Repurposing Molecular Imaging and Sensing for Cancer Image-Guided Surgery

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ABSTRACT

Gone are the days when medical imaging was used primarily to visualize anatomical structures. The emergence of molecular imaging, championed by radiolabeled fluorodeoxyglucose positron emission tomography ($^{18}$FDG PET) has expanded the information content derived from imaging to include pathophysiological and molecular processes. Cancer imaging, in particular, has leveraged advances in molecular imaging agents and technology to improve the accuracy of tumor detection, interrogate tumor heterogeneity, monitor treatment response, focus surgical resection, and enable image-guided biopsy. Surgeons are actively latching on to the incredible opportunities provided by medical imaging for preoperative planning, intraoperative guidance, and postoperative monitoring. From label-free techniques to enabling cancer-selective imaging agents, image-guided surgery provides surgical oncologists and interventional radiologists both macroscopic and microscopic views of cancer in the operating room. This review highlights the current state of molecular imaging and sensing approaches available for surgical guidance. Salient features of nuclear, optical, and multimodal approaches will be discussed, including their strengths, limitations and clinical applications. To address the increasing complexity and diversity of methods available today, this review provides a framework to identify a contrast mechanism, suitable modality, and device. Emerging low cost, portable, and user-friendly imaging systems make the case for adopting some of these technologies as the global standard of care in surgical practice.

NOTEWORTHY

1. Molecular image-guided surgery enhances real-time cancer detection and characterization.
2. A variety of contrast mechanisms highlights unique features of cancer.
3. Diverse imaging systems and contrast agents provide flexibility in the choices available to clinicians.
4. Standardization of imaging systems and reporting strategy will facilitate clinical adoption.
INTRODUCTION
Medical imaging is central to major advances witnessed in modern medicine, allowing visualization of the entire human body. With the advent of molecular imaging (MI), it is now possible to see beyond what is accessible to the naked eye, including metabolic and molecular processes. A combination of high-resolution anatomical and high-sensitivity MI allows accurate localization and in-depth interrogation of biomarker status. A notable achievement of imaging advances is the de-escalation in the need for exploratory surgery, an invasive process that is used to determine treatment planning. Until recently, there remained a dearth of information regarding the direct application of MI in the operating room (OR). Despite the availability of exquisite pre-operative images, surgeons relied on visual and tactile tissue assessment to inform surgical decisions partly due to tissue deformation that causes a significant mismatch between the visible tissue of interest and the noninvasive preoperative image. Improved adaptation of current imaging technologies in the operative room has led to a surge in image-guided surgery, particularly in surgical oncology. One of the first such integrated radiology-surgery system, Advanced Multimodality Image Guided Operating suite, was developed at Brigham and Women's Hospital in Boston (1). The platform allows collaborative medicine, where radiologists, surgeons, and oncologists can work as a team before, during, and after surgical interventions. This suite uses magnetic resonance imaging and positron emission tomography/computed tomography (PET/CT) systems as the imaging units. With recent efforts to miniaturize imaging systems and minimize the footprint in the OR, new label-free and molecular contrast-enhanced technologies for real-time intraoperative image-guided surgery have emerged (2-5). Whereas label-free approaches harness the intrinsic properties of tissue to delineate cancer from healthy tissue, contrast agent enhanced techniques amplify imaging signal in the tumor for identifying cancer boundaries.

Irrespective of the modality used, methods for intraoperative image guidance require a sensor device to capture the imaging signal. Spontaneously generated signals such as those from radionuclides do not require the additional input to produce signals, which is required for most optical (chromophore excitation) and magnetic resonance (radiofrequency perturbation) imaging methods. While spontaneous signals simplify the hardware design and improve signal quantification, the lack of control over when to generate the signal limits the depth of information that could be derived from these agents for accurate delineation of tumor boundaries. Although functional magnetic resonance imaging and other imaging systems are increasingly explored for intraoperative image guidance, we will limit this review to MI applications, focusing on optical and nuclear imaging modalities, which are widely used clinically for molecular image-guided surgery (MIGS; Supplemental Table 1). In the context of this review, we will discuss molecular imaging strategies, which cover the use of exogenous imaging agents to interrogate specific cancer biomarkers, and the sensing of endogenous or exogenous molecules that report the location or status of tumors for IGS. Nuclear imaging enables quantitative deep tissue imaging using radiotracers (3,4) and is adaptable to rapid assessment of shallow tissue in the OR. Optical imaging, however, does not interrogate deep tissue with useful spatial resolution but allows high-resolution real-time imaging at shallow depths using non-ionizing radiation (2-4). While each of these modalities can be used independently, their complementary features have led to the development of multimodal approaches that combine their unique strengths (6). We will highlight recent advances in MI modalities, contrast agents, and imaging devices that have been clinically evaluated for surgical guidance in human cancer patients and conclude with a framework to identify the most suitable intraoperative imaging approach to accomplish specific endpoints.
CONTRAST

Preoperative MI methods utilize multiple factors to uncover the presence of tumors, determine the stage of cancer, monitor treatment response, and identify residual tumors. Detecting large tumors using standard imaging methods is straightforward. However, recent advances in cancer biology have uncovered new molecular processes and imageable biomarkers that facilitate the interrogation of small tumors at the molecular level. Aided by minimally invasive biopsy and histologic validation, the goal of MI is not only to detect tumors but also to delineate malignant from benign lesions. These advances reduce the need for exploratory surgery and may improve the ability to perform more focused resections that remove less normal tissue. Instead, the task of surgical oncology is to remove tumors in an oncologically sound manner (i.e. removing the tumor and regional lymph nodes, when appropriate, to achieve a microscopically margin-negative resection). Key attributes for MI methods for surgery include a rapid display of tumors, identification of the tumor margins, detection of microscopic lesions, reproducibility, and assessment of the surgical cavity for the presence of residual tumors. How well these needs are met depends on the source of contrast between cancer and the surrounding tissue. Thus, a significant amount of effort in MI is focused on identifying the best contrast mechanism to achieve the above goals. Regardless of the source of contrast, the detected signal should improve the accuracy of cancer detection and resection without casting undue burden to the normal surgical procedure. The sources of imaging contrasts could be endogenous or exogenous to the patient (Figure 1).

< Insert Fig. 1 here>

Endogenous Contrast

Endogenous contrast consists of biomolecules that are differentially expressed in cancer compared to the surrounding tissue (3,4) (Supplemental Table 2). This mechanism is not available to nuclear imaging as the method relies on exogenously administered radioactivity for contrast. In comparison, optical and hybrid technologies can harness intrinsic signals to generate contrast and are the major beneficiaries of endogenous contrast (7-10). Many proteins play a major role in cancer survival, some of which can absorb, scatter, or emit light of specific wavelengths. This process can produce a variety of detectable imaging signals. Excitation of tissue with light of a specific wavelength range yields red-shifted autofluorescence for imaging tissue at shallow depths. Spectroscopic methods have been developed to detect specific features of some endogenous molecules. For example, Raman spectroscopy probes molecular bond vibrations by measuring inelastically scattered light, which reports molecular signatures of tissue. Similarly, diffuse reflectance spectroscopy leverages the ability of some biomolecules to scatter and absorb light in a wavelength-dependent manner to determine tissue composition and identify cancer. Another exciting method, optical coherence tomography, uses interference patterns generated by light scatter and absorption as it passes through tissue to identify tissue microstructure. Classic optical coherence tomography is not directly sensitive to molecular information in tissue, and thus a number of innovative approaches have been introduced to pair the high morphologic resolution with functional content (11). Although it has not gained acceptance for MIGS, it is only a matter of time before this powerful technique makes an impact in human surgery.

Some optical-based hybrid techniques such as photoacoustic imaging utilize molecular absorption by biological chromophores to induce localized pressure waves created by thermoelastic expansion and detected by ultrasound probes. These sources of contrast provide diverse signaling
mechanisms to improve the accuracy of cancer surgery. We will explore their applications in the surgical environment in the sections dedicated to each of these MI modalities.

Exogenous Contrast

Exogenous contrast agents are administered into the patient, typically via intravenous or intraperitoneal routes and accumulate in tumors for a few minutes or several days, depending on the intended application. The tumor uptake varies from a passive accumulation and the sensing of specific enzyme activity to the selective binding of upregulated proteins on cancer cells(1,2,12) (Supplemental Table 2). Lymph node-seeking agents such as radiocolloids are used to highlight sentinel lymph nodes as part of cancer staging (13). The multifunctionality of colloidal and nanoparticles is attractive for both active and passive targeting of tumors for image-guided surgery. The excitement about the targeting of imageable cancer biomarkers has stimulated interest in the development of many radiopharmaceuticals and fluorescent dye conjugates of tumor-targeting small molecules, peptides, and antibodies (12,14). In general, contrast agents that have short half-lives (minutes) may not be suitable for MIGS due to logistic reasons, including time from the imaging suite to the OR. An ideal agent for surgical guidance is expected to accumulate rapidly in tumors with high tumor-to-background contrast and be retained in this state for at least 4 hours. The ability to optimize the pharmacokinetics of small and medium-sized molecules such as peptides (15-17) and antibody fragments(18) makes them attractive for this purpose. Although some researchers have argued against the use of antibodies for imaging applications because of the long circulation time (up to 7 days), recent studies have leveraged the exceptional high tumor- associated receptors to identify small lesions (19). Moreover, with improved dosing regimens, these molecules can provide adequate tumor-to-background contrast within a few hours post-injection. To synergize the strengths of different imaging modalities, multimodal contrast agents are beginning to find their way into the OR (1,20). This trend bodes well for oncologic surgery, as the combination of deep tissue profiling to assess the extent of tumor infiltration and with highly sensitive detection schemes of superficial lesions will enable rapid identification of cancer boundaries. In the future, we expect the integration of endogenous with exogenous contrast mechanisms to further improve the accuracy of cancer surgery and real-time assessment of surgical margins. The clinical applications of these approaches are discussed further in the sections below.

DEVICES

Current standard of care requires surgeons to rely on visual inspection, palpation, and tactile evaluation to identify cancer. Image guidance would accelerate tumor identification and provide information about possible infiltration, thereby enhancing the ability to achieve a microscopically margin-negative (R0) resection with high reproducibility, ease of use, and enhancedthroughput. However, the evolving landscape of surgical precision and rigor requires the accurate and efficient removal of tumors with negative margins. These cells are typically microscopic, which are invisible to the naked eye. In some tumor types, intraoperative assessment of surgical margins further drives high contrast and quantitative invasive techniques to improve surgical outcomes. Sensing this opportunity, many research groups and companies are developing small footprint imaging systems for guiding oncologic surgery. Whereas gamma cameras are optimized for detecting radioactivity in the OR, detectors used in optical and ultrasound imaging are diverse, differing in the sensor and the wavelength ranges captured to the system configurations (Figure 2), which determine MIGS sensitivity, user experience, and clinical adoption potential.
<Insert Fig. 2 here>

**Standalone**

Standalone configurations are the most common design of MIGS systems (Supplemental Table 3). They typically use an articulating arm supporting illumination (in the case of optical) and detection hardware, with image-processing and display hardware integrated to a wheeled tower (19,21-23). Their fixed arm position simplifies image alignment, but only provides a top-down field of view that requires surgeons to mentally correlate the displayed molecular information to the anatomical region of interest (AROI). Remote image display also forces surgeons to transiently look away from the surgical bed, a subtle distraction that cumulatively prolongs surgery. To address these issues, systems that project molecular information directly on the AROI have been developed (24). The simple design and image display of this platform is attractive for MIGS, but the large size disrupts surgical workflow in the current form. Future miniaturization of the system will overcome this impediment and reduce cost.

**Handheld**

Handheld devices use miniature portable imaging hardware configuration that performs image-processing and display on a wheeled tower. Their design allows easier surgical workflow integration and access for hard to reach AROIs than fixed standalone systems. A variety of these systems are currently commercially available for MIGS (Supplemental Table 3). These devices are generally less expensive than the standalone systems and provide intuitive user experience (21,25-27). However, the system does not support hands-free surgery, implying that the operation will stop when used by the operating surgeon. Furthermore, when held by a surgical team member or fixed to an articulating arm, the image and surgeon’s field of view are mismatched. Familiarity with the system improves the ease of use and adoption.

**Wearable**

Wearable devices use miniaturized imaging detectors, powerful processors, and fast image-processing to display real-time molecular information to surgeons via head-mounted displays (HMDs). Direct projection of the molecular information to the surgeon’s field of view enables seamless integration to the intraoperative surgical workflow (28,29). HMDs are increasingly used for medical image visualization in mixed reality applications and their small size, low cost, and intuitive user experience make them very amenable for wide clinical adoption (Supplemental Table 3). When the illumination module is mounted on the HMD, the devices provide complete hands-free surgery, allowing the operating surgeon to have full control of the system during MIGS. Real-time display of the images in remote monitors enhances collaboration with a surgical team. Key considerations to enhance the user experience and wide adoption for HMDs include weight, size, and ergonomics.

**Minimally Invasive**

The rise and expansion of minimally invasive surgery (robotic and laparoscopic) has decreased the invasiveness and morbidity of surgery. The adaptation of imaging devices to minimally invasive platforms has been rapid and necessary to enhance imaging techniques in these procedures. Minimally invasive devices combine MI hardware with endoscopes to visualize and remove suspicious lesions inside the body (30-33). Traditional endoscopy uses white light to identify the location of tumors, but the integration of illumination modules and appropriate filters into existing or new endoscopes transforms the procedure into a theranostic platform for tumor detection, assessment, and removal.
Endoscopic surgeries are increasingly being adapted for MIGS (Supplemental Table 3). Current devices process and display images on a wheeled tower with an integrated or wall monitor. This remote analysis can distort data fidelity and decrease the detection sensitivity due to loss of signal. To overcome these challenges, newer devices incorporate sensors at the front end of the endoscope. The need to directly visualize anatomical structures during endoscopy has led to the development of computational depth perception approaches (34). However, co-registration of the image field of view with patient anatomy using preoperative structural images is complicated by tissue deformation and obtaining intraoperative structural images is challenging due to hardware complexity. To address these challenges, computational reconstruction of local 3D tissue structure has been developed for CT mapping (35). With the increasing adoption of minimally invasive surgery, there will be an increasing focus on this configuration for MIGS.

**Robotic and Remote**

Robotic systems use minimally invasive devices controlled by a surgeon through a robotic interface that controls multiple surgical ports and uses image data to guide surgical decisions (Supplemental Table 3). Such systems lend themselves naturally to remote guidance, making them popular for integration with other modalities (6,36,37). These devices are revolutionizing surgical care by improving the accuracy of tumor resection and extending the working lifespan of surgeons. They are also useful in low-resource settings by enabling remote surgical guidance. However, high cost and lengthy training time remain major limitations to wide clinical adoption.

**NUCLEAR IMAGING**

Palpable cancer is easy to detect in the OR, but some cancer types often present as non-palpable lesions. Radioguided surgery (RGS) was developed to overcome this problem (Figure 3). Not only is this technique capable of aiding tumor localization in the OR, but it also affords the opportunity to detect occult disease and sentinel lymph nodes using gamma probes or cameras (Supplemental Table 4) (38). Radiocolloid lymphoscintigraphy is the precursor to Gamma imaging RGS, serving as an alternative to guide wire localized breast surgery. In this technique, handheld scintillation probes provide an audible output when detecting low or medium energy (140 – 250 KeV) gamma rays from single-photon emission computed tomography (SPECT) radiotracers (39). RGS occult lesion localization (40) and sentinel lymph node biopsy (SLNB) (41,42) using handheld gamma probes is now the standard-of-care for breast cancer and melanoma surgery, with increasing adoption by other surgical specialties. Recently, handheld PET-probes capable of detecting high-energy (511 KeV) gamma rays have been evaluated for tumor detection (43). Lack of visual feedback in these probes led to the development of portable gamma cameras that have allowed the identification of additional SLNs, including metastatic SLNs that were missed by the conventional audio output gamma probe (44). Small field of view hybrid gamma-optical cameras (25) now allow dual-channel imaging and superimposed high-resolution nuclear-fluorescence image visualization (20). Freehand SPECT has also been used to guide SLNB during breast (45) and oral (46) cancer surgeries. When used in conjunction with cancer-targeting radiopharmaceuticals (14), RGS is an excellent method to rapidly identify cancer, guide tumor resection, and survey the cavity after bulk tumor removal to minimize residual disease. A considerable limitation of this approach is the regulatory hurdles to use and maintain radiopharmaceuticals, confining this technique to specialized surgical centers.

<Insert Fig. 3 here>
OPTICAL IMAGING

Optical imaging uses non-ionizing radiation to illuminate endogenous or exogenous sources of contrast and enables real-time, high-resolution MIGS (2-4). Many sources and mechanisms of contrast are available for optical imaging methods, leading to the development of different techniques for this modality (Figure 4). Below, we have highlighted the methods that are widely used in MIGS while acknowledging that other newer methods are equally valuable.

<Insert Fig. 4 here>

Fluorescence

**Autofluorescence.** Autofluorescence imaging takes advantage of naturally occurring fluorophores in the body, whose differential expression in healthy and cancerous tissue provides contrast for surgical guidance (Supplemental Table 5). Unlike methods that rely on exogenous contrast agents, the path to clinical use of autofluorescence-guided surgery is simpler, with instrument optimization and data analysis methods as the primary optimization task. Autofluorescence has seen strong application in detecting head and neck cancers, especially in the oral cavity by visualizing loss of signal in cancerous tissue due to altered metabolism(7). This principle enabled the use of a simple handheld autofluorescence imaging device to accurately detect oral cancer in a pilot human study with high accuracy (47). Autofluorescence-guided surgical resection of oral cancer in a large single-center trial detected occult precancerous lesions that were missed by the operating surgeon and significantly reduced the locoregional rate of recurrence in patients with high-grade and early stage oral cancer, compared to conventional surgery (48) (Figure 4). Two-channel autofluorescence of dihydronicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) has also been used to accurately delineate a boundary between oral cancer or precancerous lesions and healthy oral mucosa using NADH/FAD and redox ratio (49). An emerging application of autofluorescence is in lung cancer where differential NADH/FAD intensity ratio was observed in lung cancer versus normal tissue in preserved patient surgical samples (8). Autofluorescence video bronchoscopy has emerged as a new tool for lung cancer detection (50) and has been shown to reveal significantly larger tumor extent and influenced changes in treatment decisions in lung cancer patients when compared to conventional white light video bronchoscopy (51). This technique has been recently used for guiding surgical resection in lung cancer and was able to detect cancerous tissue with higher sensitivity compared to white light video bronchoscopy, influenced changes to surgical decisions and improved treatment outcomes (52,53). Autofluorescence of frozen brain tumor biopsy samples allowed a combination of three molecular ratios to distinguish glioblastoma from healthy control tissue with high sensitivity and specificity, which were further improved through a combination of fluorescence lifetime and with NADH/FAD and Porphyrin/NADH autofluorescence (54).

**Exogenous fluorescence.** Exogenous fluorescence imaging uses externally administered targeted fluorescent agents that accumulate in the AROI. The availability of a large selection of contrast agents (55) and devices (21) makes exogenous-MIGS widely available to clinicians (Supplemental Table 5). Its clinical impact was first demonstrated using 5-Aminolevulinic acid (5-ALA), which is a natural visible range (350-700 nm) fluorophore that accumulates in glioblastomas (56,57). 5-ALA-fluorescence MIGS increased progression-free survival in glioblastoma patients (58) and has become the standard-of-care across European countries. Acriflavine and its principal component proflavine have been used for oral cancer detection using high-resolution microendoscopy (59,60). Combination wide-field
autofluorescence imaging paired with profavine-enhanced high-resolution microendoscopy has enabled investigators to scan a wide area for suspicious lesions for loss of fluorescence, characteristic of dysplastic tissue, and then further examine nuclear-to-cytoplasmic ratio within a small area using high-resolution microendoscopy for added specificity in detecting oral neoplasia (61-63). Folate receptor-α conjugated fluorescein isothiocyanate was used to target epithelial ovarian cancers in human patients and targeted fluorescence in ovarian and breast cancer tissue was observed intraoperatively (64,65) and enabled the identification of ovarian cancer lesions not detectable by eye (65). It has also been successfully used for lung cancer identification intraoperatively (66). Fluorescein has also been used to guide resection of high-grade gliomas, where it was found to accumulate in glioma tissue (67). Visible dye conjugates for MIGS are advantageous because they are readily accessible, clinically translatable due to previous use in human patients, and the shallow penetration of light in this region allows visualization of lesions closer to the tissue surface without interference from uninvolved deep fluorescent tissue. However, the visible wavelength fluorophores did create false positive signals in both breast and ovarian cancer patients, caused by background tissue autofluorescence (65). To minimize confounding autofluorescence during MIGS, most researchers are now using NIR fluorescent dyes. Imaging in this wavelength region has the additional advantage of allowing the interrogation of deeper tissue than possible with visible dyes. Indocyanine green (ICG) is an FDA approved NIR fluorescent contrast agent that has been widely used for MIGS (55), especially for highly sensitive detection for SLNs comparable or better than standard-of-care radioactive and blue dye tracking (22,28,29) (Figure 4). A NIR folate receptor-targeted fluorophore OTL38 was recently tested in ovarian cancer patients and enabled an additional 29% of malignant lesions to be removed that were not identified by standard surgical practice (68). OTL38 also allowed highly sensitive intraoperative detection of malignant pulmonary nodules with very high sensitivity, including identification of nodules missed preoperatively (69). Development of multiple antibody-conjugated NIR dyes is underway, including epidermal growth factor receptor (EGFR)-targeting Cetuximab-IRDye800 which was safely visualized in head and neck cancer (70) and vascular endothelial growth factor-A targeting Bevacizumab-IRDye800CW which was safely visualized in breast cancer patients (71). Recently, ABY-029, an EGFR-targeted affibody conjugated to IRDye800 was reported developed for MIGS in humans (18) to take advantage of the smaller size, higher affinity and easier tissue clearance of affibodies, compared to antibodies that enable microdosing for in vivo image guidance. It is currently under investigation for first-in-human microdose studies in head and neck cancer, brain cancer and sarcoma patients, with initial reports indicating good correlation of ABY-029 fluorescence to EGFR expression (72). Additional tumor-targeted NIR fluorescent agents that are currently undergoing clinical evaluation are expected to receive regulatory approval soon (73) and may improve outcomes of oncologic surgery in centers that do not have easy access to specialist equipment, personnel, and radiotracer.

Raman Scattering

Raman scattering describes energy transfer between a photon and a vibrational mode in a molecule which results in a characteristic loss or gain of energy depending on the molecular bond and vibrational mode type. Plotting the energy difference between the incident light on the sample and the Raman scattered light creates a Raman spectrum, sometimes referred to as a “fingerprint”, with varying intensities across the spectrum based on molecular content. These information-rich spectra can be used to probe the endogenous molecular content of healthy and cancerous tissue, as well as exogenous contrast agents by comparing features such as intensity, peak width, and peak symmetry across
different regions of the spectrum. Raman spectroscopy has been successfully used to determine tissue pathology, identifying subtle changes in tissue composition in freshly excised brain tissue (74). Further application in pediatric brain cancers also demonstrated strong agreement between Raman and conventional pathology (75). The diverse sources of contrast for Raman spectroscopy allow the use of tissue-specific properties to determine which contrast source is most valuable. For example, emphasis on specific tissue Raman signatures has enabled the identification of ex vivo oral cancers (76,77), breast cancer margin assessment from in vivo (9) and ex vivo specimens (78,79), soft tissue sarcomas (80), and prostate characterization using specific Raman signatures during robotic prostate surgery (36) (Figure 4). Recently, extrinsic surface-enhanced Raman-scattering nanoparticles targeting human EGFR 2, estrogen receptor, EGFR, and CD44 antigen were successfully used for intraoperative margin assessment of excised breast tumors with high accuracy in under 15 minutes (81). Endogenous Raman imaging can take advantage of the intrinsic molecular contrast between healthy and cancerous tissue without the added barriers of translating exogenous agents; however intrinsic signal from soft tissue is very weak and requires expensive, sophisticated hardware for operation and long scanning times. Pairing Raman imaging with contrast agents allows the use of much simpler hardware while maintaining multiplexing capabilities to track multiple targets quickly. Raman-guided surgery is well poised for wide clinical adoption for intraoperative tumor margin assessment (Supplemental Table 5).

Diffuse Reflectance Spectroscopy

Diffuse reflectance spectroscopy uses specific wavelengths or a range of wavelengths to illuminate intrinsic biomolecules and uses the spectral information in the reflected light to obtain the absorption and scattering profile of the AROI. Due to altered tissue morphology in diseased tissue, reflectance spectroscopy can identify the AROI (Supplemental Table 5). Diffuse reflectance spectroscopy has been used for intraoperative breast cancer margin assessment (82-84) (Figure 4) and guiding colorectal cancer surgery (85). Hyperspectral imaging collects wide-field images at a range of wavelengths to estimate tissue properties and distinguish the AROI (10); it has been used for accurate identification of head and neck cancers in freshly excised specimens (86), intraoperative margin assessment in breast cancer (87), intraoperative detection of brain cancer (88) and to provide guidance during various gastrointestinal surgeries (89).

MULTIMODAL IMAGING

A simple MIGS system that is capable of localizing cancer preoperatively and aid cancer identification and accurate resection intraoperatively in real-time without the use of ionizing radiation is not currently available. For this reason, several researchers are developing a multimodal imaging platform that combines the strengths of two or more MI modalities to overcome the limitations of each modality and improve the accuracy of surgical guidance (Figure 5). In this context, multimodal imaging involves the use of more than one imaging method to acquire diverse data for integrated image analysis and information retrieval. A few such systems and methods are in different stages of clinical trials for MIGS (Supplemental Table 5).

<Insert Fig. 5 here>
Cerenkov Luminescence Imaging (CLI)

With the advent of highly sensitive imaging systems, it has become possible to capture the low radiance Cerenkov luminescence photons from PET radionuclides (Figure 5). The exciting opportunity to integrate noninvasive preoperative nuclear imaging with intraoperative localization of tumors using gamma or optical sensors could shorten the operating time, minimize patient discomfort, and improve surgical outcomes (Supplemental Table 6). PET radionuclides emit a broad spectrum of light with high intensity in the ultraviolet region that decreases at a rate of $1/\lambda^2$ into the visible and near-infrared wavelengths (300-900 nm). The effect is more noticeable in a dielectric medium where a charged particle travels faster than the speed of light (90). Most PET radiotracers can generate Cerenkov luminescence with maximum emission intensity at 350 nm, thus only allowing superficial imaging. Additionally, Cerenkov photons are ~1,000 times weaker than commonly used fluorophores and can only be imaged using long integration times with surgical lights turned off. CLI does not need an illumination light source, thereby enhancing the detection of the weak light from deep tissue similar to bioluminescence imaging. CLI has enabled tumor resection assessment in breast cancer patients (91) and endoscopic detection of gastrointestinal cancers (92). By leveraging the light from radionuclides used in standard of care, CLI does not add to the cost of radiopharmaceuticals. It is conceivable that the same system can be used for both preoperative and intraoperative imaging, particularly for shallow tumors. The high spatial resolution of the optical cameras could further report the extent of cancer heterogeneity, allowing the surgeon to provide additional information to a pathologist on special features of the lesion. Despite these advantages, the optical imaging sensors are optimized in the visible and NIR regions of light, where the photon counts for this technique is low. For an intraoperative device, it may be valuable to harvest the largely unused photons in the ultraviolet region via down conversion nanoparticles for improved CLI.

Photoacoustics

Photoacoustic imaging uses a short-pulsed laser to excite intrinsic or extrinsic absorbers which can undergo thermoelastic expansion after optical absorption and generate a pressure wave detectable by an ultrasound transducer (Figure 5). It combines high-resolution optical imaging with depth penetration of ultrasound (93,94). This technology has been used in the clinic for breast tumor margin assessment (93-96), SLN mapping and assessment of metastatic status in breast cancer (97), melanoma resection (98), and neurovascular bundle identification during prostate surgery (99) (Supplemental Table 6). Photoacoustic microscopy has also been used for label-free evaluation of breast tumor margins with images that were comparable with processed histology slides (96). Recent studies have extended intraoperative photoacoustic imaging to colon and ovarian cancers, yielding a wealth of information for determining whether a tumor is benign or malignant based on tumor-associated vascularity and hypoxia (100,101). The seamless interplay between contrast provided by endogenous and exogenous contrast sources further enhances the quality and content of information derived from this method. Furthermore, the rapid image processing features of new systems have boosted real-time image acquisition and display, which will accelerate clinical decisions in the OR. As newer handheld and cheaper systems continue to emerge, the full potential of this technology for image-guided surgery will be achieved. Still, the technology is more expensive than simple fluorescence MIGS systems. In addition, transmission of sound requires direct contact of the probe with tissue, a condition that may not be feasible in some surgical situations.
**Nuclear-Fluorescence**

Nuclear and fluorescence imaging methods have complementary properties to capture quantitative depth-independent nuclear information with high-resolution, real-time fluorescence images (102). This approach enables both diagnostic imaging for surgical planning and intraoperative surgical guidance (Figure 5). A variety of methods are available to achieve a complementary effect. An obvious case is to combine the exogenous radiotracers with endogenous tissue autofluorescence for presurgical or deep tissue imaging and real-time tumor boundary assessment, respectively, in the OR. Alternatively, a radiotracer can be mixed with a fluorescent molecule that targets the same tissue to achieve depth-independent cancer localization and real-time, high-resolution fluorescence-guided tumor resection. If both imaging agents are approved for clinical use, the path to clinical translation is less challenging than when new contrast agents are used (Supplemental Table 6). This method was first demonstrated using ICG-99mTc-nanocolloid for preoperative SPECT/CT, intraoperative gamma and NIR fluorescence imaging during laparoscopic SLN mapping in prostate (103,104), head and neck (104,105), melanoma (104,106), penile (104), and vulvar cancer (104) surgeries. It consistently detected more SLNs than blue dye tracking alone for a variety of cancers (104). While this method is effective for SNL identification and biopsy, the disparate biodistribution profiles of each agent may decrease the accuracy of co-localizing the images for tumor resection. A widely used approach to overcome this disparity is the conjugation of the radionuclide and the fluorescent dye to a tumor-targeting career. Recently, 111In-DOTA-girentuximab-IRDye800CW was successfully used for preoperative SPECT/CT-based tumor localization and intraoperative gamma-fluorescence imaging for tumor margin assessment of clear cell renal cell carcinoma (107). 68Ga-IRDye800CW-BBN has also allowed accurate glioblastoma detection and showed an excellent correlation between preoperative PET and intraoperative fluorescence signal localization (108). Multimodal imaging has also been adapted for robotic surgery using an innovative “drop-in” laparoscopic gamma detection that improved SNL detection sensitivity in prostate cancer patients (6). The future of this MIGS strategy is bright as more handheld or wearable devices capable of capturing both nuclear and optical signals become available, providing a single system for acquiring presurgical, intraoperative, and postsurgical images of AROI.

**Multimodal Optical**

Multimodal optical imaging combines optical modalities for accurate disease detection intraoperatively (Figure 5; Supplemental Table 6). Reflectance spectroscopy imaging was coupled with fluorescence polarization imaging for accurate delineation of basal cell carcinoma (109). Multimodal polarization, reflectance, and fluorescence imaging methods were also used for intraoperative detection of breast cancer (110). Trimodal optical imaging provided identification of oral cancer (111) using autofluorescence spectroscopy, diffuse reflectance spectroscopy, and light scattering spectroscopy. A combination of exogenous fluorescence and photoacoustic imaging has been used for intraoperative detection of pancreatic cancer (112). Multimodal optical approaches have a high potential for impact in intraoperative surgical guidance based on the simpler hardware, high resolution, and their ability to make use of many different sources of contrast depending upon the optical modality and contrast used. We expect multimodal optical approaches to continue impacting this field and enable multiplexed surgical guidance, including identification of tumors, vasculature, nerves, and other important anatomy simultaneously.
CONCLUSIONS

MIGS is already improving surgical outcomes through nuclear imaging modalities that have been the standard of care for several subspecialties. As optical imaging matures further, more optical modalities will be available to clinicians for use in their practice and research fueling the next wave of surgical innovation. Multimodal approaches may be expected to increase in adoption due to multidimensional information streams and potential combination with machine learning algorithms to gain new insights to deliver better patient care. MIGS surgery will continue to play a critical role as the state-of-the-art in surgical practice evolves in the future.

DISCLOSURE

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FIGURE 1. Sources of extrinsic and intrinsic contrast used in Molecular Image-Guided Surgery
FIGURE 2. Design configurations for Molecular Image-Guided Surgery imaging devices showing standalone (22), handheld (25), wearable (see arrow) (28), minimally invasive (30), and robotic systems (36).
FIGURE 3. Radio-Guided Surgery showing A) schematic representation and B) clinical example of gamma imaging-guided surgery (20).
FIGURE 4. Optical-Guided Surgery showing A) schematic representation and clinical examples of surgical guidance using B) autofluorescence imaging (48), C) exogenous fluorescence imaging (22), D) Raman scattering (36) and diffuse reflectance spectroscopy (84).
FIGURE 5. Multimodal guided surgery showing A) schematic representation and clinical examples of surgical guidance using B) Cerenkov luminescence imaging (91), photoacoustic imaging (97), nuclear-fluorescence imaging (105) and multimodal optical imaging (112).