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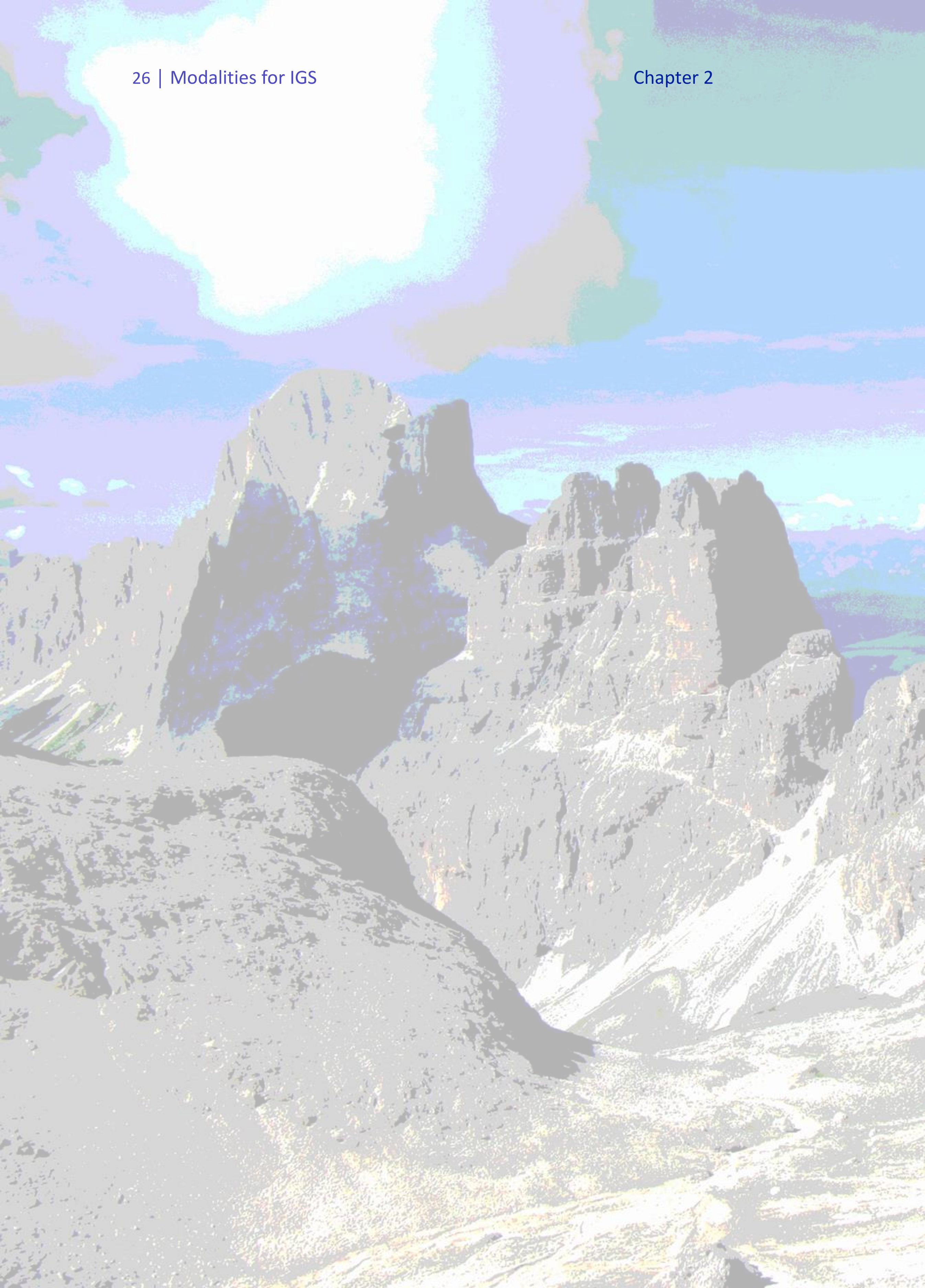
Title: Multimodal image-guided interventions using oncological biomarkers
Issue Date: 2018-05-22

Part I – Image-guided Surgery



Chapter 2

Modalities for image- and molecular-guided cancer surgery

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Adapted from: Modalities for image- and molecular-guided cancer surgery, Br J Surg 2018 Jan; 105(2): e69-e83.

Abstract

Purpose

Surgery is the cornerstone of treatment for many solid tumors. Whereas a wide variety of imaging modalities are available before surgery for staging, surgeons still primarily rely on visual and haptic cues in the operating environment. Image- and molecular-guidance might improve the adequacy of resection through enhanced tumor definition and detection of aberrant deposits. Available intra-operative modalities for image- and molecular-guided cancer surgery are reviewed here.

Procedures

Intra-operative cancer detection techniques were identified through a systematic literature search selecting peer-reviewed publications from January 2012 to January 2017. Modalities are reviewed, described and compared according to twenty-five pre-defined characteristics. To summarize the data in a comparable way, a three-point rating scale was applied to quantitative characteristics.

Results

Our search identified ten image- and molecular-guided surgery techniques, which can be divided in four different groups: conventional, optical, nuclear and endogenous reflectance modalities. The conventional modalities are the most well-known imaging modalities 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, however, their intra-operative use is limited by the use of radioactivity. The endogenous reflectance modalities provide high resolution, although with a narrow field of view.

Conclusions

Every modality has its own strengths and weaknesses, not one single modality will be suitable for all surgical procedures. Strict selection of modalities per cancer type and surgical requirements is required as well as combining modalities in order to find the most optimal balance.

Introduction

Over the last 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 our understanding of the diversity and behavior of cancer *in vivo* [2]. Despite these resources, surgeons still primarily rely on their eyes and hands as tools during surgeries [3-5]. In oncologic surgery, clean and clear demarcation of the tumor boundaries is pivotal to determine the balance between excising too little or too much tissue. Therefore, a careful examination of the tumor borders is essential [6,7]. Preoperative imaging does not always correlate well with intra-operative images due to tumor growth, deformation of soft tissue, shifting of organs or misalignment of the image display compared to the surgical field [8].

As Rosenthal et al. discussed for breast, melanoma and head-neck cancer patients, surgical excision requires 3 detection steps: initial assessment before resection; initial assessment during incision including detection of regional metastasis as well as lymph nodes; and post resection margin analysis by the pathologist[9]. Eyes and hands cannot detect the exact boundaries of a tumor or create a clear 3D morphologic or functional overview of the operative site [5]. As a result, histologic tumor involvement of the resection margins may be observed in patients with breast cancer at least 20% of the time [3,4,9,10]. In order to improve cure and complication rates, the use of intra-operative *in vivo* and real-time tools would be useful. To achieve this requires spatial resolution better the human eye, minimal interference with daily practice, operator friendly instrumentation that is time efficient [11]. To go beyond visualization of anatomic boundaries, real-time molecular information would provide additional information to optimize surgical resection.

The focus of this review was intra-operative modalities for image- and molecular-guided cancer surgery.

Methods

Twenty-five characteristics were selected to evaluate and compare the ten different IGS modalities reviewed here (**Table 1-6**). As Weissleder and Pittet state: "*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*" [2]. This statement refers to cancer imaging in general but the requirements apply equally well to image- and molecular-guided surgery in cancer patients [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, e.g., 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 (IGS) characteristics. These ratings are detailed as footnotes to the tabulated results. User friendliness was determined from discussions with end-users but differ from user to user, these were scored as easy (+), intermediate (–/+), or challenging (–).

Results

Our study identified ten modalities which could be used for image guidance during surgery. Example imaging systems for each modality, along with a representative clinical image, are visualized in **Figure 1-3**. In general, the modalities can be classified into four groups: conventional, optical, nuclear, and endogenous reflectance. Each modality is discussed below, and the characteristics of each are tabulated for comparison (conventional in **Table 1 & 4**, optical & nuclear in **Table 2 & 5** and endogenous reflectance in **Table 3 & 6** [9]).

Comparison between modalities

Modalities within each group are compared in tables below, and it is also possible to compare between groups (across multiple tables).

Table 1 provides information for conventional modalities already familiar to many practitioners, the imaging modalities are described along with the type of information that is obtained together with the surgical interference and associated risks. **Table 2** and **Table 3** provide the same information for optical and nuclear, and endogenous reflectance techniques respectively.

The same groupings are used for **Tables 4, 5 and 6**, which compare the performance of each modality during surgery, including the criteria that Weissleder and Pittet mention as being essential [2]. **Tables 4-6** additionally provide information about the clinical potential and major challenges for clinical implementation of each of the ten modalities.

Figure 4 provides a fast comparison of all ten modalities based on characteristics most interesting in clinical practice - penetration depth, resolution and acquisition. This clearly demonstrates a common trade off in image-guided surgery, a greater penetration depth often coincides with a degradation of resolution.

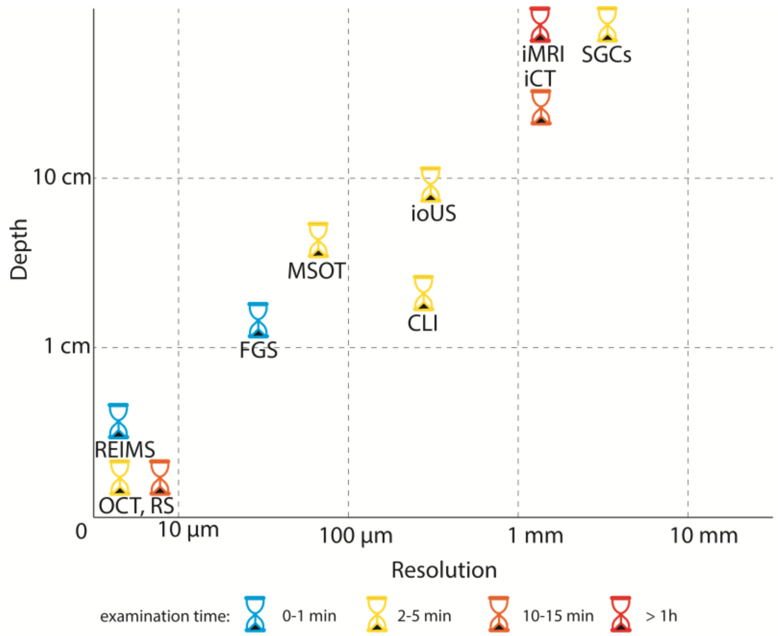

Figure 4: *Graphical representation of the IGS modalities, with respect to optimal resolution (x-axis) maximum penetration depth (y-axis) and average acquisition time (icon).*

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 golden standard consists of conventional imaging modalities that yield anatomical and macroscopic structure information. The images and information obtained with any new technologies must be compared with these established imaging modalities [35].

iMRI (intraoperative Magnetic Resonance Imaging)

To be able to use an MRI intraoperatively, MR compatibility of surgical equipment needs to be guaranteed together with special policies for safety and staff training. The implementation of these special policies can be prohibitively expensive although the costs are dependent on the field strength of the system. High field systems (>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 (< 0.3T) are cheaper since no additional requirements for the operating room (OR) are necessary and so they can be integrated into existing ORs[17]. Another advantage of using a low-field system is the availability of open systems, which is more useful during surgery. Nevertheless, the lower the field strength the lower the image quality or the longer the scan time [21,26].

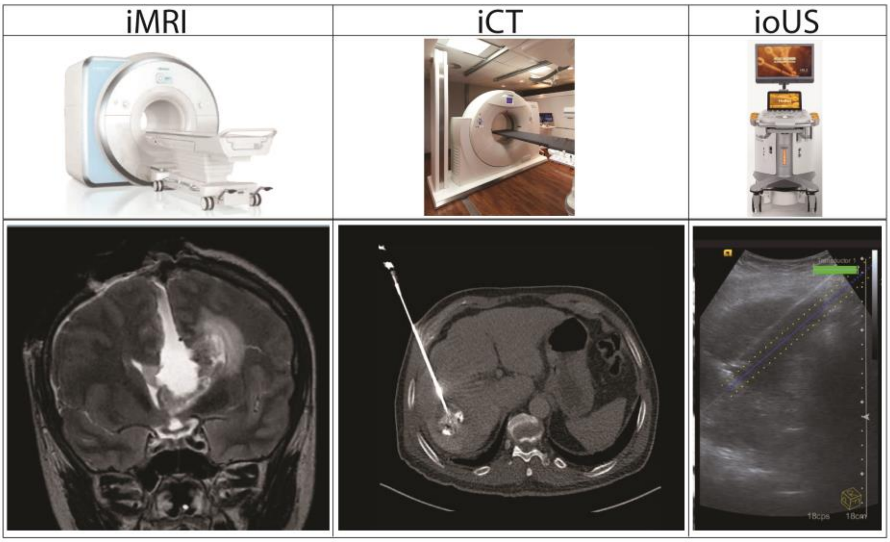

Figure 1: *Conventional image-guided surgery systems and examples of image output.*

The main reason to still make use of an MRI during surgery, despite these limitations, in neurosurgery it has been proven that the maximum amount of tumor could be removed in a safe manner [21].

iCT (intraoperative Computed Tomography)

In general CT offers high throughput with high-resolution imaging, however, this is not the case when used as an intraoperative imaging modality. Acquiring a CT during surgery takes 10-15 minutes, partly due to the interference caused by the shape of the gantry, as using a bore will cause more interference compared to a C-arm. When using the CT for assessing surgical specimens instead of the cavity, a micro-CT can be used in this way there is less interference of the surgery and a high spatial resolution of <1 µm. Nevertheless, the accuracy of margin assessment is variable due to specimen orientation and there can be a high rate of nonspecific findings due to dense parenchyma and architectural distortion due to the surgery [36].

ioUS (intraoperative Ultrasound)

Of the conventional imaging modalities ultrasound is the easiest technique to incorporate intraoperatively as it does not cause interference with surgery or logistical challenges, gives real time information and surgeons are already used to interpreting the images obtained. In addition, ioUS is one of the most sensitive imaging modalities for assessing small lesions due to the high frequency transducer which can be used. In addition to sensitivity, the specificity of discrimination between healthy tissue and residual disease is a benefit of this technique [25]. As ioUS can be used in an iterative mode one, should be aware of an essential drawback - surgical manipulation can cause artifacts so the image quality will decrease as the surgery proceeds [37].

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 which consists of a fluorophore belonging to the near infrared window (~700 nm to 900 nm) which has the largest penetration depth in tissue of optical light. In this window, penetration is one centimeter for FGS or a few centimeter with MSOT compared to only a couple of millimeters for wavelengths below 700 nm [3,4,6,19]. There is also a window above 900 nm, the so called second-window near infrared light (NIR2) ranging from 900 nm-1450 nm. This window has the advantages of even deeper tissue penetration and low tissue auto fluorescence signals which will lead to higher tumor to background ratios (TBRs). Animal study *in vivo* testing has shown a penetration depth of up to 18 mm, and simulations suggest that this might be increased to up to 10 cm [31-33]. To make use of this NIR2 window new instrumentation will be required. Specific probes for use in this range goes beyond the scope of this review, however single-walled carbon nanotubes or upconversion nanoparticles are encouraging opportunities [31-33].

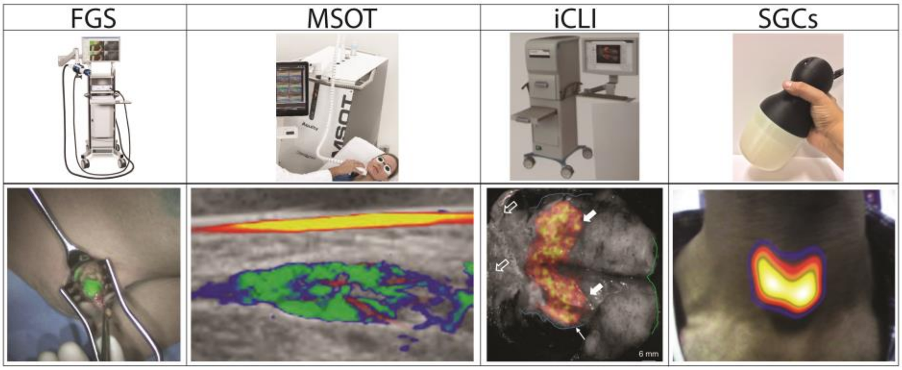

Figure 2: O*ptical and nuclear image-guided surgery systems and examples of image output SGCs* [1]*, iCLI* [2]*.*

FGS (Fluorescence Guided Surgery)

FGS has the advantage of providing real-time, relatively cheap, user friendly, and not interfering the surgical area. However, also several disadvantages exists, such as the limited penetration depth of maximum 10 mm and challenges in quantification due to other processes that are 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 (the sum of attenuation from absorption and scatter) has an exponential relationship with depth. This means practically that less than 0.0001% of the photons transmitted into tissue can be detected and that of this amount only 10-25% of the photons generated in tissue will be really recovered. This is due to the relatively small quantum yield of most fluorophores and especially NIR fluorophores. Another limitation for quantification are absorption and scatter as those characteristics are highly variable in tissue. Full correction, by measurements of the absorption, scatter and anisotropy of tissue, can lead to quantitative measurements, however this is still in its infancy [3]. Another limitation for a full clinical translation is the lack of specific contrast agents. So far only 3 tumor specific agents are registered for clinical use. Several tumor-specific agents are in the process of clinical translation however, there clinical translation is dependent on the approval of the fluorophore [3].

MSOT (Multispectral Optoacoustic Tomography)

In general, MSOT deals with the same advantages and disadvantages as FGS with the difference that MSOT has a greater penetration depth. In addition, both FGS and MSOT are based on photon delivery but in optoacoustic tomography low frequency ultrasonic pulses are also detected. Those pulses are generally unaffected by tissue absorption and scattering, essentially removing a large component of the limiting factor in 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 by determining or modeling the light propagation through the tissue, the concentration of a local chromophore can be determined. Work by both Tzoumas et al and Brochu et al has recently demonstrated that this result can be achieved both in phantoms and more importantly *in vivo*, giving a glimpse that quantitation in clinical optoacoustic tomography is a possibility [38-40].

Nuclear Imaging

Nuclear modalities use a radioactive tracer to generate images with, in general although dependent on the tracer of choice, a high sensitivity and specificity [34]. However, the use of radioactive material requires special biosafety permits, additional training, and safety procedures both for personnel and patients.

CLI (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 similar tissue penetration as optical imaging of only a centimeter. On the other hand, the advantage is that the resolution is also similar to optical imaging which means that this is higher than 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 room which makes it hardly suitable to use for open surgery, endoscopic applications would be favorable [41]. This low light level negatively influences the sensitivity which can be improved by injecting a higher amount of radioactivity. The amount of radioactivity is well correlated with the light output, radiance, though an increase in radioactivity will also lead to an increase in radiation burden.

SGCs (Small Gamma Cameras)

Gamma cameras, like single-photon emission computed tomography (SPECT) can be considered a conventional modality. However, those systems face similar drawbacks as MRI and CT in that the size and shape of the machine causes a lot of surgical interference and actually need a dedicated scanning room. To circumvent this, a handheld gamma probe is already used in clinical practice for sentinel lymph node detection. Although useful, these probes can only indicate the amount of activity within their field of view and do not have imaging capabilities. Innovative radiation detector design allow the generation of compact gamma cameras, small gamma cameras (SGCs) [42]. The differences between SPECT imaging and SGCs is that with gamma imaging the sensitivity is dependent on the tracer but independent of the depth of the tumor and for SGCs there is a tradeoff between sensitivity and spatial resolution dependent on the imaging distance. In addition, the field of view (FOV) is smaller but dependent on the detector design.

Endogenous reflectance

The last 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.

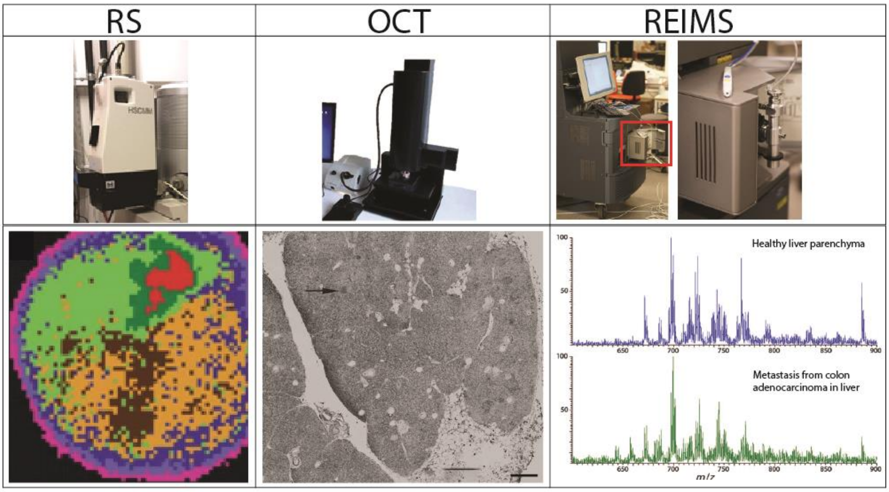

Figure 3:*Endogenous reflectance image-guided surgery systems and examples of image output. RS* [3], *REIMS* [5].

RS (Raman Spectroscopy)

In general, RS uses intrinsic properties of molecules to generate contrast which means RS 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 [22].

A possible limitation of translating RS into clinical practice is the question of how small fields of view could be applied to the validation of a tumor bed, which is relative large. A clinical trial using this technique has detected low-grade gliomas instead of the tumor bed. For this an image-resect-image technique was used in which the arm movement was predefined. This method led to an additional operation time of 10 minutes for image acquisition which was not considered obstructive to surgical workflow [22,30].

OCT (Optical Coherence Tomography)

OCT has the advantage of being analogous to US which makes the images easy to interpret for a surgeon as they are already used to those types of images. Instead of sound, OCT uses the reflections of light. This means that OCT does not need direct contact with the surgical area however, due to differences in refractive index direct contact is desirable [11,43,44]. Similarly to RS, OCT does not require a contrast agent but can use the same agents as used in optical imaging 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 [45].

REIMS (Rapid Evaporative Ionization Mass Spectrometry)

Intra-operative molecular diagnostics based on mass spectrometry have recently gained attention from the medical field as it offers the possibility of *in vivo*, *in situ*, and real-time mass spectrometric analysis of tissue [13,14]. In combination with electrosurgical devices [15], REIMS promises to guide and optimize surgical resection in real-time as it is performed within a couple of seconds. Within this timeframe, 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 keep this speed there is the need for validated tissue-specific databases which require time to generate and a large clinical cohort to account for inter-individual variability. It is expected that when this database is available any tissue can be analyzed [12,13,28]. In addition, complex molecular signatures can be identified which can increase the specificity over a single biomarker [12]. Although it is not truly an ‘imaging’ technique, REIMS has the potential to improve surgical margins by molecular sampling of them [16] comparable to Mohs surgery for skin cancer in which, during surgery, the removed specimen is examined for cancer cells [46].

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

	iMRI	iCT	ioUS
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[47].	X-rays pass through the patient, are attenuated and subsequently measured by detectors which rotate around the patient[48].	The US probe transmits ultrasound waves, which are (partially) reflected and/or scattered by tissue inhomogeneities and interfaces and sent back to the probe[49].
Type of information	Soft tissue discrimination and a multiplanar visualization[26].	Structural differences due to differences in absorbance[36, 48].	Contrast is based on scattering/ reflectance differences between different types of tissue: soft tissue, fat, and fluid[43, 49].
Anatomical information	Yes, what, is dependent on the sequence[17].	Yes[36].	Yes, although orientation is limited due to the different planes[27, 37].
2D/3D	3D.	3D.	2D, 3D with specialized transducers or a stacked 2D volume[37].
Need for contrast agent	Not necessary discrimination between two types of tissue can be improved[17, 21].	Not necessary discrimination between two types of tissue can be improved[23].	Not necessary discrimination between two types of tissue can be improved and real time vascular phase images[18, 25].
Cost machine&facility	€€€[17].	€€€[24].	€[27, 37].
Acq. Cost	€€€[50, 51].	€€[50, 51].	€[50, 51].
Time acquisition& reconstruction	Max 2h[21, 27], not real time.	10-15min[23, 27, 36], not real time.	Real time interactive information [25, 27]. Delay is dependent on the operator (max. a few minutes)[37].
Interference of surgery	Yes, highly interfering. Position wise maybe even impossible[17].	Yes, dependent on the modality and the possibility of a sliding gantry on a railtrack[23, 27].	Not in general and it gives no logistical challenges[37]. Yes, it needs direct contact with the specimen[4, 43].
Endoscopic options	No.	No.	Yes.
Safety	No, it can even detect complications in an earlier stage[17, 21, 26].	No complications or infections related to iCT and surgical complications were directly recognized[23].	Relatively safe and well tolerated[25]. It gives direct feedback to the surgeon[52].

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

	FGS	MSOT	CLI	SGCs
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 US. Molecules absorbing light undergo transient thermos-elastic expansion which generates US waves[11].	Charged particles emitted from radionuclides transfer energy as they move through a medium. If they travel faster than the speed of light, the transferred energy is released, through relaxation, as light [41].	Radionuclides introduced to the patient emit gamma radiation. Gamma photons have sufficient energy to pass relatively unimpeded through tissue to be detected by an external camera[53].
Type of information	Presence of a fluorophore or specific tissue properties in a certain area[6, 9].	The differences of optical absorption inside tissue is visualized[11].	Functional images based on the distribution of an externally administrated particle-emitting radiotracer[54].	Quantitative functional images based on the distribution of an externally administrated gamma-emitting radiotracer[55].
Anatomical information	No, however, autofluorescence provides information about tissue properties[9].	Yes, by strong endogenous absorbers like blood and melanin[11]. Interleaved with US images for mechanical contrast.	No.	No.
2D/3D	2D.	2D and 3D.	2D[41].	2D or 3D depending on the 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[41].	Yes, a gamma-emitting radiotracer.
Cost machine&facility	€[4].	€€.	€[41].	€€.
Acq. Cost	€.	€.	€€[51].	€€[50, 51].
Time acquisition& reconstruction	Real time, in the millisecond range, and related to the surgical field[3, 4].	Image generation is in real time. Possible to perform advanced analysis post process.	Several minutes[41, 53].	Depends on the amount of activity vs SNR. In general, one minute is sufficient [29, 53].
Interference of surgery	No, as there is no direct contact with the specimen as the optimal working distance is between 5-45cm[19]. A dark environment is beneficial[7].	Not in general but yes, it needs to be contact based, similar to ioUS.	Yes. Complete darkness is required for imaging. In addition, the use of radioactive tracers may have implications for working practice[41].	Not in general, there is no direct contact necessary with the specimen. However, the use of radioactive tracers, may have implications for working practice.
Endoscopic options	Yes, with the Cellvizio® system[56].	Yes.	Yes[57].	No.
Safety	NIR imaging is a safe technique only laser illumination levels needs attention [3].	Similar to US in technique, so relative safe. Only the direct contact can cause problems.	Radiation exposure for both patients and surgeons[4].	Radiation exposure for both patients and surgeons[4].

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

	RS	OCT	REIMS
Principle	Monochromatic light from 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[22, 30].	Is analogous to US, only reflections of near-infrared light are detected instead of sound [45, 58], the information is obtained by differences in reflected energy and scattering intensity [43].	An ambient ionization technique. Connected to an unmodified surgical handpiece, the system directly aspirates and analyses the smoke created by the electro-surgical 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[30].	Cross-sectional images are generated mimicking the intensity of optical backscatter of light passed through tissue[58].	The identification is based on tissue-specific libraries (molecular profiles or fingerprints) to identify the tissue type [12].
Anatomical information	Yes, dependent on the technique[30].	Yes[43, 58],tomographic images of biological tissue are generated (morphology)[11, 30, 44].	No, it is based on tissue-specific molecular signatures[28].
2D/3D	2D, 3D is possible by using stacked images or measure on a different depth.	2D and 3D depending on the detector used[45].	There is a spectrum generated and not an image[13, 15, 59].
Need for contrast agent	No, it is a label-free method as it uses intrinsic properties of molecules [22].	No, depends on the optical scattering and reflectance of tissue to generate contrast[30, 44, 45].	No, it is a label-free technique [13, 15, 59].
Cost machine&facility	€.	€.	€€[28].
Acq. Cost	€.	€.	€.
Time acquisition& reconstruction	Short (1-10 min) [30]. Spontaneous Raman scattering : acq time of 0.05 second for a single high quality spectrum. Coherent Raman imaging is faster (µs/pixel)[22, 30], with limited spectral quality.	Real time[11, 45], an image can be taken every 5s[43, 58]. Total acquisition time can be up to 5min[30, 44].	A couple of seconds (< 3s) [13, 28]. The use of a real-time recognition algorithm allows for rapid identification of tissue being analyzed.
Interference of surgery	There is no direct contact needed. [30].	Yes/No, does not require direct contact with the specimen [11, 43, 44], rapidly scan large areas of tissue[58].	No, no modification of surgical procedure is required[13]. The tissue which is measured is destroyed during this process but this is the tissue that the surgeon is cutting[12, 15].
Endoscopic options	Yes	Yes[43, 45, 58].	Yes [59].
Safety	Reasonable, due to the weak signal high levels of light energy and exposure times are often needed[60].	Relative safe, similar to ultrasound and fast image acquisition[43, 58].	Relative safe [12]. For the mass analyzer system, European norms have to be complied with.

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

	iMRI	iCT	ioUS
Resolution	Resolution around 0.3-1.3 mm[61, 62]. Improves with the scan time, and field strength[17, 21, 62].	Spatial resolution of 0.4-0.6mm[4, 27]. For micro-CT <1um[36].	High spatial and temporal resolution around 0.3-1mm[4, 25, 27, 43].
Field of View (FOV)	Up to 20cm, although the distortion increases with the FOV[63].	14cm[36].	Dependent on the transducer curved transducer > linear transducerin the range 10-60mm[4].
Iterative	Yes, but mostly one scan is obtained[21].	Yes.	Yes[37].
When to use during surgery.	Can be used for surgical (re-) orientation and as quality control of the resection cavity[17, 21, 26].	Can be used for surgical (re-) orientation and the micro-CT for lump margin assessment[23, 36].	Used for real-time surgical guidance in all stages[25, 37, 52].
Depth	Whole body.	Whole body.	Several cm[4].
Inter-operator variability	Medium [17].	Low.	High[18, 27, 52].
User friendly	-/+, depends on the familiarity of the surgeon with MR image interpretation[9].	+	+[27, 37, 52].
Availability	-/+, Due to the high price and requirement, limited [17].	+, For CT and limited use of Micro-CT[36].	++, Widely available[27, 37, 52].
Status Machine	Clinical.	Clinical.	Clinical.
Status contrast agent.	Non-tumor specific are available.	Non-tumor specific are available.	Three agents available in Europe[18, 27].
Quantification of size/ signal	Yes, absolute[2].	Yes, absolute[2].	Yes, absolute[2].
Cancer type	Neuro[4, 9, 17, 21, 26].	Lump margin assessment[36], neuro[23], spinal[20].	Abdomen[18, 25 9], H&N area[37, 52], breast[9], neuro[9].
Artifacts/ Limitations	-Vascularized tumors will lead to poorly visualized operation fields - hematomas that produces imaging artifacts[17].	-Dense parachyma and architectural distortion making margin assessment difficult[36]. -Bone anatomy is well visualized but limited on the lesion itself[20]. - Radiation exposure[24].	-Cirrhosis, can be improved by using CA[25]. -Steatosis (induced by chemo)[18]. - lack of anatomical orientation[37].
Sensitivity, specificity of the system.	Increases with the field strength[21, 26, 34].	. The specificity is > 90% but sensitivity only 60%[36].	Both the sensitivity and specificity are high[25].

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

	FGS	MSOT	CLI	SGCs
Resolution	10um[4], dependent on the camera system[19].	Dependent on the detector. Typically higher resolution is achieved with a depth trade off 15um till 3mm, 200-300um up to 3cm[11].	Fundamental spatial resolution limit of 0.3mm, further degraded by scattering in tissue[41, 64].	Spatial resolution can range from 3mm – 30mm[4, 29, 53].
Field of View (FOV)	Dependent on the camera system between 20-250mm[19].	Similar to ioUS, when the resolution increases the FOV is decreasing.	Typical endoscopic FOVs. An open field FOV is 80×80mm [65].	Dependent on the camera system, between 40-120mm is typical. Can vary with pinhole cameras [29, 53].
Iterative	Yes.	Yes.	Yes, though limited by the half-life of the radiotracer used.	Yes, though limited by the half-life of the radiotracer used.
When to use during surgery.	Used for tumor margin/SLN localization and quality control of the resection cavity[3,4,19].	Usage is similar to FGS but with more anatomical information[11, 66].	Mostly used for quality control of the resection cavity and lump assessment[41, 67].	Used for SLN detection, for surgical orientation and as quality control of the resection cavity[29, 68].
Depth	0.5-2cm[3, 4].	Several cm[4].	1 cm, dependent on the radiotracer used[41, 69].	No limit[4].
Inter-operator variability	Low.	Medium.	Low.	Low.
User friendly	+, NIR light does not alter the appearance of the surgical field [3, 30].	+	+, does not alter the appearance of the surgical field. -, Radiation burden. -, exclusion of all ambient light.	+, does not alter the appearance of the surgical field. - , Radiation burden.
Availability	+, Available[3].	-/+, Available in limited centers.	-/+, Available in limited centers.	+, Available.
Status Machine	Clinical[3, 19].	Clinical trials ongoing.	Clinical trials, some systems available for clinical use[41].	Some systems available for clinical use, some undergoing trials[53].
Status contrast agent.	Only 3 tumor a-specific CA are registered for clinical use[3].	Likely that the agents under investigation for FGS will also be studied for MSOT[4].	Clinically available for the available PET tracers and more tumor-specific tracers are in clinical development.	Clinically available for the available SPECT tracers more tumor-specific tracers are in clinical development.
Quantification of size/ signal	Relative, absorption and scatter limit the ability for absolute quantifcation[2].	Yes, via the amount of signal in an area[38-40].	Relative absorption and scatter limit the ability for absolute quantification[41].	Absolute or relative depending on camera design[2].
Cancer type	Primary tumor, lymph nodes, vascularization, metastases[3].	Hollow organs for endoscopic else all solid tumors[11].	Broad range of solid tumors.	For numerous cancer types; SLN, parathyroid, colon[29].
Artifacts/ Limitations	-Attenuation correction of the excitation light can help with target detection, over-compensation can cause false-positives[3]. - Penetration depth[3, 19].	Requires surface contact.	-High radiation burden. -Exclusion of ambient light is essential, endoscopic applications would be favorable[41]. - Scattering can cause signal to be visualized in the incorrect area[64].	-Radiation burden -Tradeoffs between dose, acquisition time, sensitivity, spatial resolution and FOV.
Sensitivity, specificity of the system.	Superficial tissue can be detected with a high sensitivity. Sensitivity is decreased with the depth[4, 19].	Nanomolar sensitivity with high specificity based on multispectral imaging.	- Lack of sensitivity due to low light levels [41]. - Specificity is dependent on the tracer [34].	- An increase in imaging distance degrades sensitivity and spatial resolution [29, 53]. - Are dependent on the system and tracer[34].

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

	RS	OCT	REIMS
Resolution	High, in the submicron range[22].	High, 1-15um, limited by the depth penetration which is, depending on the tissue, up to 5mm[11, 30, 43-45].	Not applicable, as it does not generate an image but a profile.
Field of View (FOV)	~ 0.1 mm[37], at highest far-field optical resolution [22, 30].	Around 1cm[2,30].	Around a surgical dissection rate of 1mm/s which leads to a FOV of 1mm[3 13].
Iterative	Yes[30]	Yes[43, 45, 58].	Yes, however not on the same piece of tissue[12, 28].
When to use during surgery.	Mostly used for quality control of the resection cavity and lump assessment[22].	Mostly used for quality control of the resection cavity and lump assessment[30, 45].	Mostly used for quality control of the resection cavity and lump assessment[12, 28].
Depth	Hundreds of micrometer [22, 30].	0.2cm[30, 43, 45, 58].	Not applicable/ limited.
Inter-operator variability	Low, when incorporated in a robotics system	High/medium[45].	Low as a reference library is used for feedback and tissue classification [13].
User friendly	+, when incorporated in a robotics system, otherwise low.	+[45]	+, does not change the procedure of electrosurgical dissections[13].
Availability	-/+, Available in limited number of centers.	-/+, Not in routine clinical use for surgery available for other approaches[11, 44, 45].	-/+, Available in limited centers for research purpose only.
Status Machine	Mostly *ex vivo* studies, only one study *in vivo* so far published[30].	Mostly ex vivo, *in vivo* clinical trials are needed[43]. Handheld probes are in development [11, 30].	Clinical research, mainly on *ex vivo* tissue and few papers reporting *in vivo* tissue analysis[12, 13, 28].
Quantification of size/ signal	Relative quantification[2]	Yes, absolute.	Only relative, comparison based on different molecular fingerprints from one tissue type to another[13].
Cancer type	Neurology[22, 30], gastrointestinal, bladder, cervical[22]	Bladder, prostate, kidney[43] breast[45, 58], melanoma, thyroid[45], ovarium[44].	All solid tumors like breast, liver, colorectal,brain[13]
Artifacts/ Limitations	- interrogate a small region of tissue, - SNR can be a limiting factor - the intrinsic weak signals can be partly solved by high quality instruments [22, 30].	-limited penetration depth[43]. -optical scattering and coherent speckle artifacts from cellular structures limits the visualization of small cells[58].	-The tissue needs to be disrupted for analysis and cannot be measured again[12]. -The need for validated tissue-specific databases[13].
Sensitivity, specificity of the system.	Accuracy, Sensitivity and Specificity >90% to distinguish normal brain from tumor invaded brain[22].	High, sensitivity rates between 80-100% could be found and specificity 60-100% [11, 30, 45].	High, >90% depends on the accuracy of the classification library

Discussion

Tumor removal is an incremental and iterative process so there should also be the possibility to obtain intra-operative images linked to those obtained by initial staging scans [12]. This may require merging of more than one modality. US is a well-established technique for interventional procedures but is rarely the choice for definitive staging. In comparison, single-photon emission computed tomography (SPECT) and positron emission tomography (PET) may be used to perform tumor staging but cannot be used during surgery due to size limits whereas portable SGCs may suffice [11]. For this purpose, a conventional anatomic technique (e.g. MRI, CT or US) can be combined with a biological imaging modality such as optical or nuclear imaging, with the use of a targeted tracer. Or else a technique used during surgery for (re-)orientation can be combined with a technique which is used for quality control of the resection cavity or lump assessment as mentioned in **Table 4-6**. Another option is the use of a technique with a high penetration depth but a somewhat lower resolution complementary to one of the imaging modalities of the endogenous reflectance group to compensate for the loss of resolution. Both options will lead to more complete overview of the actual situation in a patient. **Figure 4** visualizes the differences between the techniques in relation to depth, resolution and acquisition time [2,70]. One has to be aware that techniques which are further apart from each other in the figure may gain the most in combination. So far, the biggest challenge remains the fusion of the images generated by different techniques which can lead to a certain degree of uncertainties, the greater the distance between two modalities in **Figure 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 [71]. The overall goal of imaging is to provide a better outcome. It should be noted that a “better outcome” can be defined—and may often differ—from different perspectives, i.e., from the patient, surgeon, instrument manufacturer, and society [16]. In iMRI, for example, surgeons appreciate the fact that they have a better visualisation and a higher chance of a complete resection of the tumor but, in contrast, they prefer shorter procedure times and with the use of iMRI these can be increased up to two hours [21,27]. In addition, a reduction of complications, like tumor-bed hematoma formation may be achieved with iMRI detection [17,21]. From a manufacturing standpoint, iMRI is viewed as successful due to the reputation and competitive benefits from good system performance in an operating room [16].

For the imaging modalities discussed above, 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 most ideal situation. With augmented reality the imaging results are projected onto the operative field which allows the visualization of different types of images merged with each other. Those images can be obtained pre-operatively, which allow 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 tumors associated with bone structures [8]. However, the challenges for minimal invasive surgery are shifted to limited depth perception and haptic feedback leading to a disconnection between the hand and eye [72]. With augmented reality a patient-specific virtual model can be created for open or minimal invasive surgery to assist surgeons in maintaining 3D interpretations as in robotic procedures [8,73].

It should be noted that none of the modalities described provide comprehensive medical information. Due to improvements in conventional imaging modalities the expectations placed on imaging systems have increased and none of them are without any limitations [74,75]. Hybrid or multimodality imaging is commonly employed in diagnostics (e.g. PET-CT or SPECT) to combine functional and anatomical information.

Is it necessary to have the amount of signal intensity or contrast agent in each cubic centimeter or is the signal intensity/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 linear obtained results. What data is necessary for a particular medical/clinical outcome? Does an improved clinical outcome rely on absolute numbers during surgery? And can this data be generated in sufficient time for the patient/surgeon? Most imaging modalities are unable to provide absolute quantification due 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 therapy-response monitoring, 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. Especially in case of quantification, standardization is a prerequisite. To achieve images which 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 of the contrast agent and the heterogeneity of every patient. The technical factors can be standardized relatively easily with the use of standard operating protocols (SOPs) and an accurate quality assurance program, including validated libraries or calibration curves for the contrast agent. As an example, the REMARK study gave recommendations for how to report results about tumor 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.

In conclusion, every modality has its own strengths and no single modality will be suitable for all surgical procedures and fields. Strict selection of modalities per cancer type and surgical requirements is required as well as combining modalities in order to increase visibility 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.

Acknowledgment/Disclosure of funding

This research has been 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 M4I was made possible with the support of the LINK program of the Dutch Province of Limburg. The financial support for this study did not have any influence on the collection, analysis, and interpretation of the data as the authors declare that there is no conflict of interest. We are grateful for the assistance of Alan Chan, Hang Nguyen, Ron Heeren, Alan Perkins and John Lees with the manuscript. We thank Siemens Healthcare for providing the images shown in **Figure 1**.

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