even deeper tissue penetration and low tissue autofluorescence signals, which lead to higher tumour to background ratios. *In vivo* testing in experimental studies has shown a penetration depth of up to 18 mm, and simulations suggest that this might be increased to up to 10 cm[65–67]. To make use of this NIR2

Table 1 Description of conventional intraoperative image-guided surgery modalities and interference with surgical workflow

	Intraoperative MRI	Intraoperative CT	Intraoperative ultrasonography
Principle	MRI is based on the different spin relaxation rates of atoms within tissue under a static magnetic field and radiofrequency pulses via the excitation of hydrogen nuclei[16]	X-rays pass through the patient, are attenuated and subsequently measured by detectors which rotate around the patient[17]	The probe transmits ultrasound waves, which are (partially) reflected and/or scattered by tissue inhomogeneities and interfaces and sent back to the probe[18]
Type of information	Soft tissue discrimination and multiplanar visualization[19]	Structural differences owing to differences in absorbance[17,20]	Contrast is based on scattering/reflectance differences between different types of tissue: soft tissue, fat and fluid[18,21]
Anatomical information	Yes, depending on the sequence[22]	Yes[20]	Yes, although orientation is limited owing to the different planes[23,24]
2D/3D	3D	3D	2D, 3D with specialized transducers or a stacked 2D volume[23]
Need for contrast agent	Not necessary; discrimination between two types of tissue can be improved with the use of contrast agent and it gives the opportunity to acquire real-time vascular-phase images[22,25]	Not necessary; discrimination between two types of tissue can be improved[26]	Not necessary; discrimination between two types of tissue can be improved and real-time vascular-phase images acquired[27,28]
Cost (machine and facility)	€€€[22]	€€€[29]	€[23,24]
Acquisition cost	€€€[30,31]	€€[30,31]	€[30,31]
Time (acquisition and reconstruction)	Maximum 2 h[24,25], not real time	10–15 min[20,24,26], not real time	Real-time interactive information[24,27]. Delay is dependent on operator (maximum a few minutes)[23]
Interference with surgery	Yes, highly interfering. Positionwise may even be impossible[22]	Yes, depending on the modality and the possibility of a sliding gantry on a railtrack[24,26]	Not in general and presents no logistical challenges[23]. Yes, it needs direct contact with specimen[4,21]
Endoscopic options	No	No	Yes
Safety	Safe; it can even detect complications at an earlier stage[19,22,25]	No complications or infections related to intraoperative CT and surgical complications directly recognized[26]	Relatively safe and well tolerated[27]. It gives direct feedback to the surgeon[32]

2D, two-dimensional; 3D, three-dimensional.

window, new instrumentation is required. Specific probes for use in this range go beyond the scope of this review; however, single-walled carbon nanotubes or upconversion nanoparticles are encouraging opportunities[65–67].

Fluorescence-guided surgery

FGS has the advantage of providing real-time imaging, being relatively cheap and user friendly, and not interfering with the surgical area. However, it also has several disadvantages, such as the limited penetration depth (maximum 1 cm), and challenges in quantification owing to other processes associated with the use of light, such as photobleaching, transmission and reflection changes. Light in general is attenuated by absorption and scatter in tissue; the total attenuation (sum of attenuation from absorption and scatter) has an exponential relationship with depth. Practically, this means that fewer than 0·0001 per cent of the photons transmitted into tissue can be detected and, of this amount, only 10–25 per cent of the photons generated in tissue will really be recovered. This is due to the relatively small quantum yield of most fluorophores and especially near-infrared fluorophores. Other limitations for quantification are absorption and scatter as these characteristics are highly variable in tissue. Full correction, by measurement of the absorption, scatter and anisotropy of tissue, can lead to quantitative measurements; however, this is still in its infancy[3]. Another limitation to full clinical translation is the lack of specific contrast agents. So far only three tumour-specific agents have been registered for clinical use. Several other tumour-specific agents are in the process of clinical translation, but this is dependent on the fluorophore being approved[3].

Multispectral optoacoustic tomography

In general, MSOT has the same advantages and disadvantages as FGS, except that it has a greater penetration

Table 2 Description of optical and nuclear image-guided surgery modalities and interference with surgical workflow

	Fluorescence-guided surgery	Multispectral optoacoustic tomography	Cherenkov luminescence imaging	Small γ camera
Principle	An injected/endogenous fluorophore is excited at a specific wavelength and the emitted fluorescent photons are detected[6]	Light is used as input energy and acoustics for signal detection, similar to ultrasonography. Molecules absorbing light undergo transient thermoelastic expansion which generates ultrasound waves[11]	Charged particles emitted from radionuclides transfer energy as they move through a medium. If the particles are travelling faster than the speed of light in that medium, the transferred energy is released, through relaxation, as light[33]	Radionuclides introduced to the patient emit γ radiation. γ photons have sufficient energy to pass relatively unimpeded through tissue to be detected by an external camera[34]
Type of information	Presence of a fluorophore or specific tissue properties in a certain area[6,9]	Differences in optical absorption inside tissue are visualized[11]	Functional images based on the distribution of an externally administered particle-emitting radiotracer[35]	Quantitative functional images based on the distribution of an externally administered γ-emitting radiotracer[36]
Anatomical information	No; however, autofluorescence provides information about tissue properties[9]	Yes, by strong endogenous absorbers like blood and melanin[11]. Interleaved with ultrasound images for mechanical contrast	No	No
2D/3D	2D	2D and 3D	2D[33]	2D or 3D depending on detector system used
Need for contrast agent	Yes typically; however, when using endogenous fluorescence signal a contrast agent is not necessary[4,9]	Yes/no; detects endogenous tissue absorbers or exogenous contrast agents[4]	Yes, a particle-emitting radiotracer[33]	Yes, a γ-emitting radiotracer
Cost (machine and facility)	€[4]	€€	€[33]	€€
Acquisition cost	€	€	€€[31]	€€[30,31]
Time (acquisition and reconstruction)	Real time, in the millisecond range, and related to the surgical field[3,4]	Image generation in real time. Possible to perform advanced analysis after the process	Several minutes[33,34]	Depends on amount of activity *versus* signal to noise ratio. An acquisition time of 1 min sufficient in the majority of patients[34,37]
Interference with surgery	No, there is no direct contact with the specimen as the optimal working distance is between 5 and 45 cm[38]. A dark environment (overhead lights off) is beneficial[7]	Not in general but it needs to be contact-based, similar to intraoperative ultrasonography	Yes, complete darkness required for imaging. Use of radioactive tracers may have implications for working practice[33]	Not in general; there is no direct contact necessary with specimen. However, use of radioactive tracers may have implications for working practice
Endoscopic options	Yes, with the Cellvizio® system[39]	Yes	Yes[40]	No
Safety	Near-infrared imaging is a safe technique; only laser illumination levels need attention[3]	Similar to ultrasonography in technique, so relatively safe. Only the direct contact can cause problems	Overexposure to radiation for both patients and surgeons[4]	Radiation exposure for both patients and surgeons[4]

2D, two-dimensional; 3D, three-dimensional. Cellvizio® (Mauna Kea Technologies, Paris, France).

depth. In addition, both FGS and MSOT are based on photon delivery, but low-frequency ultrasonic pulses are also detected in optoacoustic tomography. These pulses are generally unaffected by tissue absorption and scattering, essentially removing a large component of the limiting factor in the development of quantitative methods for fluorescence-based imaging at depth. Given that the strength of an optoacoustic signal within a pixel is a function of both the diffusive light reaching that pixel and the concentration of absorber present, it is apparent that the concentration of a local chromophore can be determined by measuring or modelling light propagation through the tissue. Recent work by Tzoumas and colleagues[61,62] and Brochu and co-workers[63] has demonstrated that this result can be achieved both in phantoms and, more importantly, *in vivo*, giving a glimpse that quantitation is a possibility in clinical optoacoustic tomography.

Nuclear imaging

Nuclear modalities use a radioactive tracer to generate images, with a high sensitivity and specificity in general, although this is dependent on the tracer of choice[55]. However, the use of radioactive material requires special biosafety permits, additional training, and safety procedures for both personnel and patients.

Table 3 Description of endogenous reflectance image-guided surgery modalities and interference with surgical workflow

	Raman spectroscopy	Optical coherence tomography	Rapid evaporative ionization mass spectrometry
Principle	Monochromatic light of a certain wavelength illuminates tissue and scatters light with new wavelengths. The energy related to the wavelength shift is a function of the vibrational energies of molecular bonds in tissues[41,42]	Analogous to ultrasonography, but reflections of near-infrared light are detected instead of sound[43,44]; the information is obtained by differences in reflected energy and scattering intensity[21]	An ambient ionization technique. Connected to an unmodified surgical handpiece, the system directly aspirates and analyses the smoke created by the electrosurgical device from the surface of the tissue
Type of information	Cellular structures can be distinguished based on the chemically specific Raman spectrum of metabolites, lipids, proteins, DNA[41]	Cross-sectional images are generated, mimicking the intensity of optical backscatter of light passed through tissue[43]	Identification is based on use of tissue-specific libraries (molecular profiles or fingerprints) to identify the tissue type[45]
Anatomical information	Yes, depending on technique[41]	Yes[21,43], tomographic images of biological tissue are generated (morphology)[11,41,46]	No, it is based on tissue-specific molecular signatures[47]
2D/3D	2D, 3D is possible by using stacked images or measuring at a different depth	2D and 3D depending on detector used[44]	A spectrum is generated, not an image[15,48,49]
Need for contrast agent	No, it is a label-free method as it uses intrinsic properties of molecules[42]	No, depends on optical scattering and reflectance of tissue to generate contrast[41,44,46]	No, it is a label-free technique[15,48,49]
Cost (machine and facility)	€	€	€€[47]
Acquisition cost	€	€	€
Time (acquisition and reconstruction)	Short (1–10 min)[41]. Spontaneous Raman scattering: acquisition time 0·05 s for a single high-quality spectrum. Coherent Raman imaging is faster (μs/pixel)[41,42], with limited spectral quality	Real time[11,44]; an image can be taken every 5 s[21,43]. Total acquisition time up to 5 min[41,46]	A couple of seconds (< 3 s)[15,47]. Use of real-time recognition algorithm allows rapid identification of tissue being analysed
Interference with surgery	No direct contact needed[41]	Yes/no; does not require direct contact with specimen[11,21,46]; can rapidly scan large areas of tissue[43]	No modification of surgical procedure required[15]. The tissue measured is destroyed during this process, but the surgeon is cutting this tissue[45,49]
Endoscopic options	Yes	Yes[21,43,44]	Yes[48]
Safety	Reasonable; owing to the weak signal, high levels of light energy and long exposure times are often needed[50]	Relatively safe; similar to ultrasonography and rapid image acquisition[21,43]	Relatively safe[45]. For the mass analyser system, European norms have to be complied with

2D, two-dimensional; 3D, three-dimensional.

Cherenkov luminescence imaging

Cherenkov luminescence imaging (CLI) is actually a combination of optical and nuclear imaging as the radioactive tracer in CLI is used to create optical photons. A drawback of this is that CLI has a tissue penetration of only 1 cm, similar to that of optical imaging. On the other hand, the resolution is also similar to that of optical imaging, which is higher than that of any other nuclear imaging modality. Nevertheless, the intensity of the optical photons generated is about a billion times lower than the illumination in an operating theatre, which makes it hardly suitable for use during open surgery; endoscopic applications would be favourable[33]. This low light level negatively influences the sensitivity, which can be improved by injecting a larger amount of radioactivity. The amount of radioactivity correlates well with the light output (radiance), although an increase in radioactivity will also lead to an increase in radiation burden.

Small γ cameras

Like single-photon emission CT (SPECT), γ cameras can be considered a conventional modality. However, these systems face similar drawbacks to MRI and CT in that the size and shape of the machine causes a lot of surgical interference, and a dedicated scanning room is needed. To circumvent this, a handheld γ probe is already used in clinical practice for sentinel lymph node detection. Although useful, these probes can only indicate the amount of activity

Table 4 Performance and clinical potential of conventional intraoperative image-guided surgery modalities

	Intraoperative MRI	Intraoperative CT	Intraoperative ultrasonography
Resolution	Resolution around 0·3–1·3 mm[51,52]. Improves with scan time and field strength[22,25,52]	Spatial resolution 0·4–0·6 mm[4,24]; < 1 μm for micro-CT[20]	High spatial and temporal resolution around 0·3–1 mm[4,21,24,27]
FOV	Up to 20 cm, although distortion increases with FOV[53]	14 cm[20]	Dependent on transducer; curved transducer > linear transducer in range 10–60 mm[4]
Iterative	Yes, but mostly one scan obtained[25]	Yes	Yes[23]
When to use during surgery	Can be used for surgical (re)orientation and for quality control of resection cavity[19,22,25]	Can be used for surgical (re)orientation, and micro-CT for lump margin assessment[20,26]	Used for real-time surgical guidance at all stages[23,27,32]
Depth	Whole body	Whole body	Several centimetres[4]
Interoperator variability	Medium[22]	Low	High[24,28,32]
User friendly	−/+, Depends on familiarity of surgeon with magnetic resonance image interpretation[9]	+	+[23,24,32]
Availability	−/+, Limited owing to high price and room/surgery requirements[22]	+, For CT and limited use of micro-CT[20]	++, Widely available[23,24,32]
Status of machine	Clinical	Clinical	Clinical
Status of contrast agent	Non-tumour-specific agents available	Non-tumour-specific agents available	Three agents available in Europe[24,28]
Quantification of size/signal	Yes, absolute[2]	Yes, absolute[2]	Yes, absolute[2]
Cancer type	Brain[4,9,19,22,25]	Lump margin assessment[20], brain[26], spinal[54]	Abdomen[9,27,28], head and neck area[23,32], breast[9], brain[9]
Artefacts/ limitations	Vascularized tumours and haematomas lead to poorly visualized operative fields as they produce imaging artefacts[22]	Dense parenchyma and architectural distortion make margin assessment difficult[20]. Bone anatomy well visualized but limited on the lesion itself[54]. Radiation exposure[29]	Cirrhosis and steatosis (induced by chemotherapy); imaging of cirrhotic or steatotic liver can be improved by using contrast agent[27,28]. Lack of anatomical orientation[23]
Sensitivity, specificity of system	Increases with field strength[19,25,55]	Specificity > 90%, but sensitivity only 60%[20]	Sensitivity and specificity both high[27]

FOV, field of view.

within their field of view and do not have imaging capabilities. Innovative radiation detector design has allowed the generation of compact γ cameras[68]. The difference between SPECT and use of small γ cameras is that with γ imaging the sensitivity is dependent on the tracer but independent of the depth of the tumour; for small γ cameras there is a trade-off between sensitivity and spatial resolution, depending on the imaging distance. In addition, the field of view is smaller but dependent on the detector design.

Endogenous reflectance

The final group of techniques encompasses a variety of endogenous reflectance/signals modalities. The advantage of this group is that no additional contrast agents are necessary to generate relevant and very detailed information based on the characteristics of the tissue itself. Nevertheless, creating high-resolution output may require substantial acquisition times.

Raman spectroscopy

In general, Raman spectroscopy uses the intrinsic properties of molecules to generate contrast, which means that is not limited to a certain tissue type, although it requires a more specialized approach for skin pigments such as in melanoma. To create additional contrast, plasmonic particles or organic polymers coupled with antibodies could be used. Stimulated Raman scattering can be used to monitor dynamic changes, alterations in tissue cellularity, axonal density and protein/lipid ratio[42].

A possible limitation of translating Raman spectroscopy into clinical practice is the question of how small fields of view could be applied to the validation of a tumour bed, which is relatively large. A clinical trial[42] using this technique detected low-grade gliomas instead of the tumour bed. For this, an image–resect–image technique was used in which the arm movement was predefined. This method led to an additional operating time of 10 min for image acquisition, which was not considered obstructive to surgical workflow[41,42].

Table 5 Performance and clinical potential of optical and nuclear image-guided surgery modalities

	Fluorescence-guided surgery	Multispectral optoacoustic tomography	Cherenkov luminescence imaging	Small γ camera
Resolution	10 μm[4], depending on camera system[38]	Dependent on detector. Resolution is decreased when imaging depth is increased. Resolution at 3 mm depth is 15 mm, and at 3 cm depth decreases to 200–300 mm[11]	Fundamental spatial resolution limit 0·3 mm, further degraded by scattering in tissue[33,56]	Spatial resolution can range from 3 to 30 mm[4,34,37]
FOV	Between 20 and 250 mm, depending on camera system[38]	Similar to intraoperative ultrasonography; when resolution increases, FOV decreases	Typical endoscopic FOVs. One open-field camera has an 80 × 80-mm FOV[57]	Between 40 and 120 mm is typical, depending on camera system. Can vary with use of pinhole cameras[34,37]
Iterative	Yes	Yes	Yes, but limited by half-life of radiotracer used	Yes, but limited by half-life of radiotracer used
When to use during surgery	Used for tumour margin/SLN localization and quality control of resection cavity[3,4,38]	Use similar to fluorescence-guided surgery but with more anatomical information[11,58]	Mostly used for quality control of resection cavity and lump assessment[12,33]	Used for SLN detection, surgical orientation and for quality control of resection cavity[37,59]
Depth	1 cm[3,4]	Several centimetres[4]	1 cm, depending on radiotracer used[33,60]	No limit[4]
Interoperator variability	Low	Medium	Low	Low
User friendly	+, Near-infrared light does not alter appearance of surgical field[3,4,10]	+	+, Does not alter appearance of surgical field −, Radiation management −, Exclusion of all ambient light	+, Does not alter appearance of surgical field −, Radiation management
Availability	+, Available[3]	−/+, Available in limited centres	−/+, Available in limited centres	+, Available
Status of machine	Clinical[3,38]	Clinical trials ongoing	Clinical trials; some systems available for clinical use[33]	Some systems available for clinical use, some undergoing trials[34]
Status of contrast agent	Only three tumour-specific agents registered for clinical use[3]	Agents under investigation for fluorescence-guided surgery also likely to be studied for multispectral optoacoustic tomography[4]	PET tracers clinically available but none specific to Cherenkov luminescence imaging; more tumour-specific tracers are in clinical development	SPECT tracers clinically available but none specific to γ camera imaging; more tumour-specific tracers are in clinical development
Quantification of size/signal	Only relative; absorption and scatter limit ability for absolute quantification[2]	Yes, via amount of signal in an area[61–63]	Only relative; absorption and scatter limit ability for absolute quantification[33]	Absolute or relative depending on camera design[2]
Cancer type	Primary tumour, lymph nodes, examination of vital structures, metastases[3]	Broad range of solid tumours eventually examined via endoscopy[11]	Broad range of solid tumours	Numerous cancer types; SLN, parathyroid, colon[37]
Artefacts/ limitations	Attenuation correction of excitation light can help with target detection, although overcompensation can also cause false-positives[3]. Penetration depth[3,38]	Requires surface contact	High radiation burden. Exclusion of ambient light is essential; endoscopic applications would be favourable[33]. Scattering can cause signal to be visualized in incorrect area[56]	Radiation burden. Trade-offs between dose, acquisition time (sensitivity), spatial resolution and FOV
Sensitivity, specificity of system	Superficial tissue can be detected with high sensitivity. Sensitivity decreases with depth[4,38]	Nanomolar sensitivity with high specificity based on multispectral imaging	Lack of sensitivity owing to very low light levels[33]. Specificity dependent on tracer used[55]	Increase in imaging distance degrades sensitivity and spatial resolution[34,37]. Sensitivity and specificity dependent on system and tracer[55]

FOV, field of view; SLN, sentinel lymph node; SPECT, single-photon emission CT.

Optical coherence tomography

Optical coherence tomography (OCT) has the advantage of being analogous to ultrasonography, which makes the images easy for surgeons to interpret as they are already familiar with such images. Instead of sound, OCT uses the reflections of light. This means that OCT does not need direct contact with the surgical area; however, owing to differences in refractive index, direct contact is desirable[11,21,46]. Similar to Raman spectroscopy, OCT does not require a contrast agent, but the agents used in optical

Table 6 Performance and clinical potential of endogenous reflectance intraoperative image-guided surgery modalities

	Raman spectroscopy	Optical coherence tomography	Rapid evaporative ionization mass spectrometry
Resolution	High, in submicron range[42]	High, 1–15 μm, limited by depth of penetration which is up to 5 mm, depending on the tissue[11,21,41,44,46]	Not applicable; it does not generate an image but a profile
FOV	Approximately 0·1 mm[23], at highest far-field optical resolution[41,42]	Around 1 cm[2,41]	Surgical dissection rate of around 1 mm/s, leading to FOV of 1 mm[3,15]
Iterative	Yes[41]	Yes[21,43,44]	Yes, but not on the same piece of tissue[45,47]
When to use during surgery	Mostly for quality control of resection cavity and lump assessment[42]	Mostly for quality control of resection cavity and lump assessment[41,44]	Mostly for quality control of resection cavity and lump assessment[45,47]
Depth	Hundreds of micrometres[41,42]	0·2 cm[21,41,43,44]	Not applicable/limited
Interoperator variability	Low, when incorporated in a robotics system	High/medium[44]	Low as a reference library is used for feedback and tissue classification[15]
User friendly	+, When incorporated in a robotics system; otherwise low	+[44]	+, Does not change the procedure of electrosurgical dissections[15]
Availability	−/+, Available in limited number of centres	−/+, Not in routine clinical use for surgery; available for other approaches (opthalmology)[11,44,46]	−/+, Available in limited centres for research purposes only
Status of machine	Mostly *ex vivo* studies, only one *in vivo* study so far published[41]	Mostly *ex vivo*; *in vivo* clinical trials needed[21]. Handheld probes are in development[11,41]	Clinical research, mainly on *ex vivo* tissue; a few papers have reported *in vivo* tissue analysis[15,45,47]
Quantification of size/signal	Relative quantification[2]	Yes, absolute	Only relative; comparison based on different molecular fingerprints from one tissue type to another[15]
Cancer type	Neurological[41,42], gastrointestinal, bladder, cervical[42]	Bladder, prostate, kidney[21], breast[43,44], melanoma, thyroid[44], ovary[46]	All solid tumours, e.g. breast, liver, colorectal, brain[15]
Artefacts/ limitations	Interrogate a small region of tissue. Signal to noise ratio can be a limiting factor. Intrinsic weak signals can be partly solved by use of high-quality instruments[41,42]	Limited penetration depth[21]. Optical scattering and coherent speckle artefacts from cellular structures limit visualization of small cells[43]	Tissue needs to be disrupted for analysis and cannot be measured again[45]. Need for validated tissue-specific databases[15]
Sensitivity, specificity of system	Accuracy, sensitivity and specificity > 90% to distinguish normal brain from tumour-invaded brain[42]	High, sensitivity rates between 80 and 100%, specificity 60–100%[11,41,44]	High, > 90% depending on accuracy of classification library

FOV, field of view.

imaging can be utilized to generate additional contrast if needed. This opportunity to image without a contrast agent shortens the pathway towards full clinical translation as the regulatory issues and risks associated with contrast agents can be circumvented[44].

Rapid evaporative ionization mass spectrometry

Intraoperative molecular diagnostics based on mass spectrometry have recently gained attention from the medical field as they offer the possibility of *in vivo*, *in situ* and real-time mass spectrometric analysis of tissue[15,69]. In combination with electrosurgical devices[49], rapid evaporative ionization mass spectrometry (REIMS) promises to guide and optimize surgical resection in real time as it is performed within a couple of seconds. Within this time frame, the smoke generated by electrocautery is aspirated through tubing and a chemical analysis takes place, followed by real-time data processing and finally quasi-instant visual feedback. Nevertheless, to maintain this speed, there is the need for validated tissue-specific databases which require time to generate and for a large clinical cohort to account for interindividual variability.

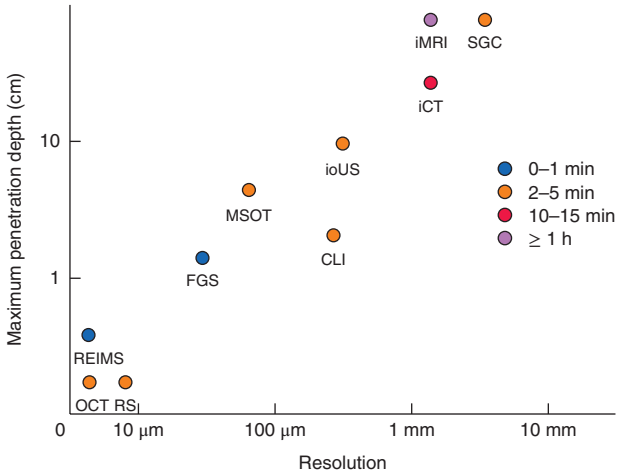

Fig. 4 Graphical representation of the image-guided surgery modalities, with respect to optimal resolution, maximum penetration depth and average acquisition time. CLI, Cherenkov luminescence imaging; FGS, fluorescence-guided surgery; iCT, intraoperative CT; iMRI, intraoperative MRI; ioUS, intraoperative ultrasonography; MSOT, multispectral optoacoustic tomography; OCT, optical coherence tomography; REIMS, rapid evaporative ionization mass spectrometry; RS, Raman spectroscopy; SGC, small γ camera

When such a database becomes available, it is expected that any tissue can be analysed[15,45,47]. In addition, complex molecular signatures can be identified, which can increase the specificity over that achieved with a single biomarker[45]. Although it is not a true 'imaging' technique, REIMS has the potential to improve surgical margins by molecular sampling of margins[70], comparable to that in Mohs surgery for skin cancer, in which the resected specimen is examined for cancer cells during the procedure[71].

Discussion

Tumour removal is an incremental and iterative process, so there should also be the possibility to obtain intraoperative images linked to those obtained by initial staging scans[45]. This may require merging more than one modality. Ultrasonography is a well established technique for interventional procedures, but is rarely the choice for definitive staging. In comparison, SPECT and PET may be used for tumour staging, but cannot be utilized during surgery owing to size limits, whereas portable small γ cameras may suffice[11]. For this purpose, a conventional anatomical technique, such as MRI, CT or ultrasonography, can be combined with a biological imaging modality, such as optical or nuclear imaging, with the use of a targeted tracer. Alternatively, a technique used during surgery for (re)orientation can be combined with one used for quality control of the resection cavity or lump assessment (*Tables 4–6*). Another option is the use of a technique with a high penetration depth but somewhat lower resolution complementary to one of the imaging modalities in the endogenous reflectance group to compensate for the loss of resolution. Both options will lead to a more complete overview of the actual situation in a patient. *Fig. 4* shows differences between the techniques in relation to depth, resolution and acquisition time[2,72]. Techniques that are plotted further apart from each other in the figure may gain the most in combination. So far, the biggest challenge remains fusion of the images generated by different techniques, which can lead to a degree of uncertainty; the greater the distance between two modalities in *Fig. 4*, the greater the challenge.

Over the past decade, imaging has broadened from the conventional anatomical overview to state-of-the-art methods giving a molecular description of structure or function[73]. The overall goal of imaging is to provide a better outcome. It should be noted that better outcome can be defined from different perspectives, that of the patient, surgeon, instrument manufacturer and society, and these may often differ[70]. With intraoperative MRI, for example, surgeons appreciate the fact that they have better visualization and a higher chance of complete tumour resection, but they prefer shorter procedure times and use of intraoperative MRI can increase these by up to 2 h[24,25]. In addition, a reduction in complications, such as tumour-bed haematoma formation, may be achieved with intraoperative MRI detection[22,25]. From a manufacturing standpoint, intraoperative MRI is viewed as successful owing to its reputation and competitive benefits from good system performance in an operating room[70].

For the imaging modalities discussed here, when used in open surgery, the surgeon must look away from the operative field to review the images on a screen; this is not the ideal situation. With augmented reality, the imaging results are projected on to the operative field, which allows visualization of different types of image merged with each other. These images can be obtained before surgery, allowing more detailed planning of the operation beforehand. The major limitation with this approach is the deformation of soft tissue during the surgical procedure, and the orientation of the image display in relation to the surgical field. The application of augmented reality is most promising in the treatment of tumours associated with bone structures[8]. However, the challenges for minimally invasive surgery are shifted to limited depth perception and haptic feedback, leading to a disconnection between the hand and eye[74].

With augmented reality, a patient-specific virtual model can be created for open or minimally invasive surgery to assist surgeons in maintaining 3D interpretations, as in robotic procedures[8,75].

It should be noted that none of the modalities described provide comprehensive medical information. As a result of improvements in conventional imaging modalities, the expectations placed on imaging systems have increased and none are without any limitations[76,77]. Hybrid or multimodal imaging is commonly employed in diagnostics (such as PET–CT or SPECT) to combine functional and anatomical information.

Is it necessary to know the signal intensity or amount of contrast agent in each cubic centimetre, or is having the signal intensity or amount of contrast agent in arbitrary units per pixel/voxel sufficient? Surgical decisions are generally based on visual interpretation of data, which gives only an impression and does not lead to quantitative results. What data are necessary for a particular medical/clinical outcome? Does improved clinical outcome rely on absolute numbers during surgery? Can these data be generated in sufficient time for the patient/surgeon? Most imaging modalities are unable to provide absolute quantification owing to noise, scattering and motion, or the absence of a standard. All ten modalities reviewed here allow relative quantification, assuming that the signals are independent of the position in the sample and no motion artefacts are present. Although absolute quantification is preferred, particularly in monitoring response to therapy, relative quantification is sufficient in practice and for most other indications. The future of medical imaging is in the transfer of images to data with a high negative power and a focus on sensitivity.

Finally, standardization is necessary to achieve reproducible and reliable information, which makes interinstitutional comparisons feasible and facilitates the implementation of new techniques from one site to another. Standardization is a prerequisite, especially in case of quantification. To achieve images that are intuitive to interpret, reproducibility and reliability are key parameters. Each modality requires technical standardization for both signal acquisition and image reconstruction, and to account for the biological factors associated with the contrast agent and patient heterogeneity. Technical factors can be standardized relatively easily with the use of standard operating protocols and an accurate quality assurance programme, including validated libraries or calibration curves for the contrast agent. As an example, the REMARK (REporting recommendations for tumour MARKer prognostic studies) study gave recommendations on how to report results about tumour markers in a standardized way for assessment of the quality and generalizability for further research[78]. A similar protocol should be developed for imaging and molecular modalities used in surgery.

Each imaging modality has its own strengths, and no single technique will be suitable for all surgical procedures and fields. Strict selection of modalities by cancer type and surgical requirements is required, as well as combining techniques in order to increase visualization and decrease noise. The range of available modalities at differing levels of development makes comparison necessarily qualitative. Eventually, standardization of data across the different imaging and molecular modalities will enable data to be compared in an equipollent manner.

Acknowledgements

The authors thank A. Chan, H. Nguyen, R. Heeren, A. Perkins and J. Lees for assisting with the manuscript; and Siemens Healthcare for providing the images shown in *Fig. 1*. This research was made possible with the support of a European Union H2020-MSCA-RISE grant (644373 – PRISAR) and by the Bas Mulder Award (UL2015-7665) from the Alpe d'Huzes Foundation/Dutch Cancer Society. The research performed at Maastricht MultiModal Molecular Imaging Institute was made possible with the support of the LINK programme of the Dutch Province of Limburg. The financial supporters did not have any influence on the collection, analysis or interpretation of the data.
Disclosure: The authors declare no conflict of interest.

References

1 Mondal SB, Gao S, Zhu N, Liang R, Gruev V, Achilefu S. Real-time fluorescence image-guided oncologic surgery. *Adv Cancer Res* 2014; **124**: 171–211.
2 Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 2008; **452**: 580–589.
3 Vahrmeijer AL, Hutteman M, van der Vorst JR, van de Velde CJ, Frangioni JV. Image-guided cancer surgery using near-infrared fluorescence. *Nat Rev Clin Oncol* 2013; **10**: 507–518.
4 de Boer E, Harlaar NJ, Taruttis A, Nagengast WB, Rosenthal EL, Ntziachristos V *et al.* Optical innovations in surgery. *Br J Surg* 2015; **102**: e56–e72.
5 Tempany CM, Jayender J, Kapur T, Bueno R, Golby A, Agar N *et al.* Multimodal imaging for improved diagnosis and treatment of cancers. *Cancer* 2015; **121**: 817–827.
6 Keereweer S, Van Driel PB, Snoeks TJ, Kerrebijn JD, Baatenburg de Jong RJ, Vahrmeijer AL *et al.* Optical image-guided cancer surgery: challenges and limitations. *Clin Cancer Res* 2013; **19**: 3745–3754.

7 Benckert C, Bruns C. The surgeon's contribution to image-guided oncology. *Viszeralmedizin* 2014; **30**: 232–236.
8 Nijmeh AD, Goodger NM, Hawkes D, Edwards PJ, McGurk M. Image-guided navigation in oral and maxillofacial surgery. *Br J Oral Maxillofac Surg* 2005; **43**: 294–302.
9 Rosenthal EL, Warram JM, Bland KI, Zinn KR. The status of contemporary image-guided modalities in oncologic surgery. *Ann Surg* 2015; **261**: 46–55.
10 Sanguinetti A, Lucchini R, Santoprete S, Bistoni G, Avenia S, Triola R *et al.* Surgical margins in breast-conserving therapy: current trends and future prospects. *Ann Ital Chir* 2013; **84**: 595–606.
11 Sarantopoulos A, Beziere N, Ntziachristos V. Optical and opto-acoustic interventional imaging. *Ann Biomed Eng* 2012; **40**: 346–366.
12 Grootendorst MR, Cariati M, Pinder S, Kothari A, Douek M, Kovacs T *et al.* Intraoperative assessment of tumor resection margins in breast-conserving surgery using 18F-FDG Cerenkov luminescence imaging – a first-in-human feasibility study. *J Nucl Med* 2017; **58**: 891–898.
13 Bugby SL, Lees JE, Ng AH, Alqahtani MS, Perkins AC. Investigation of an SFOV hybrid gamma camera for thyroid imaging. *Phys Med* 2016; **32**: 290–296.
14 Liszka BM, Rho HS, Yang Y, Lenferink ATM, Terstappen LWMM, Otto C. A microfluidic chip for high resolution Raman imaging of biological cells. *RSC Adv* 2015; **5**: 49 350–49 355.
15 Balog J, Sasi-Szabo L, Kinross J, Lewis MR, Muirhead LJ, Veselkov K *et al.* Intraoperative tissue identification using rapid evaporative ionization mass spectrometry. *Sci Transl Med* 2013; **5**: 194ra193.
16 Wu B, Warnock G, Zaiss M, Lin C, Chen M, Zhou Z *et al.* An overview of CEST MRI for non-MR physicists. *EJNMMI Phys* 2016; **3**: 19.
17 Seeram E. *Computed Tomography, Physical Principles, Clinical Applications, and Quality Control* (4th edn). Elsevier: St Louis, 2016.
18 Khokhlova TD, Haider Y, Hwang JH. Therapeutic potential of ultrasound microbubbles in gastrointestinal oncology: recent advances and future prospects. *Therap Adv Gastroenterol* 2015; **8**: 384–394.
19 Patel KS, Yao Y, Wang R, Carter BS, Chen CC. Intraoperative magnetic resonance imaging assessment of non-functioning pituitary adenomas during transsphenoidal surgery. *Pituitary* 2016; **19**: 222–231.
20 Tang R, Buckley JM, Fernandez L, Coopey S, Aftreth O, Michaelson J *et al.* Micro-computed tomography (Micro-CT): a novel approach for intraoperative breast cancer specimen imaging. *Breast Cancer Res Treat* 2013; **139**: 311–316.
21 Gupta M, Su LM. Current and evolving uses of optical coherence tomography in the genitourinary tract. *Curr Urol Rep* 2015; **16**: 15.
22 Buchfelder M, Schlaffer SM. Intraoperative magnetic resonance imaging during surgery for pituitary adenomas: pros and cons. *Endocrine* 2012; **42**: 483–495.
23 Moiyadi AV, Shetty P. Direct navigated 3D ultrasound for resection of brain tumors: a useful tool for intraoperative image guidance. *Neurosurg Focus* 2016; **40**: E5.
24 Prada F, Del Bene M, Moiraghi A, Casali C, Legnani FG, Saladino A *et al.* From grey scale B-mode to elastosonography: multimodal ultrasound imaging in meningioma surgery – pictorial essay and literature review. *Biomed Res Int* 2015; **2015**: 925729.
25 Ginat DT, Swearingen B, Curry W, Cahill D, Madsen J, Schaefer PW. 3 Tesla intraoperative MRI for brain tumor surgery. *J Magn Reson Imaging* 2014; **39**: 1357–1365.
26 Hosoda T, Takeuchi H, Hashimoto N, Kitai R, Arishima H, Kodera T *et al.* Usefulness of intraoperative computed tomography in surgery for low-grade gliomas: a comparative study between two series without and with intraoperative computed tomography. *Neurol Med Chir (Tokyo)* 2011; **51**: 490–495.
27 Joo I. The role of intraoperative ultrasonography in the diagnosis and management of focal hepatic lesions. *Ultrasonography* 2015; **34**: 246–257.
28 Cantisani V, Grazhdani H, Fioravanti C, Rosignuolo M, Calliada F, Messineo D *et al.* Liver metastases: contrast-enhanced ultrasound compared with computed tomography and magnetic resonance. *World J Gastroenterol* 2014; **20**: 9998–10 007.
29 Ji S, Fan X, Paulsen KD, Roberts DW, Mirza SK, Lollis SS. Intraoperative CT as a registration benchmark for intervertebral motion compensation in image-guided open spinal surgery. *Int J Comput Assist Radiol Surg* 2015; **10**: 2009–2020.
30 Sistrom CL, McKay NL. Costs, charges, and revenues for hospital diagnostic imaging procedures: differences by modality and hospital characteristics. *J Am Coll Radiol* 2005; **2**: 511–519.
31 Saini S, Seltzer SE, Bramson RT, Levine LA, Kelly P, Jordan PF *et al.* Technical cost of radiologic examinations: analysis across imaging modalities. *Radiology* 2000; **216**: 269–272.
32 Clayburgh DR, Byrd JK, Bonfili J, Duvvuri U. Intraoperative ultrasonography during transoral robotic surgery. *Ann Otol Rhinol Laryngol* 2016; **125**: 37–42.
33 Das S, Thorek DL, Grimm J. Cerenkov imaging. *Adv Cancer Res* 2014; **124**: 213–234.
34 Perkins AC, Lees JE. *Gamma Cameras for Interventional and Intraoperative Imaging*. CRC Press: Boca Raton, 2016.
35 King MT, Carpenter CM, Sun C, Ma X, Le QT, Sunwoo JB *et al.* β-Radioluminescence imaging: a comparative evaluation with Cerenkov luminescence imaging. *J Nucl Med* 2015; **56**: 1458–1464.
36 Herrmann K, Nieweg OE, Povoski SP. *Radioguided Surgery: Current Applications and Innovative Directions in Clinical Practice*. Springer: Cham, 2016.

37 Tsuchimochi M, Hayama K. Intraoperative gamma cameras for radioguided surgery: technical characteristics, performance parameters, and clinical applications. *Phys Med* 2013; **29**: 126–138.

38 Chi C, Du Y, Ye J, Kou D, Qiu J, Wang J *et al.* Intraoperative imaging-guided cancer surgery: from current fluorescence molecular imaging methods to future multi-modality imaging technology. *Theranostics* 2014; **4**: 1072–1084.

39 Pavlov V, Meyronet D, Meyer-Bisch V, Armoiry X, Pikul B, Dumot C *et al.* Intraoperative probe-based confocal laser endomicroscopy in surgery and stereotactic biopsy of low-grade and high-grade gliomas: a feasibility study in humans. *Neurosurgery* 2016; **79**: 604–612.

40 Kothapalli SR, Liu H, Liao JC, Cheng Z, Gambhir SS. Endoscopic imaging of Cerenkov luminescence. *Biomed Opt Express* 2012; **3**: 1215–1225.

41 Valdes PA, Roberts DW, Lu FK, Golby A. Optical technologies for intraoperative neurosurgical guidance. *Neurosurg Focus* 2016; **40**: E8.

42 Hollon T, Lewis S, Freudiger CW, Sunney Xie X, Orringer DA. Improving the accuracy of brain tumor surgery via Raman-based technology. *Neurosurg Focus* 2016; **40**: E9.

43 Boppart SA, Luo W, Marks DL, Singletary KW. Optical coherence tomography: feasibility for basic research and image-guided surgery of breast cancer. *Breast Cancer Res Treat* 2004; **84**: 85–97.

44 Erickson-Bhatt SJ, Nolan RM, Shemonski ND, Adie SG, Putney J, Darga D *et al.* Real-time imaging of the resection bed using a handheld probe to reduce incidence of microscopic positive margins in cancer surgery. *Cancer Res* 2015; **75**: 3706–3712.

45 Calligaris D, Norton I, Feldman DR, Ide JL, Dunn IF, Eberlin LS *et al.* Mass spectrometry imaging as a tool for surgical decision-making. *J Mass Spectrom* 2013; **48**: 1178–1187.

46 Peters IT, Stegehuis PL, Peek R, Boer FL, van Zwet EW, Eggermont J *et al.* Noninvasive detection of metastases and follicle density in ovarian tissue using full-field optical coherence tomography. *Clin Cancer Res* 2016; **22**: 5506–5513.

47 Santagata S, Eberlin LS, Norton I, Calligaris D, Feldman DR, Ide JL *et al.* Intraoperative mass spectrometry mapping of an onco-metabolite to guide brain tumor surgery. *Proc Natl Acad Sci U S A* 2014; **111**: 11 121–11 126.

48 Alexander J, Gildea L, Balog J, Speller A, McKenzie J, Muirhead L *et al.* A novel methodology for *in vivo* endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife. *Surg Endosc* 2017; **31**: 1361–1370.

49 Takats Z, Strittmatter N, McKenzie JS. Ambient mass spectrometry in cancer research. *Adv Cancer Res* 2017; **134**: 231–256.

50 Bauer NJ, Hendrikse F, March WF. *In vivo* confocal Raman spectroscopy of the human cornea. *Cornea* 1999; **18**: 483–488.

51 Krishnamurthy U, Neelavalli J, Mody S, Yeo L, Jella PK, Saleem S *et al.* MR imaging of the fetal brain at 1·5T and 3·0T field strengths: comparing specific absorption rate (SAR) and image quality. *J Perinat Med* 2015; **43**: 209–220.

52 Dula AN, Pawate S, Dortch RD, Barry RL, George-Durrett KM, Lyttle BD *et al.* Magnetic resonance imaging of the cervical spinal cord in multiple sclerosis at 7T. *Mult Scler* 2016; **22**: 320–328.

53 Torfeh T, Hammoud R, McGarry M, Al-Hammadi N, Perkins G. Development and validation of a novel large field of view phantom and a software module for the quality assurance of geometric distortion in magnetic resonance imaging. *Magn Reson Imaging* 2015; **33**: 939–949.

54 D'Andrea K, Dreyer J, Fahim DK. Utility of preoperative magnetic resonance imaging coregistered with intraoperative computed tomographic scan for the resection of complex tumors of the spine. *World Neurosurg* 2015; **84**: 1804–1815.

55 Liu J, Chen Z, Wang T, Liu L, Zhao L, Guo G *et al.* Influence of four radiotracers in PET/CT on diagnostic accuracy for prostate cancer: a bivariate random-effects meta-analysis. *Cell Physiol Biochem* 2016; **39**: 467–480.

56 Yamamoto S, Hamamura F, Watabe T, Ikeda H, Kanai Y, Watabe H *et al.* Development of a PET/Cerenkov-light hybrid imaging system. *Med Phys* 2014; **41**: 092504.

57 Grootendorst MR, Cariati M, Kothari A, Tuch DS, Purushotham A. Cerenkov luminescence imaging (CLI) for image-guided cancer surgery. *Clin Transl Imaging* 2016; **4**: 353–366.

58 He H, Wissmeyer G, Ovsepian SV, Buehler A, Ntziachristos V. Hybrid optical and acoustic resolution optoacoustic endoscopy. *Opt Lett* 2016; **41**: 2708–2710.

59 Lees JE, Bugby SL, Alqahtani MS, Jambi LK, Dawood NS, McKnight WR *et al.* A multimodality hybrid gamma–optical camera for intraoperative imaging. *Sensors (Basel)* 2017; **17**: E554.

60 Ma X, Yang W, Zhou S, Ma W, Hu Z, Liang J, Jing W. Study of penetration depth and resolution of Cerenkov luminescence emitted from ^{18}F-FDG and ^{131}I. *J Nucl Med* 2012; **53**(Suppl 1): 55.

61 Tzoumas S, Nunes A, Deliolanis NC, Ntziachristos V. Effects of multispectral excitation on the sensitivity of molecular optoacoustic imaging. *J Biophotonics* 2015; **8**: 629–637.

62 Tzoumas S, Nunes A, Olefir I, Stangl S, Symvoulidis P, Glasl S *et al.* Eigenspectra optoacoustic tomography achieves quantitative blood oxygenation imaging deep in tissues. *Nat Commun* 2016; **7**: 12121.

63 Brochu FM, Brunker J, Joseph J, Tomaszewski MR, Morscher S, Bohndiek SE. Towards quantitative evaluation of tissue absorption coefficients using light fluence correction in optoacoustic tomography. *IEEE Trans Med Imaging* 2016; **36**: 322–331.

64 Kircher MF, Willmann JK. Molecular body imaging: MR imaging, CT, and US. Part I. principles. *Radiology* 2012; **263**: 633–643.

65 Ghosh D, Bagley AF, Na YJ, Birrer MJ, Bhatia SN, Belcher AM. Deep, noninvasive imaging and surgical guidance of submillimeter tumors using targeted M13-stabilized single-walled carbon nanotubes. *Proc Natl Acad Sci U S A* 2014; **111**: 13 948–13 953.

66 Welsher K, Sherlock SP, Dai H. Deep-tissue anatomical imaging of mice using carbon nanotube fluorophores in the second near-infrared window. *Proc Natl Acad Sci U S A* 2011; **108**: 8943–8948.

67 Gao W, Wang Z, Lv L, Yin D, Chen D, Han Z *et al.* Photodynamic therapy induced enhancement of tumor vasculature permeability using an upconversion nanoconstruct for improved intratumoral nanoparticle delivery in deep tissues. *Theranostics* 2016; **6**: 1131–1144.

68 Ng AH, Blackshaw PE, Alqahtani MS, Jambi LK, Bugby SL, Lees JE *et al.* A novel compact small field of view hybrid gamma camera: first clinical results. *Nucl Med Commun* 2017; **38**: 729–736.

69 Balog J, Szaniszlo T, Schaefer KC, Denes J, Lopata A, Godorhazy L *et al.* Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Anal Chem* 2010; **82**: 7343–7350.

70 Jolesz FA, Kettenbach J, Grundfest WS. Cost-effectiveness of image-guided surgery. *Acad Radiol* 1998; **5**(Suppl 2): S428–S431.

71 Highsmith JT, Highsmith MJ, Monheit GD. Histologic accuracy of mohs micrographic surgery. *Dermatol Surg* 2017; [Epub ahead of print].

72 Tichauer KM, Wang Y, Pogue BW, Liu JT. Quantitative *in vivo* cell-surface receptor imaging in oncology: kinetic modeling and paired-agent principles from nuclear medicine and optical imaging. *Phys Med Biol* 2015; **60**: R239–R269.

73 Blasberg R, Piwnica-Worms D. Imaging: strategies, controversies, and opportunities. *Clin Cancer Res* 2012; **18**: 631–637.

74 Schostek S, Ho CN, Kalanovic D, Schurr MO. Artificial tactile sensing in minimally invasive surgery – a new technical approach. *Minim Invasive Ther Allied Technol* 2006; **15**: 296–304.

75 Hallet J, Soler L, Diana M, Mutter D, Baumert TF, Habersetzer F *et al.* Trans-thoracic minimally invasive liver resection guided by augmented reality. *J Am Coll Surg* 2015; **220**: e55–e60.

76 Culver J, Akers W, Achilefu S. Multimodality molecular imaging with combined optical and SPECT/PET modalities. *J Nucl Med* 2008; **49**: 169–172.

77 Garcia-Allende PB, Glatz J, Koch M, Ntziachristos V. Enriching the interventional vision of cancer with fluorescence and optoacoustic imaging. *J Nucl Med* 2013; **54**: 664–667.

78 McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, Statistics Subcommittee of the NCI–EORTC Working Group on Cancer Diagnostics. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer* 2005; **93**: 387–391.