Advanced Review

Image-guided surgery using multimodality strategy and molecular probes

Lei Xi[1] and Hubei Jiang[1,2]*

The ultimate goal of cancer surgery is to maximize the excision of tumorous tissue with minimal damage to the collateral normal tissues, reduce the postoperative recurrence, and improve the survival rate of patients. In order to locate tumor lesions, highlight tumor margins, visualize residual disease in the surgical wound, and map potential lymph node metastasis, various imaging techniques and molecular probes have been investigated to assist surgeons to perform more complete tumor resection. Combining imaging techniques with molecular probes is particularly promising as a new approach for image-guided surgery. Considering inherent limitations of different imaging techniques and insufficient sensitivity of nonspecific molecular probes, image-guided surgery with multimodality strategy and specific molecular probes appears to be an optimal choice. In this article, we briefly describe typical imaging techniques and molecular probes followed by a focused review on the current progress of multimodal image-guided surgery with specific molecular navigation. We also discuss optimal strategy that covers all stages of image-guided surgery including preoperative scanning of tumors, intraoperative inspection of surgical bed and postoperative care of patients.

How to cite this article:
WIREs Nanomed Nanobiotechnol 2015. doi: 10.1002/wnan.1352

INTRODUCTION

Imaging techniques are playing increasingly important roles in clinical cancer treatment, especially in cancer surgery. These imaging techniques include ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon-emission computed tomography (SPECT), and numerous novel optical imaging techniques.[1] Of all these modalities, optical imaging techniques open new possibilities and show promising potential in cancer care.[2] Coupled with molecular probes, these imaging techniques offer the ability of surgical molecular navigation, which allows for more complete tumor resection while preserving important structures.[3,4]

Today, molecular imaging strategies have been primarily associated with whole-body imaging techniques for cancer diagnosis and treatments.[3,4] The most successful and widely used is PET/CT where PET utilizes ^{18}F-fluorodeoxyglucose to depict the metabolic discrepancies between malignant and normal cells and CT provides anatomic structures.[5,6] For intraoperative applications, a hand-held PET detection probe has been developed to localize tumor lesions and identify margins in breast cancer surgery.[7] However, the low spatiotemporal resolution, ionization, and high cost present major limitations to PET.[7] Owing to its high resolution, MRI is gaining increasing attention for researchers to investigate effective molecular probes such as iron oxide nanoparticles (IONPs).[8] However, MRI is a preoperative scanning imaging technique and has restrictions in intraoperative applications. Optical imaging modalities reveal significant advantages in molecular imaging, and many

*Correspondence to: hjiang@bme.ufl.edu

[1]School of Physical Electronics, University of Electronic Science and Technology of China, Chengdu, China

[2]Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA

Conflict of interest: The authors have declared no conflicts of interest for this article.

molecular probes have been investigated for different optical imaging techniques including fluorescence imaging (FI), Raman spectroscopy, fluorescence molecular tomography (FMT), and photoacoustic imaging (PAI).[2,9] Most applications of optical imaging in clinical oncology lie in cancer surgery. While these optical imaging techniques possess various advantages, each has its own limitations. For example, FI and Raman spectroscopy have limited penetration depth, and the imaging field-of-view of PAI is limited.[10–13] In addition, in PAI, the transmission of high-frequency acoustic waves needs coupling medium that may increase the infection risk in open surgical area.[13]

An important growing area attracting considerable attention is the development of specific multifunctional nanoprobes, which allows the use of multimodal imaging approaches.[9] For example, Yang's group developed near-infrared (NIR) dye-labeled specific IONPs to enhance the contrast of both MRI and FI.[14,15] Gambhir's group reported multifunctional targeted nanoparticles that can serve as the contrast agent for MRI, Raman spectroscopy, and PAI.[16] Zhen's group constructed nano gold tripods labeled with radionuclide ^{64}Cu for dual PET and PAI.[17] Our group proposed and validated a new strategy of applying NIR dye-labeled specific IONPs for PAI, FMT, and FI during the resection of tumors.[12,13,18] This review outlines currently available molecular probes and imaging modalities for image-guided surgery and focuses on multimodal imaging approaches with the aid of specific multifunctional nanoprobes. In conclusion, we propose a new strategy covering all aspects of image-guided surgery including preoperative scanning of tumors, intraoperative inspection of surgical area and postoperative care of patients.

MOLECULAR PROBES

Molecular probes are the key for surgical molecular navigation. While numerous different molecular probes are available for image-guided surgery, in this review, special focus will be given to probes closely associated with intraoperative guidance for tumor resection and evaluation using FI, MRI, and PAI. Specifically, two different types of molecular probes are described: NIR fluorochromes for FI and metallic nanoparticles for PAI and MRI.

Targeting Strategies

Combining molecular probes with given tumor biomarkers will enable probes to specifically target certain cancer cells. The targeting strategy of molecular imaging can significantly improve the accuracy and sensitivity of image-guided surgery. Currently, there are two major targeting strategies: passive targeting and active targeting.[19,20]

The most widely used is passive targeting strategy that delivers the probes via enhanced permeability and retention (EPR) effect.[21] Indocyanine green (ICG) has been successfully used in clinical applications for *in vivo* mapping of the lymph nodes and detection of gliomas, liver cancers, and breast cancers.[22–25] The other example is 5-aminolevulinic acid (5-ALA) that has been demonstrated in cancer staging and resection of intracranial tumors, spinal meningioma, bladder tumors, and gastric cancers.[26–29] The efficiency of passive targeting strategy depends on both the properties of nanoprobes (dimension, surface modification, and circulation half-life) and tumors (leakiness of the neovasculature and the degree of tumor angiogenesis). Nevertheless, the use of EPR effect in clinical applications is restricted by the insufficient evaluation of the heterogeneity of this effect among different tumors.

The strategy of active targeting has the potential to improve the targeting efficiency of molecular probes to cancerous tissues. The active targeting strategy involves the conjugation of molecular probes with cancer targeting ligands such as small molecules, peptides, proteins, antibodies, affibodies, and aptamers.[20] The first clinical study of active targeting strategy is to use fluorescein isothiocyanate (FITC)-labeled folate to localize folate receptor in ovarian cancers.[30] Other active targeting strategies include but are not limited to: $\alpha v\beta 3$ integrin and vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and epithelial cell adhesion molecule (EpCAM).[31] An important active targeting strategy is to target activatable probes that are designed to be silent until they interact with targeted proteins, certain microenvironment, or reactive species in the tumor. One example of such active targeting strategy is enzymatic activation. The urokinase plasminogen activator (uPA), which can target its receptor in cell surface, is considered to be promising for detecting tumor margins. One representative study is to label NIR fluorescence dyes to the recombinant peptides of the amino terminal fragment (ATF) of the receptor-binding domain of the uPA for intraoperative mapping of breast cancer margins.[14] Another typical protease is matrix metalloproteinase (MMP), such as MMP2 and MMP9. Olson and colleagues have successfully performed preclinical evaluation of a specific molecular imaging bioconjugate composed of chlorotoxin (CTX) and Cy5.5. They found that the binding

of this bioconjugate to cancer cells was facilitated by MMP2. The mouse studies showed that CTX:Cy5.5 has the potential to fundamentally prove intraoperative detection and resection of malignancies.[32]

Fluorescent Probes

FI is currently the most popular imaging technique used in image-guided surgery. Conventional FI techniques use fluorochromes in the visible light spectrum from 400 to 600 nm. However, it suffers from a low penetration depth of a few millimeters because of high light absorption by biological chromospheres and a high level of background light/autofluorescence that results in a low signal to background ratio (SBR).[33] To obtain high sensitivity and deep penetration depth, wavelengths are best confined in the well-known first NIR window, 650–900 nm (NIR-I), which results in less absorption, low autofluorescence, high spatial resolution, and high sensitivity.[33] Organic small molecular fluorophores are the major source for synthesis of current NIR-I fluorochromes, such as boron-dipyrromethene dyes, crynine dyes, and their derivatives.[34,35] The only two Food and Drug Administration (FDA) approved NIR dyes for clinical use are ICG and methylene blue (MB). ICG and MB are nontoxic and can be excreted from human body rapidly. Hence, they have the potential to be used in image-guided surgery in the near future.

Recently, fluorophores that emit light within the second NIR window, 1000–1700 nm (NIR-II), have been reported to significantly improve the imaging quality of imaging-guided surgery. Fluorescence emission light in the NIR-II window has less autofluorescence interference, reduced photon scattering and absorption. Also, longer wavelengths provide deeper anatomic feature definition of tissues compared with NIR-I fluorochromes.[36,37] There are also some nanoparticle-based NIR fluorescence probes and quantum dots, applications of that will not be included in this review.

Metallic Nanoparticles

Metallic nanoparticles have been extensively utilized in molecular imaging for cancer diagnosis and treatments. Owing to the tunable optical absorption mechanism of gold nanoparticles, various nanoconstructs have been studied including gold nanorods, gold nanoshells, gold nanostars, gold nanocages, gold nanotripods, and nanovesicles.[38] For image-guided surgery, a modified gold nanoparticle, composed of a 60 nm gold core coated with the Raman molecular tag, silica protection layer, and Gd^3, has been used to map the margins of brain tumors during the resection through three imaging modalities (MRI, PAI, and Raman imaging).[16] In another study, gold nanocages conjugated with [Nle4, D-Phe7]-α-melanocyte-stimulating hormone were used for photoacoustic detection of melanomas, demonstrating the potential to clinically map the margins of skin cancer.[39] Besides gold nanoparticles, superparamagnetic IONPs, approved by the FDA for clinical applications, were intensively used to enhance the contrast in MRI for the detection of hepatic tumors and lymph node metastasis.[40,41] Recently, IONPs have been demonstrated in applications of nodal staging and surgical residual disease detection via PAI.[42] Although various metallic nanoparticles have been used in animal model and preclinical demonstrations of image-guided surgery, clinical use is still limited because of several fundamental obstacles such as the uncertain cytotoxicity of the heavy metal ingredients or surface-coated materials, poor biodistribution caused by reticuloendothelial system (RES) and high preparation expense.

The Delivery of Molecular Probes to the Tumor Site

For surgical molecular navigation, the specific molecular probes need to be delivered to the cancerous tissue and interact with the targets to realize successful binding. Before the nonspecific probes are cleared from the body, the binding probes must be retained in the targeted sites. However, many probes with characterization of low hydrophilicity tend to accumulate in the tumor site nonspecifically that will result in high background noise.[43] To minimize this effect, either suitable probes with less stickiness to cells or proper chemical modifications are required.

Generally, there are two major ways for the target-specific probes to be delivered to the cancerous tissues: systemic administration and local injection. Compared with local injection, systemic administration where the probes are delivered through intravenous injection has the ability to mark possible satellite lesions around the main tumor via whole-body circulation, although it will increase the risk of systemic side effect. With systemic administration, the abnormal neovascularization of tumors allows the probes to pass through the blood vessel walls by EPR effect, and then the probes encounter the extracellular matrix (ECM) tissues that surround the tumor cells. The only way for the probes to cross the ECM is by diffusion that may be prevented by high hydrostatic pressure and cause inhomogeneous diffusion of the probes. Once the probes successfully pass through the ECM, the interaction between the

probes and their targets will occur. Both the dose of the probes and the antigen expression level determine the delivery efficiency. However, it is difficult for the probes to be effectively delivered to the small tumor lesions, which are less than 2 mm in size, and hypovascular tumors because these tissue types have not experienced neoangiogenesis.[44,45] One possible solution to this challenge is to utilize MMPs, cathepsin cysteine proteases, or hypoxia markers in the development of new probes.[46–48] Another strategy is to relieve vessel compression by breaking the ECM scaffold.[49] Although local injection owns low delivery efficiency, sacrifices discrimination of deeper structures and may miss some satellite lesions, it is much safer and still widely used for intraoperative molecular imaging.

MOLECULAR IMAGING MODALITIES

Computed Tomography, Positron Emission Tomography, and Ultrasonography

CT is the most commonly used imaging technique in cancer diagnosis today due to its speed, high resolution, and cost-effectiveness. However, for molecular imaging, CT has some limitations. For instance, the contrast of soft tissue is limited without contrast agent, and to achieve sufficient sensitivity, a dose of several millimolar contrast agent concentrations is required, which is several orders greater than that needed in MRI and optical imaging techniques. Another limitation is the potential toxicity of the iodine-based CT contrast agents used in large amounts.[50] The development of nanomaterials, such as bismuth sulfide nanoparticles and their applications in atherosclerotic plaques and macrophage-rich organs, has revealed new possibilities for molecular CT imaging.[51,52] Currently, however, the application of molecular CT techniques in image-guided surgery is limited.

PET imaging, often used in combination with CT, is the only molecular imaging modality clinically used for the diagnosis and staging of cancer. It involves the detection of γ rays emitted by positron-emitting nuclide. One commonly used radionuclide, fludeoxyglucose (^{18}F-FDG), is sensitive to malignant cells via overexpressed glucose transporters of cancer cells.[53] ^{18}F-FDG works due to the elevated glucose consumption of malignant cancer cells in many cancer types such as lung, GI-tract, neck and head, ovarian, and breast.[54,55] Most recent research shows that intraoperative portable PET probe technology is promising for the localization of various tumors during the surgery.[7] However, the specificity of PET is somewhat limited by the characteristics of the molecular tracers because it targets other noncancerous tissues with an increased uptake of glucose. For instance, increased ^{18}F-FDG uptake occurs in inflammation and hyperplastic bone marrow. Also, the spatial resolution of PET is relatively low so it is difficult to use for accurate resection of primary tumor lesions and possible residual tumors.

US is a high spatiotemporal, nonionization, and low-cost imaging modality.[56] It is based on the detection of the reflected sound waves generated by single/multiple transducers. Microbubbles, a major US imaging probe, are gas–liquid emulsions with a 1–4 μm gas core that causes a high echogenic response and leads to a higher contrast in images. Microbubbles are often used to target markers on tumor vascular endothelial cells such as vascular endothelial growth factor receptor 2 (VEGFR2). Dual targeting microbubbles that can bind both VEGFR2 and $\alpha v\beta 3$ integrin to enhance the imaging contrast have also been developed.[57] The other major type of US imaging probes, nonmicrobubble molecular probes, consists of liquid or solid colloids. They have a size in the nanometer range and can exit vasculature through EPR effect. These probes can not only enhance the US imaging contrast but also carry drugs for cancer treatment.[58] Despite promising results of molecular US imaging and various intraoperative applications of conventional US imaging such as image-guided therapy and biopsy, the applications of molecular US imaging in image-guided surgery is limited.

Magnetic Resonance Imaging

The principle of MRI is based on creating a magnetic field surrounding the patients where the protons in tissue (i.e., hydrogen atoms) are used to generate signals that are processed into a reconstructed image of the whole body. MRI has high spatial resolution and ability to recover anatomical details of whole human body without exposure to radiation. Even without the use of molecular probes, new techniques such as diffusion-weighted (DW) MRI can provide functional information in cancer diagnosis.[59] For example, DW MRI shows the same accuracy as PET/CT in the detection of lung cancer metastases.[60] For molecular MRI imaging, there are two major contrast agents: superparamagnetic IONPs for T2-weighted images and Gadolinium-based (Gd-based) molecular probes for T1-weighted images.[8,61] IONPs have a polymer coat that may be modified to be conjugated with specific targeting ligands such as uPA and HER2.[14,15] IONPs have been used for molecular MRI in various types of cancers such as prostate, ovarian, and breast.[14,15] However, IONPs are negative contrast agents that reduce the signals of target structures. This

disadvantage limits their use in low signal regions of the body. Recent advances reported several Gd-based probes that can be activated by enzymatic cleavage such as MMPs associated with the invasiveness of cancer cells.[62] Even though molecular MRI techniques are mostly used in cancer detection and diagnosis, their applications in image-guided surgery are focused on preoperative scanning.

Optical Imaging

Optical imaging encompasses various imaging modalities including autofluorescence imaging, FI using visible or NIR light, optical coherence tomography (OCT), diffuse optical tomography (DOT), FMT, Raman imaging, two-photon microscopy, super-resolution microscopy, and PAI that is a hybrid imaging modality combining optical excitation and ultrasound emission. Among these, NIR FI, Raman imaging, and PAI have demonstrated potential application in image-guide surgery for various cancers.

Over the past decades, along with the rapid development of NIR fluorophores and nanomaterials, the concept of using NIR light has played a crucial role in intraoperative image-guided surgery. In the operating room, tumor-specific signals of NIR FI provide significant discrimination from the surrounding normal tissues. FI has inherent advantages that make it the most suitable and widely used technique for surgical molecular navigation. It is a nonionizing, high spatial resolution, real-time modality. It is noncontact and can cover a large field-of-view, so it is particularly suited to inspect the open surgical wound during the surgery. Also, it is easily integrated with other imaging modalities to achieve multimodality strategies. However, the FDA has not approved any tumor-specific NIR molecular probes for clinical use. Fluorescence-Assisted Resection and Exploration (FLARE)/Mini FLARE developed by Harvard Medical School is one common NIR fluorescence image-guide surgery system.[63] Recently, Mini FLARE has been successfully used in breast cancer surgery with intravenous injection of ICG to map sentinel lymph nodes (SLNs). In this study, a total of 35 SLNs were detected and the optimal dose of ICG adsorbed to human serum albumin (ICG:HSA) ranged from 400 to 800 μM without any adverse reactions. Until now, only nonspecific fluorescent probes such as ICG and fluorescein are FDA-approved for clinical use. For targeted-specific fluorescence probes, only one clinical trial has been reported that used folate receptor-α (FR-α) as the target and folate FITC as the probe to intraoperatively guide the resection of human ovarian cancer.[30] From this clinical trial, it appears that folate FITC is safe as an intravenously injected molecular probe for image-guided surgery in humans. In this study, the surgeon found numerous tumor lesions that were invisible to white light camera or naked eyes. This study shows the potential of image-guided surgery with specific molecular probe to improve the complete resection of human ovarian cancers and significantly reduce the recurrence rate (Figure 1(a)). Although ICG has been approved by FDA for clinical use and demonstrated its safety via 30-year human trials, the conjugation chemistry of ICG is difficult because of its amphophilicity and few functional groups. To solve this problem, new idea was proposed to combine ICG with FDA-approved monoclonal antibodies (mAb) such as daclizimab, panitumumab, and trastuzumab, and develop ICG-mAb conjugates as activatable *in vivo* molecular imaging probes.[64] Despite this exciting study, clinically usable molecular fluorescent probes are still limited and further efforts are required to promote the translational applications. Besides the detection of primary tumors, another potential benefit of NIR FI is to detect residual disease and potential metastatic lesions. Our group reported the use of uPA receptor-targeted NIR dye and NIR FI to inspect surgical wounds and detect possible residual disease.[13–15] From animal experiments, we found that mice with surgical molecular navigation had lower local recurrence and higher survival rate compared to mice with conventional surgery. In a separate study, IntegriSense 680 (VisEn Medical, Woburn, MA, USA) has been used to detect colorectal liver metastases and mesenteric metastases during the surgery by targeted imaging of integrin $\alpha v\beta 3$ expression.[65] In a lumpectomy, the distance between the margin of the tumor and surrounding tissue is critical to evaluate the surgical outcome. If the border of the tumor mass is more than 2 mm away from the surrounding normal tissue, it is considered to be a negative margin. Otherwise, the woman requires additional, sometimes multiple, operations or re-excisions. Akrotome Imaging Inc. developed a compact molecular probe platform that combined FI with specific NIR probe to detect tumor margins with 90% sensitivity and specificity (http://www.akrotome.com/home.html). Although NIR FI has various advantages, the biggest challenge is the limited penetration depth that is normally less than 5 mm and the dramatic decrease in spatial resolution with increasing penetration depth.

The principle of Raman spectroscopy is based on the shift of frequency caused by the interaction between excitation photons and target tissues. The vibrational frequencies of the biomolecules result in changes in the emergent light that shift the emission wavelength above and below the wavelength of the

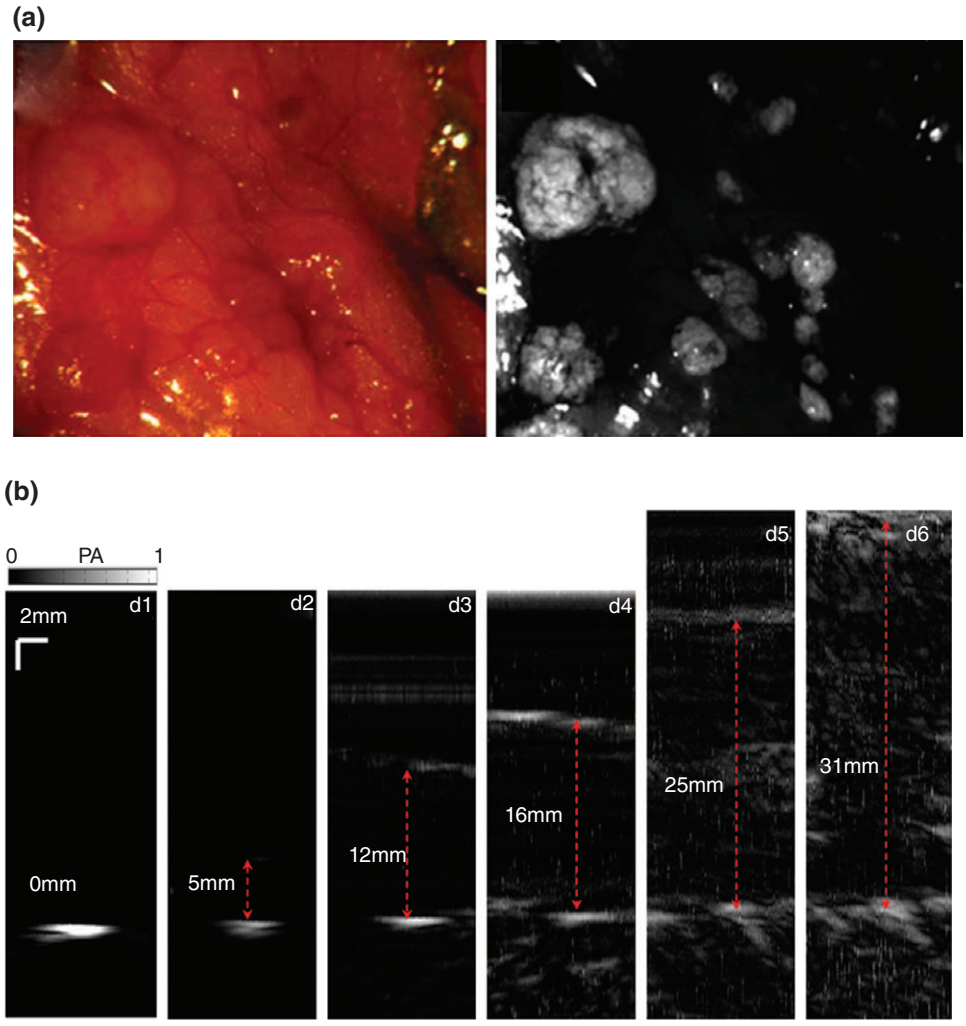

FIGURE 1 | (a) The first clinical trial of fluorescence image-guided surgery in ovarian cancer. Color image of abdominal cavity (left) and the corresponding fluorescence image (right). (Reprinted with permission from Ref 30. Copyright 2011 Nature Publishing Group) (b) *In vivo* photoacoustic images of breast tumor lesions with different thickness (5, 12, 16, 25, and 31 mm) of chicken breast added between the detector and tumor at 24 h post the injection of near-infrared (NIR) labeled iron oxide nanoparticles (IONPs) (Reprinted with permission from Ref 18. Copyright 2014 John Wiley & Sons)

excitation light. The Raman spectrum is independent of the optical properties of the tissue, and it does not require the use of dyes, nanoparticles, or other contrast agents. It has been successfully used to identify cancerous tissues from normal surrounding tissues in skin, mucosal surfaces, and solid organs.[66] However, the intrinsic contrast of Raman spectroscopy is quite low that limits clinical applications. With the development of nanomaterials, various surface-enhanced Raman spectroscopy (SERS) nanoprobes have been developed to increase Raman spectroscopy contrast capabilities. In addition, several optical fiber-based Raman probes have been investigated to assess specific tissues especially cancerous tissue *in vivo*.[67] In one study, a hand-held spectroscopic device was demonstrated for *in vivo* intraoperative tumor detection in small animals.[11] Both the primary tumor and the potential residual disease in the surgical wound were targeted using ICG via EPR effect. After the resection of the primary tumor guided by FI, the spectroscopic device was used to detect the residual tumor lesions. The results showed that the sensitivity of spectroscopic device was significantly higher than that of conventional NIR FI. Unfortunately, Raman spectroscopy has even lower penetration depth than FI. This inherent challenge may ultimately limit its potential clinical applications.

PAI, probably the most rapidly developed hybrid imaging modality in the past decade, is an imaging technique that detects wide-band acoustic waves

generated by tissue absorption of ultra-short laser pulses. It has the advantages of rich optical contrast as well as increased ratio of imaging depth to spatial resolution. Also, the resolution and maximum penetration depth can be adjusted with different ultrasonic frequencies. In PAI, the optical-to-acoustic conversion efficiency in the generation of acoustic waves determines the imaging contrast. Our group has developed a miniaturized PAI system for intraoperative detection of tumor margins using a small animal model of surgery.[68] It was found that the error between the margin by PAI and the true margin from histology was less than 10%. However, because of limited intrinsic photoacoustic contrast of cancerous tissue and relatively small field-of-view used, the imaging depth was less than 3 mm. Photoacoustic signal from tumor is typically two to four times higher compared to the healthy tissue. In order to enhance the photoacoustic contrast, several specific and nonspecific contrast agents have been used to generate acoustic transients for applications in the detection of lymph nodes, melanomas, angiogenesis, the cerebral cortex, and brain tumors. A study by our group has indicated the potential of the combination of PAI and receptor-targeted NIR dye-labeled uPA receptor-targeted IONPs as a clinical tool for image-guided surgery. In this study, it was found that systemic delivery of the specific nanoprobe produced 10-fold enhancement in photoacoustic signals in the tumor and allowed imaging of tumors located 3.1 cm beneath the surface[18] (Figure 1(b)). In another study, a lymph node located 8 mm under the surface was detected with high spatial resolution through the use of FDA-approved nonspecific ICG.[69] However, there is currently no FDA-approved specific nanoprobe for clinical use in PAI or other optical imaging methods.

MULTIMODALITY IMAGING

While MRI, CT, and PET offer whole-body imaging ability because of the unlimited penetration depth, it is challenging to develop their compact and real-time models required for intraoperative guidance of surgery. While NIR FI and Raman spectroscopy are capable of real-time imaging, they have limited penetration depth and cannot provide volumetric or depth information. While US and PAI provide three-dimensional (3D)images, they may not be suitable for use in surgical wounds because the requirement of coupling medium may lead to the risk of infection. In image-guided surgery, the first stage is to gain preoperative information of primary tumor and/or metastatic lesions such as sizes, locations, and even margins where whole-body molecular imaging modalities are required. In the operating room, a real-time, portable imaging system with sufficient imaging depth is preferred to guide the resection of the primary tumors and examine the associated lymph nodes. After the first resection, a noncontact imaging modality with high temporal resolution and large field-of-view is required to detect potential residual disease in the surgical wound. In addition, the margins of the resected tumor lesions need to be confirmed to ensure the success of the surgery. After the surgery, postoperative follow-up imaging is required to monitor the possible recurrence. It is apparent that not a single imaging modality is able to cover all of these stages. A possible solution is to develop multimodality imaging approaches that have their own features and advantages in different stages. Multimodal molecular probes can be achieved by either mixing two or more separate chemical entities or exploiting a single entity that owns multifunctionality.

Hybrid Nuclear and Fluorescence Imaging

The integration of nuclear and FI combines the advantages of both radioguidance and FI and coordinates preoperative whole-body molecular screening diagnosis with intraoperaitive guidance of surgery. The characteristics of high sensitivity and unlimited penetration depth of PET (and SPECT) complement the limited penetration depth of NIR FI. Most hybrid nuclear and FI employs dual-labeled NIR/PET (and SPECT) imaging probes such as ICG-99 m Tc-nanocolloid that has been successfully applied in SLN biopsy of 65 patients with penile cancer.[70] In that study, after the administration of imaging probes, SPECT/CT was used to map the SLN before the surgery and a γ camera and NIR FI system were utilized to confirm the resection of SLN postsurgery (Figure 2). The results showed that FI enabled a clear visualization of the borders of the SLN. In other preclinical studies using animal models, specific dual-labeled NIR/PET (SPECT) agents have been used such as $(^{64}\text{Cu-DOTA})_n$-trastuzumab-$(\text{IRDye 800CW})_m$ in HER2 positive breast cancer and $(\text{DOTA})_n$–anti-EpCAM–$(\text{IRDye 800})_m$ for prostate cancer.[71,72]

Hybrid Magnetic Resonance and Fluorescence Imaging

Compared to nuclear imaging such as PET and SPECT, MRI can provide higher spatial resolution structural information. Also, it is possible to synthesize a dual modality probe for both MRI and FI. One dual modality probe was prepared by conjugating HER-2 affibodies with NIR-830 dye and IONPs to enhance

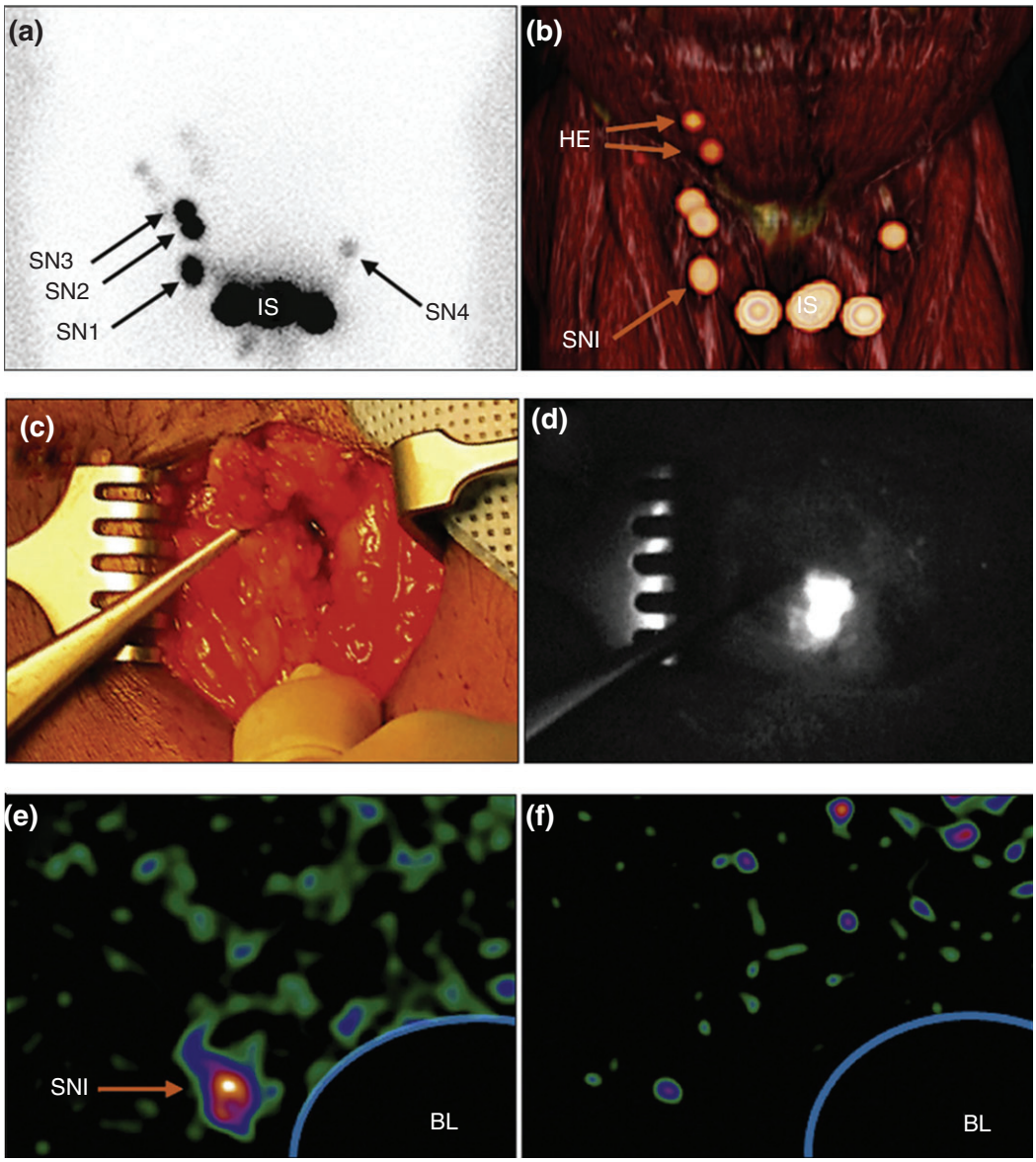

FIGURE 2 | Intraoperative hybrid nuclear and fluorescence identification of sentinel node using indocyanine green (ICG)-99mTc-nanocolloid. (a) Preoperative mapping of metastases in sentinel lymph node (SLN). There are four SLNs around the injection site (IS). (b) Integrated three-dimensional (3D) rendered single-photon-emission computed tomography (SPECT) and computed tomography (CT) showed that the most caudal SLN on the right side was located in an inferior Daseler zone. (c, d) Intraoperative detection of metastases in SLN using color camera (c) and fluorescence imaging (FI) (d). (e, f) Post inspection of the excision area using portable γ camera after removing three SLN (e) and the remaining SLN (f). (Reprinted with permission from Ref 70. Copyright 2014)

the contrast of T2-weighted MRI and serve as the emission source for NIR FI[15] (Figure 3). This probe showed an effective binding rate for both primary and disseminated ovarian tumors in the peritoneal cavity of mice. In the experiments, before the surgery, MRI provided both structural and molecular information for screening and diagnosis that could be used in the surgery. After the surgery, NIR FI was used to evaluate the completeness of the surgery by detecting both residual disease and metastasis.[13] This result showed the potential of this technique for improved image-guided surgery.

Hybrid Nuclear and PAI

While NIR FI is powerful for intraoperative applications as indicated before, it is limited to several millimeters in penetration depth and cannot provide 3D visualization. Recently, a novel multifunctional molecular probe for both nuclear imaging and

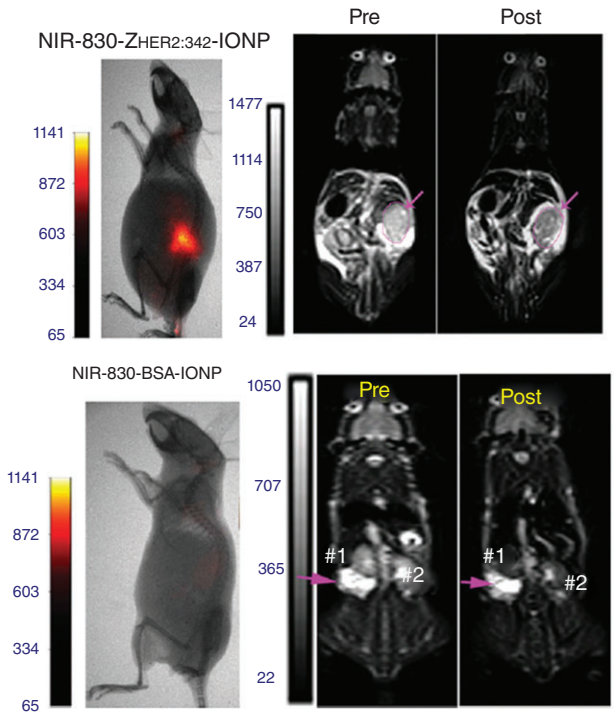

FIGURE 3 | Imaging of SKOV3 tumor-bearing mice by hybrid magnetic resonance imaging (MRI) and near-infrared (NIR) fluorescence imaging (FI). NIR FI of the primary tumor after the administration of specific and nonspecific NIR-labeled iron oxide nanoparticles (IONPs) (left column) and corresponding MRI of the same mice before and after the injection of the nanoprobes. (Reprinted with permission from Ref 15. Copyright 2014 John Wiley & Sons)

photoacoustic contrast enhancement was reported. The PEGylated Au-tripods was conjugated with $\alpha_v\beta_3$ integrins targeting peptide and cyclic Arg-Gly-Asp-D-Phe-Cys (RGDfC) peptide was used as a photoacoustic contrast agent. In addition, ^{64}Cu was labeled with this novel nanoprobe as a radionuclide for PET.[17] After intravenous administration of this probe, tumor-bearing mice showed threefold higher photoacoustic contrast in tumors than the blocking group (Figure 4). With the information provided by PET, PAI could provide volumetric information of tumors with high sensitivity and spatial resolution. Using a miniaturized, compact PAI system, surgeons could obtain more critical information of tumors during the surgery. This study suggested that this multifunctional probe could serve as a new platform with high specificity and sensitivity for dual nuclear and PAI in image-guided surgery.

Hybrid Photoacoustic and Ultrasound Imaging

PAI can be combined with ultrasound imaging without adding additional instruments because the receiver of photoacoustic signals could serve as the transmitter of ultrasound imaging. A representative application of this combination is to guide the biopsy of SLN.[73] After administration of nonspecific MB, PAI showed the 3D visualization of the accumulation of MB in the SLN. Through the co-registration of ultrasound imaging, both molecular information and surrounding anatomy were depicted (Figure 5). In addition to mapping the SLN, this probe can be used to guide the movement of biopsy needles in the operating room. This dual modality probe was modified from a clinical US scanner that makes it more convenient for clinical translation.

Tri-Modal MRI, PAI, and Raman Imaging

Owing to the unique signature of the SERS spectrum, Raman imaging allows for ultra-sensitive detection of SERS contrast agents. It is ideal to develop a multifunctional nanoprobe allowing for both deep tumor visualization and highly sensitive and specific detection of tumor margins in the preoperative and intraoperative stages of cancer surgery. A new trimodality enabled nanoprobe was developed for MRI, PAI, and Raman imaging of brain tumor.[16] This trimodality strategy can achieve whole brain imaging for tumor localization before surgery by using MRI, high spatial resolution, and volumetric imaging of tumors via PAI, and highly sensitive and specific imaging of surface tumor margins through Raman imaging (Figure 6). This multifunctional nanoprobe has a longer retention time compared to conventional contrast agents, which allows for repeated imaging and fewer injections. In addition, the accumulation of this nanoprobe relies on the nonspecific EPR effect allowing it to be used to image other types of cancers with intrinsic EPR effects, such as lung cancer, melanoma, and renal cancer.

Integrated Photoacoustic and Near-Infrared Fluorescence Imaging

Compared to FI, PAI has higher spatial resolution and deeper penetration depth but lower sensitivity and smaller field-of-view. The combination of these two modalities would be an improved strategy for image-guided surgery. However, conventional FI is unable to provide depth information making it more suitable for inspecting the surgical wound after the first resection. Even with the information provided by whole-body imaging techniques such as MRI, CT, and PET, it is difficult to locate the tumor after the patient moves to the operating room. The limited field-of-view prevents PAI from visualization of tumors before surgery because the size of most organs,

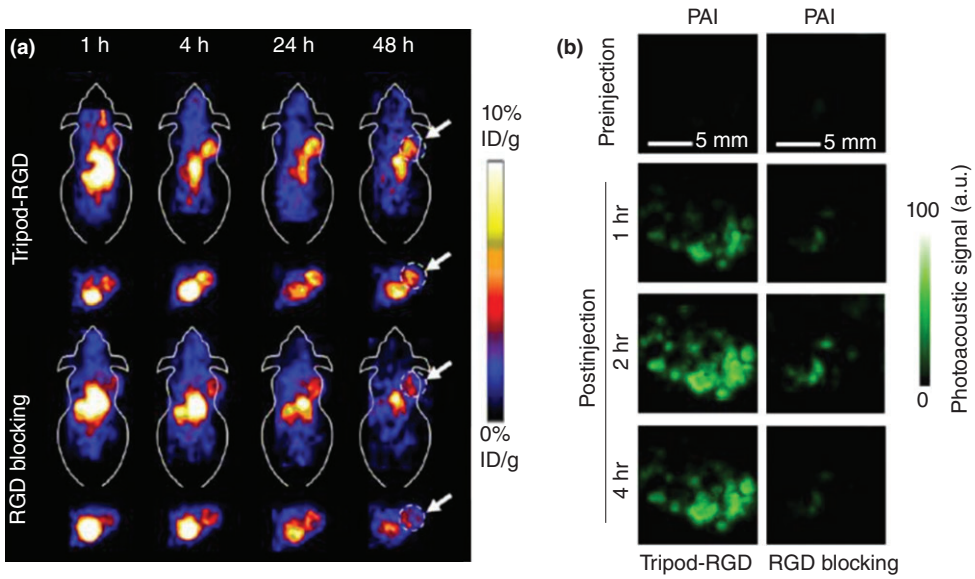

FIGURE 4 | Small animal positron emission tomography (PET) (a) and photoacoustic imaging (PAI) (b) images of U87MG human glioblastoma tumors at 1, 4, 24, and 48 h post injection of RGD-functionalized and RGD-blocking tripods. The tumors of the mice with injection of RGD-functionalized tripods have stronger signals in both PET and photoacoustic (PA) images compared with the animal group with injection of RGD-blocking tripods. (Reprinted with permission from Ref 17. Copyright 2014 American Chemical Society)

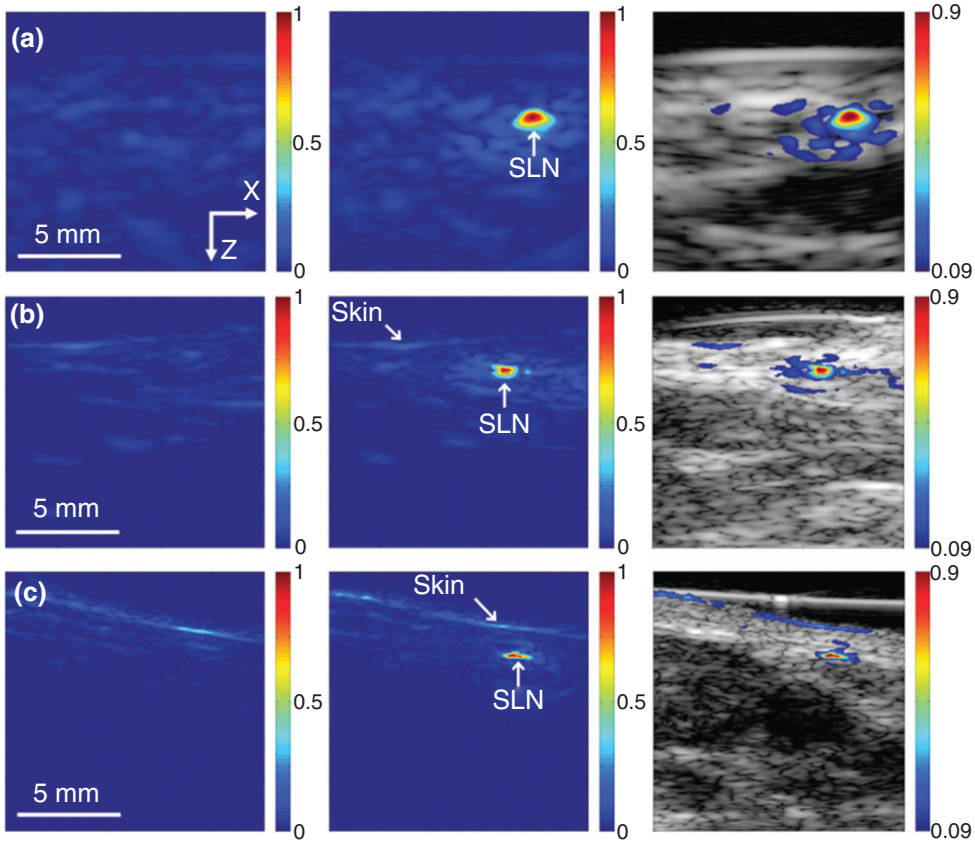

FIGURE 5 | Dual modal ultrasonography (US) and photoacoustic (PA) images of the sentinel lymph node (SLN) with S5-1 probe (80 elements, 1–5 MHz) (a), L8-4 (128 elements, 4–8 MHz) (b), and L15-7io (128 elements, 7–15 MHz) (c) before (left images of a–c) and 20 min after (middle images of a–c) the injection of Indocyanine green (ICG). The right images of (a–c) are the co-registered photoacoustic and US images. (Reprinted with permission from Ref 73. Copyright 2010)

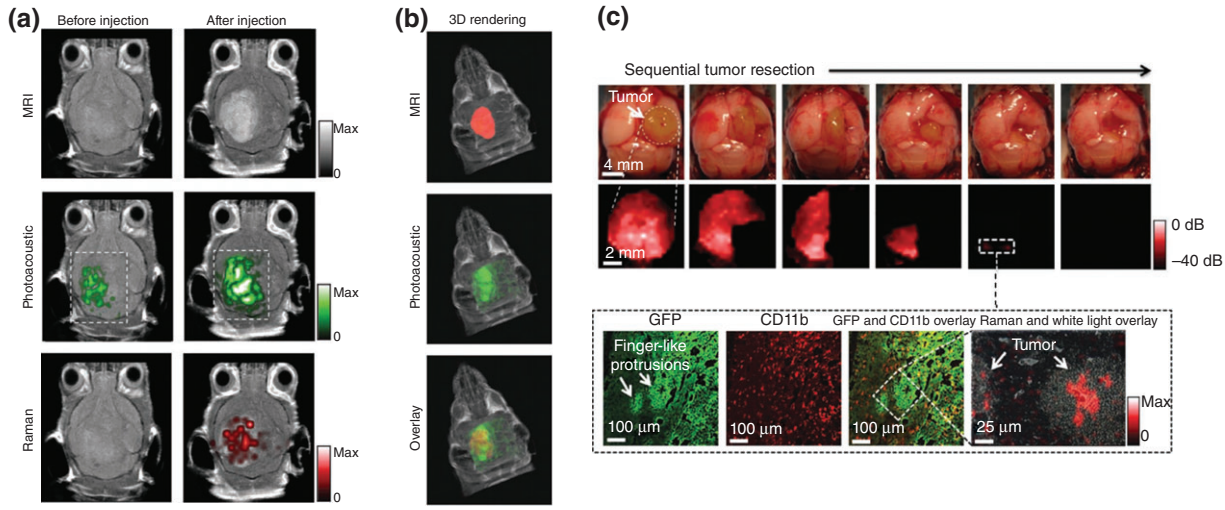

FIGURE 6 | Triple modality detection and Raman guided resection of brain tumors with injection of multifunctional nanoprobes. (a) Two-dimensional (2D) magnetic resonance imaging (MRI), photoacoustic imaging (PAI), and Raman imaging of brain tumors before and after the injection of nanoprobes. (b) Three-dimensional (3D) MRI, PAI, and Raman imaging of brain tumors before and after the injection of nanoprobes. (c) Intraopearive Raman imaging was performed after each resection of the brain tumor. After the gross removal of the major tumor, several small residual diseases were detected by Raman imaging. The histological analysis of the residual diseases was included in the dashed rectangle. (Reprinted with permission from Ref 16. Copyright 2012 Nature Publishing Group)

such as ovaries and breasts, is much larger than the field-of-view of PAI. FMT is an inexpensive and fast 3D optical imaging modality that has been employed for molecular imaging. In contrast to conventional two-dimensional (2D) FI, it provides extra depth and spatial information of a tumor. It was found that FMT was more sensitive and specific to detect low molecular concentration within tissues compared with PAI. An even better solution is to combine three different imaging modalities including FMT, PAI, and FI.[12,13,18] Using an animal model of surgery coupled with targeted-specific NIR dye-IONPS probe, the benefits of combining the three imaging modalities were demonstrated. Specifically, before the surgery, following the whole-body scan of mice by MRI that confirmed the delivery of the probe to the tumor sites, hand-held FMT device was used to quickly scan the tumor bed in 3D. Then, the photoacoustic scan was carried out to three-dimensionally confirm the suspicious tumor bed indicated by FMT with better image quality. After the first resection of the primary tumor guided by PAI, real-time planar FI was utilized to inspect the surgical wound to detect any residual disease (Figure 7). If residual disease was detected, the second surgery would be performed. All the mice were kept for up to 30 days after surgery, and weekly bioluminescence imaging was used to monitor the possible local recurrence. The results of this study showed that the group of mice that received molecular navigation had much lower local recurrence (8%) than the group that received conventional surgeries (33%). This study demonstrated the potential use of this strategy for optimized image-guided surgery.

CONCLUSION AND OUTLOOK

In order to successfully treat and cure patients with solid cancer, it is crucial to detect the tumor early followed by accurate staging and complete removal of the tumor. Molecular imaging techniques have the potential to play a critical role in all the stages associated with the cancer management, especially in image-guided cancer surgery.

The aim of image-guided surgery is to completely remove cancerous tissues while preserving healthy tissues/structures. A single modality simply cannot fulfill to the requirements involved in preoperative, intraoperative, and postoperative stages. As described in Section *Multimodality Imaging*, these requirements/desires at different stages are very different. Multimodal approaches appear to be the solution. Having examined the strengths/weaknesses of the existing and emerging modalities, we believe hybrid or integrated photoacoustic and diffuse optical imaging has the potential to be an optimal approach for preoperative and postoperative scanning of patients because this approach is fast and low in cost, offers high resolution, and uses nonionization radiation. It provides tissue functional parameters such as blood volume and oxygen saturation without the use of any contrast agents. In the intraoperative stage, hand-held

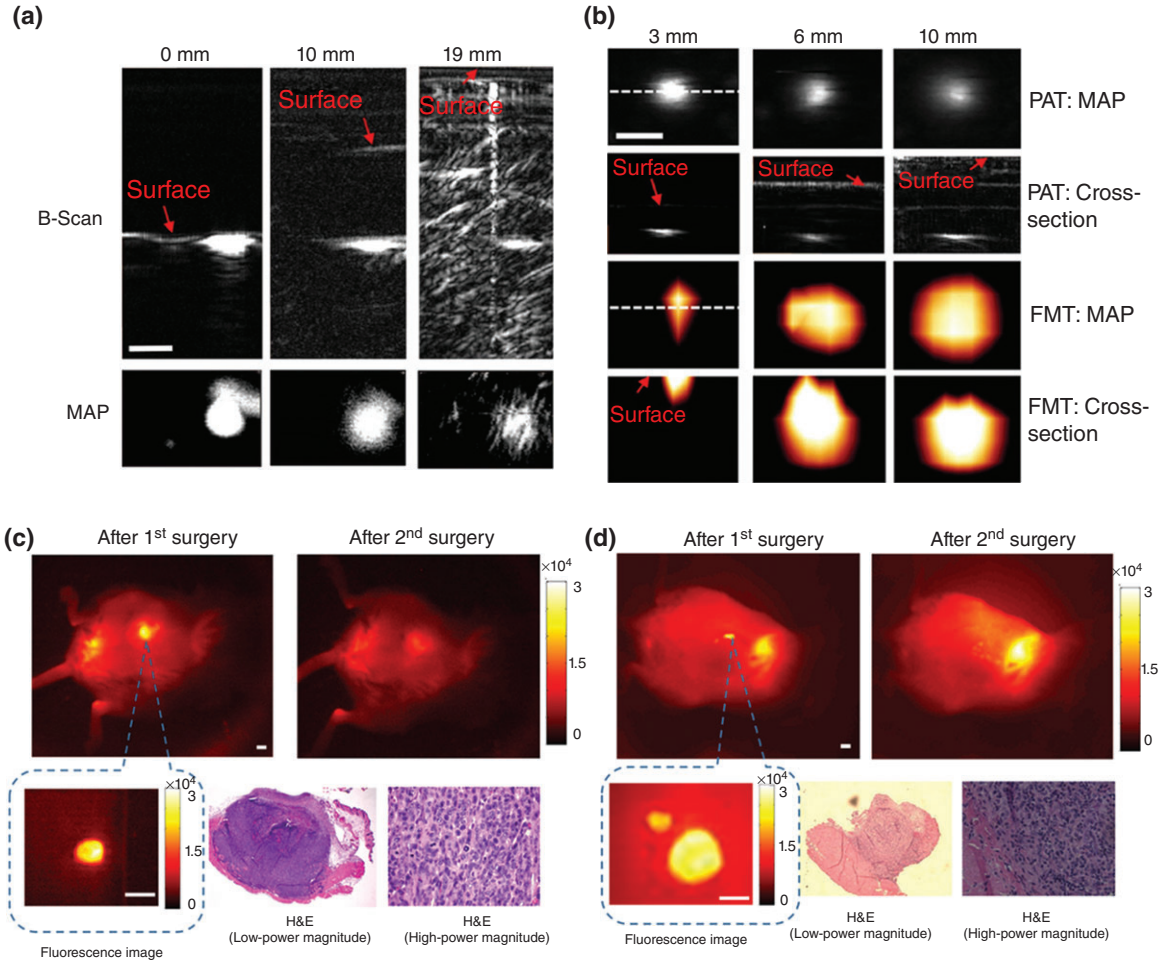

FIGURE 7 | Demonstration of multimodality image-guided surgery using photoacoustic imaging (PAI), fluorescence molecular tomography (FMT), and fluorescence imaging (FI). (a) B-scan (top row) and maximum amplitude projection (MAP) (bottom row) PAI images of a tumor in a representative mouse that received near-infrared (NIR)-labeled iron oxide nanoparticles (IONPs). The back flank of the mouse was overlaid with 0, 10, and 19 mm thickness of chicken breast to mimic the normal tissue in humans. (b) Dual modality imaging of the same tumor located at different depth (3, 6, and 10 mm). From the MAP and cross sections of PAI and FMT, both the lateral and axial resolutions of PAI were higher than that of FMT (Reprinted with permission from Ref 12. Copyright 2014 Elsevier). (c, d) Fluorescent detection of residual disease in the surgical bed after incomplete resection. The residual diseases that could not be visualized by surgeons were clearly detected by the FI and no residual disease was detected after the second surgery. The histological analysis showed the residual nodule interface. (Reprinted with permission from Ref 13. Copyright 2014 Springer)

FMT and PAI can optimally visualize the tumors and map the SLN with high sensitivity and high resolution. After the surgery, planar FI and Raman imaging are probably the most suitable combination for inspecting the surgical wound and detecting residual disease with ultrahigh sensitivity. Finally, ultrasound imaging and portable PET may complement optical techniques in different stages.

REFERENCES

1. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 2008, 452:580–589.
2. Keereweer S, Kerrebijn JDF, van Diel PB, Xie B, Kaijzel EL, Snoeks TJA, Que I, Hutteman M, van der Vorst JR, Mieog JSD, et al. Optical image-guided surgery-where do we stand? *Mol Imaging Biol* 2011, 13:199–207.
3. Hussain T, Nguyen QT. Molecular imaging for cancer diagnosis and surgery. *Adv Drug Deliv Rev* 2014, 66C:90–100.
4. Kircher MF, Willmann JK. Molecular body imaging: MR imaging, CT, and US. Part I. Principles. *Radiology* 2012, 263:633–643.

5. Kapoor V, McCook BM, Torok FS. An introduction to PET-CT imaging. *Radiographics* 2004, 24:523–543.
6. Buck AK, Herrmann K, Stargardt T, Dechow T, Krause BJ, Schreyögg J. Economic evaluation of PET and PET/CT in oncology: evidence and methodologic approaches. *J Nucl Med* 2010, 51:401–412.
7. Strong VE, Humm J, Russo P, Jungbluth A, Wong WD, Daghighian F, Old L, Fong Y, Larson SM. A novel method to localize antibody-targeted cancer deposits intraoperatively using handheld PET β and γ probes. *Surg Endosc* 2008, 22:386–391.
8. Islam T, Josephson L. Current state and future applications of active targeting in malignancies using superparamagnetic iron oxide nanoparticles. *Cancer Biomark* 2009, 5:99–107.
9. Bu L, Shen B, Cheng Z. Fluorescent imaging of cancerous tissues for targeted surgery. *Adv Drug Deliv Rev* 2014, 76:21–38.
10. Keereweer S, Van Driel PB, Snoeks TJ, Kerrebijn JD, De Jong Baatenburg RJ, Vahrmeijer AL, Sterenborg HJ, Löwik CW. Optical image-guided cancer surgery: challenges and limitations. *Clin Cancer Res* 2013, 19:3745–3754.
11. Nijssen A, Koljenovic S, Bakker Schut TC, Caspers PJ, Puppels GJ. Towards oncological application of Raman spectroscopy. *J Biophotonics* 2009, 2:29–36.
12. Xi L, Satpathy M, Zhao Q, Qian W, Yang L, Jiang H. HER-2/neu targeted delivery of a nanoprobe enables dual photoacoustic and fluorescence tomography of ovarian cancer. *Nanomedicine* 2014, 10:669–677.
13. Xi L, Zhou G, Gao N, Yang L, Gonzalo DA, Hughes SJ, Jiang H. Photoacoustic and fluorescence image-guided surgery using a multifunctional targeted nanoprobe. *Ann Surg Oncol* 2014, 21:1602–1609.
14. Yang L, Peng XH, Wang A, Wang X, Cao Z, Ni C, Karna P, Zhang X, Wood WC, Gao X, et al. Receptor targeted nanoparticles for in vivo imaging of breast cancer. *Clin Cancer Res* 2009, 15:4722–4732.
15. Satpathy M, Wang L, Zielinski R, Qian W, Lipowska M, Capala J, Lee GY, Xu H, Wang YA, Mao H, et al. Active targeting using HER-2-affibody-conjugated nanoparticles enabled sensitive and specific imaging of orthotopic HER-2 positive ovarian tumors. *Small* 2014, 10:544–555.
16. Kircher MF, De La Zerda A, Jokerst JV, Zavaleta CL, Kempen PJ, Mittra E, Pitter K, Huang R, Campos C, Habte F, et al. A brain tumor molecular imaging strategy using a new triple-modality MRI-photoacoustic –Raman nanoparticle. *Nat Med* 2012, 18:829–834.
17. Cheng K, Kothapalli SR, Liu H, Koh AL, Jokerst JV, Jiang H, Yang M, Li J, Levi J, Wu JC, et al. Construction and validation of nano gold tripods for molecular imaging of living subjects. *J Am Chem Soc* 2014, 136:3561–3571.
18. Xi L, Grobmyer SR, Zhou G, Qian W, Yang L, Jiang H. Molecular photoacoustic tomography of breast cancer using receptor targeted magnetic iron oxide nanoparticles as contrast agents. *J Biophotonics* 2014, 7:401–409.
19. Dass CR. Tumour angiogenesis, vascular biology and enhanced drug delivery. *J Drug Target* 2004, 12:245–255.
20. Lofblom J, Feldwisch J, Tolmachev V, Carlsson J, Stahl S, Frejd FY. Affibody molecules: engineered proteins for therapeutic, diagnostic and biotechnological applications. *FEBS Lett* 2010, 584:2670–2680.
21. Prabhakar U, Maeda H, Jain RK, Sevick-Muraca EM, Zamboni W, Farokhzad OC, Barry ST, Gabizon A, Grodzinski P, Blakey DC. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res* 2013, 73:2412–2417.
22. Crane LM, Themelis G, Pleijhuis RG, Harlaar NJ, Sarantopoulos A, Arts HJ, van der Zee AG, Ntziachristos V, van Dam GM. Intraoperative multispectral fluorescence imaging for the detection of the sentinel lymph node in cervical cancer: a novel concept. *Mol Imaging Biol* 2011, 13:1043–1049.
23. Haglund MM, Berger MS, Hochman DW. Enhanced optical imaging of human gliomas and tumor margins. *Neurosurgery* 1996, 38:308–317.
24. Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, Hasegawa K, Beck Y, Fukayama M, Kokudo N. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. *Cancer* 2009, 115:2491–2504.
25. Pallone MJ, Poplack SP, Avutu HB, Paulsen KD, Barth RJ. Supine breast MRI and 3D optical scanning: a novel approach to improve tumor localization for breast conserving surgery. *Ann Surg Oncol* 2014, 21:2203–2208.
26. Grossman R, Nossek E, Shimony N, Raz M, Ram Z. Intraoperative 5-aminolevulinic acid-induced fluorescence in primary central nervous system lymphoma. *J Neurosurg* 2014, 120:67–69.
27. Muroi C, Fandino J, Coluccia D, Berkmann S, Fathi AR, Landolt H. 5-aminolevulinic acid fluorescence-guided surgery for spinal meningioma. *World Neurosurg* 2013, 223:e221–e223.
28. Draga RO, Grimbergen MC, Kok ET, Jonges TN, van Swol CF, Bosch RJ. The quality of 5-aminolevulinic acid-induced photodynamic diagnosis and transurethral resection of bladder tumors: does the urologist play a role? *Urol Int* 2012, 89:326–331.
29. Murayama Y, Ichikawa D, Koizumi N, Komatsu S, Shiozaki A, Kuriu Y, Ikoma H, Kubota T, Nakanishi M, Harada Y, et al. Staging fluorescence laparoscopy for gastric cancer by using 5-aminolevulinic acid. *Anticancer Res* 2012, 32:5421–5427.
30. Van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJ, van der Zee AG, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer

by folate receptor-α targeting: first in-human results. *Nat Med* 2011, 17:1315–1319.

31. Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2002, 2:91–100.
32. Veiseh M, Gabikian P, Bahrami SB, Veiseh O, Zhang M, Hackman RC, Ravanpay AC, Stroud MR, Kusuma Y, Hansen SJ, et al. Tumor paint: a chlorotoxin:cy5.5 bioconjugate for intraoperative visualization of cancer foci. *Cancer Res* 2007, 67:6882–6888.
33. Miyawaki A. Fluorescence imaging in the last two decades. *J Microsc* 2013, 62:63–68.
34. Terai T, Nagano T. Small- molecule fluorophores and fluorescent probes for bioimaging. *Pflugers Arch* 2013, 465:347–359.
35. Luo S, Zhang E, Su Y, Cheng T, Shi C. A review of NIR dyes in cancer targeting and imaging. *Biomaterials* 2011, 32:7127–7138.
36. Hong G, Lee JC, Robinson JT, Raaz U, Xie L, Huang NF, Cooke JP, Dai H. Multi-functional in vivo vascular imaging using near-infrared II fluorescence. *Nat Med* 2012, 18:1841–1846.
37. 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.
38. Nie L, Chen X. Structural and functional photoacoustic molecular tomography aided by emerging contrast agents. *Chem Soc Rev* 2014, 43:7132–7170.
39. Kim C, Cho EC, Chen J, Song KH, Au L, Favazza C. In vivo molecular photoacoustic tomography of melanomas targeted by bioconjugated gold nanocages. *ACS Nano* 2010, 4:4559–4564.
40. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N Engl J Med* 2003, 348:2491–2499.
41. Wunderbaldinger P, Josephson L, Bremer C, Moore A, Weissleder R. Detection of lymph node metastases by contrast-enhanced MRI in an experimental model. *Magn Reson Med* 2002, 47:292–297.
42. Grootendorst DJ, Jose J, Fratila RM, Visscher M, Velders AH, Ten Haken B, Van Leeuwen TG, Steenbergen W, Manohar S, Ruers TJ. Evaluation of superparamagnetic iron oxide nanoparticles (Endorem®) as a photoacoustic contrast agent for intra-operative nodal staging. *Contrast Media Mol Imaging* 2013, 8:83–91.
43. Adams KE, Ke S, Kwon S, Liang F, Fan Z, Lu Y, Hirschi K, Mawad ME, Barry MA, Sevick-Muraca EM. Comparison of visible and near-infrared wavelength-excitable fluorescent dyes for molecular imaging of cancer. *J Biomed Opt* 2007, 12:024017.
44. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971, 285:1182–1186.
45. Arnold F. Tumour angiogenesis. *Ann R Coll Surg Engl* 1985, 67:295–298.
46. Burstin JV, Eser S, Seidler B, Meining A, Bajbouj M, Mages J, Lang R, Kind AJ, Schnieke AE, Schmid RM, et al. Highly sensitive detection of early-stage pancreatic cancer by multimodal near-infrared molecular imaging in living mice. *Int J Cancer* 2008, 123:2138–2147.
47. Keliher EJ, Reiner T, Earley S, Klubnick J, Tassa C, Lee AJ, Ramaswamy S, Bardeesy N, Hanahan D, Depinho RA, et al. Targeting cathepsin E in pancreatic cancer by a small molecule allows in vivo detection. *Neoplasia* 2013, 15:684–693.
48. Youssif BG, Okuda K, Kadonosono T, Salem OI, Hayallah AA, Hussein MA, Kizaka-Kondoh S, Nagasawa H. Development of a hypoxia-selective near-infrared fluorescent probe for non-invasive tumor imaging. *Chem Pharm Bull* 2012, 60:402–407.
49. Provenzano PP, Cuevas C, Chang AE, Goel VK, Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012, 21:418–429.
50. Shilo M, Reuveni T, Motiei M, Popovtzer R. Nanoparticles as computed tomography contrast agents: current status and future perspectives. *Nanomedicine* 2012, 7:257–269.
51. Rabin O, Perez JM, Grimm J, Wojtkiewicz G, Weissleder R. An X-ray computed tomography imaging agent based on long-circulating bismuth sulphide nanoparticles. *Nat Mater* 2006, 5:118–122.
52. Hyafil F, Cornily JC, Feig JE, Gordon R, Vucic E, Amirbekian V, Fisher EA, Fuster V, Feldman LJ, Fayad ZA. Noninvasive detection of macrophages using a nanoparticulate contrast agent for computed tomography. *Nat Med* 2007, 13:636–641.
53. Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol* 2005, 202:654–662.
54. Conti PS, Lilien DL, Hawley K, Keppler J, Grafton ST, Bading JR. PET and [18 F]-FDG in oncology: a clinical update. *Nucl Med Biol* 1996, 23:717–735.
55. Juweid ME, Cheson BD. Positron-emission tomography and assessment of cancer therapy. *N Engl J Med* 2006, 354:496–507.
56. Deshpande N, Needles A, Willmann JK. Molecular ultrasound imaging: current status and future directions. *Clin Radiol* 2010, 65:567–581.
57. Willmann JK, Kimura RH, Deshpande N, Lutz AM, Cochran JR, Gambhir SS. Targeted contrast-enhanced ultrasound imaging of tumor angiogenesis with contrast microbubbles conjugated to integrin-binding knottin peptides. *J Nucl Med* 2010, 51:433–440.
58. Rapoport N, Gao Z, Kennedy A. Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy. *J Natl Cancer Inst* 2007, 99:1095–1106.
59. Kircher MF, Hricak H, Larson SM. Molecular imaging for personalized cancer care. *Mol Oncol* 2012, 6:182–195.

60. Ohno Y, Koyama H, Onishi Y, Takenaka D, Nogami M, Yoshikawa T, Matsumoto S, Kotani Y, Sugimura K. Non-small cell lung cancer: whole-body MR examination for M-stage assessment—utility for whole-body diffusion-weighted imaging compared with integrated FDG PET/CT. *Radiology* 2008, 248:643–654.
61. Aime S, Cabella C, Colombatto S, Geninatti Crich S, Gianolio E, Maggioni F. Insights into the use of paramagnetic Gd(III) complexes in MR-molecular imaging investigations. *J Magn Reson Imaging* 2002, 16:394–406.
62. Xu X, Wang Y, Chen Z, Sternlicht MD, Hidalgo M, Steffensen B. Matrix metalloproteinase-2 contributes to cancer cell migration on collagen. *Cancer Res* 2005, 65:130–136.
63. Mieog SD, Troyan S, Hutteman M, Donohoe K, van der Vorst J, Stockdale A, Liefers GJ, Choi HS, Gibbs-Strauss SL, Putter H, et al. Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer. *Ann Surg Oncol* 2011, 18:2483–2491.
64. Ogawa M, Kosaka N, Choyke P, Kobayashi H. In vivo molecular imaging of cancer with a quenching near-infrared fluorescent probe using conjugates of monoclonal antibodies and indocyanine green. *Cancer Res* 2009, 15:1268–1272.
65. Coleman PJ, Brashear KM, Askew BC, Hutchinson JH, McVean CA, Duong le T, Feuston BP, Fernandez-Metzler C, Gentile MA, Hartman GD. Nonpeptide $\alpha v\beta 3$ antagonists. Part 11: discovery and preclinical evaluation of potent $\alpha v\beta 3$ antagonists for the prevention and treatment of osteoporosis. *J Med Chem* 2004, 47:4829–4837.
66. Shetty G, Kendall C, Shepherd N, Stone N, Barr H. Raman spectroscopy: elucidation of biochemical changes in carcinogenesis of oesophagus. *Br J Cancer* 2006, 94:1460–1464.
67. Madjewski B, Judy BF, Mouchli A, Kapoor V, Holt D, Wang MD, Nie S, Singhal S. Intraoperative near-infrared imaging of surgical wounds after resections can detect residual disease. *Clin Cancer Res* 2012, 18:5741–5751.
68. Xi L, Grobmyer SR, Wu L, Chen R, Zhou G, Gutwein LG, Sun J, Liao W, Zhou Q, Xie H, et al. Evaluation of breast tumor margins in vivo with intraoperative photoacoustic imaging. *Opt Express* 2012, 20: 8726–8731.
69. Kim C, Song KH, Gao F, Wang LV. Sentinel lymph nodes and lymphatic vessels: noninvasive dual-modality in vivo mapping by using indocyanine green in rats—volumetric spectroscopic photoacoustic imaging and planar fluorescence imaging. *Radiology* 2010, 255:442–450.
70. Brouwer OR, van den Berg NS, Matheron HM, van der Poel HG, van Rhijn BW, Bex A, van Tinteren H, Valdés Olmos RA, van Leeuwen FW, Horenblas S. A hybrid radioactive and fluorescent tracer for sentinel node biopsy in penile carcinoma as a potential replacement for blue dye. *Eur Urol* 2014, 65: 600–609.
71. Sampath L, Kwon S, Hall MA, Price RE, Sevick-Muraca EM. Detection of cancer metastases with a dual-labeled near-infrared/positron emission tomography imaging agent. *Transl Oncol* 2010, 3:307–317.
72. Hall MA, Kwon S, Robinson H, Lachance PA, Azhdarinia A, Ranganathan R, Price RE, Chan W, Sevick-Muraca EM. Imaging prostate cancer lymph node metastases with a multimodality contrast agent. *Prostate* 2012, 72:129–146.
73. Erpelding TN, Kim C, Pramanik M, Jankovic L, Maslov K, Guo Z, Margenthaler JA, Pashley MD, Wang LV. Sentinel lymph nodes in the rat: noninvasive photoacoustic and US imaging with a clinical US system. *Radiology* 2010, 256:102–110.