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Emerging Intraoperative Imaging Modalities to Improve Surgical Precision

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Author Comments:	Stanford, April 14th, 2018 To: Dr. Gibson Editor-in-chief Molecular Imaging and Biology Dear Dr. Gibson, Please find enclosed a state-of-the-art review entitled 'Emerging Intraoperative Imaging Modalities to Improve Surgical Precision'. This manuscript was written on behalf of the Intra-Operative Study Group of the European Society of Molecular Imaging and in response to the invitation of Prof. Tony Lahoutte and Prof. Bertrand Tavitian to publish a bundle of hot-topic reviews in the June issue of Molecular Imaging and Biology. All authors have reviewed the manuscript and have given final approval. The paper is not under consideration elsewhere. Thank you for your time and kind consideration. Yours Sincerely, Stephan Rogalla & Sophie Hernot Chairs of Intra-Operative Imaging Study Group of ESMI E-mail: srogalla@stanford.edu Tel: 001-650-3025243
Suggested Reviewers:	Christopher H Contag, PhD Professor and Chair, Michigan State University contagch@egr.msu.edu Dr. Contag is a pioneer in the field of optical imaging and molecular imaging in general. He has been past president of WMIS.
	Alexander L Vahrmeijer, M.D. Ph.D. Professor, Universiteit Leiden A.L.Vahrmeijer@lumc.nl Dr. Vahrmeijer is a physician scientist and an expert in the field of intraoperative imaging. He is head of the research group and former chair and founder of the surgical navigation group at ESMI.

Emerging Intraoperative Imaging Modalities to Improve Surgical Precision

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Abstract

Intraoperative imaging (IOI) is performed to guide delineation and localization of regions of surgical interest. While oncological surgical planning predominantly utilizes computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US), intraoperative guidance mainly remains on surgeon interpretation and pathology for confirmation. Over the past decades however, intraoperative guidance has evolved significantly with the emergence of several novel imaging technologies, including fluorescence-, Raman, photoacoustic- and radio-guided approaches. These modalities have demonstrated the potential to further optimize precision in surgical resection and improve clinical outcomes for patients. Not only can these technologies enhance our understanding of the disease, they can also yield large imaging datasets intraoperatively that can be analyzed by deep learning approaches for more rapid and accurate pathological diagnosis. Unfortunately, many of these novel technologies are still under preclinical or early clinical evaluation. Organizations like the Intra-Operative Imaging Study Group of the European Society for Molecular Imaging (ESMI) support interdisciplinary interactions with the aim to improve technical capabilities in the field, an approach that can succeed only if scientists, engineers, and physicians work closely together with industry and regulatory bodies to resolve roadblocks to clinical translation. In this review we provide an overview of a variety of novel IOI technologies, discuss their challenges and present future perspectives on the enormous potential of IOI for oncological surgical navigation.

Keywords:

Intraoperative imaging, Surgical navigation, Image-guided Surgery, Fluorescence imaging, Raman spectrometry, Photoacoustic imaging, Optoacoustic imaging, Thermoacoustic imaging, Radioguided surgery, Deep learning

Introduction

For decades, surgical resection has been guided by the naked eye, and despite the surgeon's training, experience, manual dexterity, and meticulous observations, more precise and objective technologies are needed to support surgical procedures [1]. The information required by the surgeon in the operating room (OR) can vary significantly. Oncologic surgeons need to assess tumor margins, the borders between healthy and cancerous tissue and note critical landmarks such as nerves or blood vessels in order to optimize resection of malignant tissue while minimizing harm. Otherwise, in cardiovascular surgery, vulnerable atherosclerotic plaques need to be identified and localized to optimize treatment. Several imaging techniques are applied in the OR with the aim of supporting the surgeon. Ultrasound (US) imaging, for example, is used to re-assess liver tumor location intraoperatively, X-Ray imaging used to confirm catheter positioning, and computed tomography (CT) and magnetic resonance imaging (MRI) are used in neurosurgery for tissue shift correction. However, US suffers from limited contrast imaging capabilities while MRI and CT are challenging to use in the OR due to their size and geometric constraints. Moreover, MRI and CT are costly, cannot yet image in real-time to facilitate rapid intraoperative decision-making, and they present challenges in maintaining a sterile surgical field and may interrupt the workflow.

In recent years, a number of imaging modalities have been successfully utilized for intraoperative guidance, including optical (fluorescence and Raman), acoustic (photoacoustics and radiofrequency (RF)-acoustics), and nuclear imaging-based approaches. These approaches have improved the signal-to-noise ratio of diseased tissue, even in deep-seated regions, thereby augmenting intraoperative identification beyond the limitations of direct visual inspection and palpation. These modalities can be used in standalone fashion to detect intrinsic tissue signals or in conjunction with imaging agents to increase contrast and specificity. Furthermore, they afford synchronous visualization of anatomical and molecular parameters, empowering the surgeon to maximally extract information pertinent to a successful intervention and an improved clinical outcome. However, analyzing the tremendous amount of real-time imaging data acquired by modern IOI technologies is beyond the processing capacity of a human being, and will require the assistance of emerging computational methods, such as deep learning and artificial intelligence.

Optical imaging techniques

Fluorescence-guided surgery

The main goal of intraoperative imaging (IOI) is to aid in surgical navigation. One approach is to have the specific tissue or lesion of interest literally 'light up', for example using fluorescence. Fluorescence imaging is a simple, low-cost, and contact-free method in which fluorophores are excited by an appropriate light source and emitted photons are detected (Fig. 1a). Although the native fluorescence signature of tumors may be different from that of normal tissues [2], the signal's sensitivity and specificity are often too limited to accurately delineate cancer lesions. Therefore, the development of exogenous agents has become an exciting field of research as an attempt to enhance fluorescent contrast and provide additional functional or molecular information. Fluorescence imaging is highly compatible with the intraoperative setting, as it enables real-time imaging at form factors that can be integrated into the surgical routine, and offers superior sensitivity compared to preoperative imaging or visual inspection and palpation during surgery.[3]

While fluorescence techniques employed in microscopy of cells or naturally transparent organisms (C. elegans, zebrafish etc.) have relied mostly on the use of visible light, near-infrared (NIR) wavelengths in the so-called 'tissue optical window' (650-900 nm) are usually preferred for *in vivo* imaging because of lower autofluorescence, deeper tissue penetration (several mm to a cm), and reduced light scattering [4]. Since NIR fluorescence is invisible to the human eye, and to enable detection of small administered doses operating under microdosing conditions, a sensitive photodetector, such as a charge coupled device camera with high spatial and temporal resolution, is indispensable [5-6]. Many fluorescence imaging systems with an integrated light source and camera have already been employed for intraoperative use and several are commercially available [7]. These systems differ in their operational characteristics, i.e. their sensitivity and specificity for certain fluorophores, rejection of ambient light, or ability to image multiple fluorophores simultaneously. Furthermore, depending on the design, they can be applied to open surgeries, minimally-invasive and robotic surgeries, or endoscopic examinations [8]. Wearable display goggles and novel projection strategies have been proposed to obviate standard monitor displays that require the surgeon to divert his gaze from the operative field [9-10].

However, scattering of NIR light by sub-cellular organelles and other microscopic tissue constituents, greatly limits penetration into deep-seated targets and degrades spatial resolution [11]. Fluorescence imaging in the NIR-II window (1000-1700 nm) is currently being investigated in an attempt to increase spatial resolution since tissue exhibits less light scattering at longer wavelengths [12]. Yet, due to the increased attenuation of light in the NIR-II window by lipids and water, the signal-to-noise achieved may be reduced compared to NIR light. Agents that emit in the NIR-II range include single-walled carbon nanotubes and quantum dots, in addition to a few small-molecule-based dyes. These agents are still in preclinical development and their clinical translatability remains to be assessed. Furthermore, advancements in the sensitivity and affordability of detector systems are needed for widespread adoption of NIR-II imaging.

The most commonly used NIR fluorescent agent is indocyanine green (ICG), a clinically approved NIR dye used widely in angiography, perfusion imaging, and more recently sentinel lymph node (SLN) mapping [13]. ICG has also been used for the intraoperative detection of tumors, where it passively accumulates via the enhanced permeability and retention (EPR) effect [14]. Despite its sensitivity, however, ICG has seen limited use due to its lack of specificity. Alternatively, 5-ALA (Gliolan) and its derivate 5-ALA hexyl ester (Hexvix) are two clinically approved pro-drugs used for the visualization of high-grade gliomas and bladder cancer, respectively. They elicit synthesis of protoporphyrin IX, a red-fluorescent protein which accumulates preferentially in malignant tissues. Randomized controlled Phase III clinical trials not only showed that 5-ALA-mediated fluorescence-guidance enabled more effective tumor detection and resection but also improved progression-free survival [15-16].

Most efforts to highlight tumors during surgery over the last decade have focused on improving specificity through receptor-targeted fluorescent agents. These agents specifically recognize membrane-bound biomarkers overexpressed by tumor cells (e.g. EGFR, folate receptor-α, CEA, EpCAM, c-MET, CAIX) or stroma (e.g. VEGF, $\alpha_v\beta_3$, uPAR) [17]. So-called 'pan-cancer biomarkers' may be used for detection of multiple cancer types, thereby enabling broad clinical application while avoiding patient preselection.

In 2011, a pioneering first-in-human study demonstrated the feasibility of visualizing tumor burden with FITC-labeled folate during ovarian cancer debulking surgery [18]. Subsequently, several clinical trials, some of which are still ongoing, have investigated the application of fluorescently-

labeled monoclonal antibodies as IOI tracers for a variety of cancer types [8, 19-20]. In many cases, antibodies that are already clinically available as targeted therapeutics are modified with a fluorophore, thereby minimizing regulatory issues associated with the development of a completely new binder. These studies have demonstrated the successful assessment of tumor margins *in situ* during surgery (Fig. 1b) as well as *ex vivo* on excised specimens at both the macroscopic and microscopic level (Fig. 1c) [21-22]. Such back-table fluorescence examination can guide sampling and hasten the pathology report during surgery [23-24]. Nevertheless, further studies are needed to establish objective and standardized criteria for differentiating diseased and healthy tissue, and to demonstrate the impact of fluorescence-guidance on surgical efficacy (clear margin rates, surgery duration, length of hospital stay, complications) and patient outcomes (survival, recurrence-rates, esthetics, and quality of life).

Smaller targeting agents - incorporating peptides, protein scaffolds, or antibody-fragments - with improved pharmacokinetic properties and specificity, have also been developed and are undergoing evaluation [25-29]. For example, fluorescent peptides have been clinically translated that were shown to improve the detection of polyps and neoplastic lesions in humans during an endoscopic examination at early time points [30-31]. Another approach that was shown to be safe and feasible is the use of activatable tracers that become fluorescent upon cleavage of their peptide backbone by tumor-specific enzymes [32]. This strategy is still awaiting thorough clinical investigation.

Finally, during complex surgeries where lesions of interest reside in close proximity to blood vessels and nerves (e.g. in head and neck cancers or prostate cancer), highlighting these vital structures could improve the surgeon's ability to avoid or preserve them. Thus far, direct visualization of nerves with fluorescent agents remains limited to a few preclinically tested nerve-binding fluorophores, fluorescent peptides, and antibodies [33-34]. Color-coding tumors and critical structures for simultaneous viewing will require multi-spectral imaging.

Raman spectroscopy

As a highly specific and potentially label-free modality, Raman spectroscopy can provide molecular information by analyzing the so-called inelastic scattering of photons upon excitation

(Fig. 1d). When a laser beam interacts with a molecule, photons are scattered and the inelastic scattering can be measured using complex and expensive systems consisting of fiber bundles, lenses, spectrometers, and photomultiplier tubes (PMT). However, as label-free Raman imaging is time consuming, the low signal intensity can be overcome by the use of surface-enhanced Raman nanoparticles (SERS) to amplify the signal [35-36]. One limitation in fluorescence imaging is an often reduced signal *in vivo* compared to *ex vivo* imaging of the same specimen. Furthermore, apart from signal differences due to overlying tissues or camera-to-tissue distances, excitation within cavities (e.g. oral cavity) may be hindered, causing the imaging beam to not ideally excite the fluorophore. This is especially problematic in open field fluorescent systems, but also occurs in very tight and narrow cavities of the human body, where maneuvering may be hindered [37]. Raman imaging, on the other hand, does not require the laser beam to hit the target at a specific angle when the signal is enhanced by SERS [38]. In addition, fluorescence imaging is limited in its multiplexing capabilities due to the overlapping spectra of fluorophores, narrowing the number of pathological questions that can be answered in a single procedure. In order to scan multiple disease stages in the same tissue, such as low-grade and high-grade dysplasia, malignancy, and inflammation, multiplexing can be achieved by using disease stage-specific targets with distinct SERS labels. Simultaneously, untargeted SERS of known intensity can be delivered to calibrate the ratiometric image [39]. SERS agents possess a number of features that are attractive for imaging, such as high brightness relative to endogenous Raman signals, having complex and narrow emission spectra that can be used as molecular fingerprints, and affording the capability of multiplexing ten or more parameters. Unlike fluorescence imaging, where autofluorescence contributes significantly to noise, the background in Raman imaging is greatly reduced due to the high specificity of the Raman spectra [40]. SERS agents consist of a gold nanoparticle core coated with a Raman active compound and a silica shell. Although the gold nanoparticles have been shown to persist in organs and tissues long after intravenous injection of SERS agents, no immediate toxic effects have been observed thus far in preclinical testing [41]. For increased specificity, peptides or antibodies can be conjugated to the SERS nanoparticle surface [40] for targeting of cell surface proteins. Due to their size (5-100 nm), SERS agents are not amenable to intracellular targeting [42]. While intravenous administration usually requires several hours for targeting and plasma clearance, topical applications requires very short incubation times and the particles' individual spectral fingerprints can be unmixed immediately [43]. Non-targeted controls

and ratiometric imaging can be used to control for nonspecific pooling and optimization of signal *in vivo* [35]. The utility of Raman imaging for surgical resection has been reported by Liu *et. al*., who topically applied a suspension of five different SERS particles (four targeted and one non-targeted) for 5 minutes on resected xenograft tumor tissue, followed by washing. They were able to clearly distinguish cancer tissue from healthy tissue using this approach and could rule out non-specific binding [39]. Garai *et al*. reported the development, testing, and clinical feasibility of a circumferential scanning Raman micro-endoscopy system that can image the entire colonic lumen. The device has been tested on bench-top colon phantoms, *in vivo* in a porcine model with submucosally injected SERS, and in a human trial in which the topology of the colon was scanned in the absence of SERS [35]. This device can be inserted into the working channel of currently available clinical endoscopes. Since each pixel of a circumferential scan during a controlled retraction of the colonoscope can be mapped, a reconstruction of the topology of the colon wall can be displayed. On top of that image a map showing the location of tumor-targeting SERS nanoparticles displayed in various ways. Location of SERS can be shown as pure spectra, the signal can be shown as a colored patch in a topology phantom or directly overlayed into the white-light endoscopy (Fig. 1e) [35]. This can be used to guide biopsies or removal of residual cancer. In addition, several groups are using Raman for intraoperative imaging or to successfully distinguish between cancer and healthy tissue. Harmsen *et al*. were able to detect tumor cells that were undetectable by common imaging modalities (e.g. CT) in preclinical testing [43]. Thus, powerful and sensitive devices in combination with highly specific SERS agents are rapidly innovating clinical diagnostics and surgical navigation.

Photoacoustics and thermoacoustic imaging

To overcome the effects of light scattering in optical imaging of tissues, photoacoustic imaging (also known as optoacoustic imaging) combines the favorable characteristics of both optical and acoustical aspects. The photoacoustic effect is based on pressure transients generated by absorption of pulsed or modulated light [11]. These pressure waveforms experience substantially less scattering than light, as they propagate from absorption sites within the tissue to the tissue boundary. Photoacoustic imaging can be performed over a wide range of depths and resolutions. Similar to optical microscopy, optical-resolution photoacoustic microscopy achieves sub-micron

resolution at sub-mm penetration depths, while acoustic-resolution photoacoustic tomography achieves sub-millimeter resolution at a depth of up to several centimeters [11]. Novel laser technologies and reconstruction schemes allow real-time imaging [44]. The use of multiple wavelengths can allow, in principle, quantification of the various chromophore concentration and thus provide molecular-specific contrast.

Photoacoustic imaging is often combined with ultrasonography whereby the combined approach presents improved anatomical, functional and molecular information. Thus, photoacoustics is ideally suited for integration into the standard surgical workflow. Since certain molecules such as hemoglobin and melanin strongly absorb light, they serve as ideal endogenous contrast agents for photoacoustic imaging of highly vascularized, rich, or pigmented tissues [45]. Quantitative spectral separation of these molecules is challenging due to spectral coloring effects with depth, but it has been recently shown that the unmixing accuracy can be significantly improved by proper algorithmic treatment of data [46]. Anatomical imaging of vascularity combined with functional imaging of hemoglobin levels and oxygen saturation, all performed with photoacoustics, were shown to assist in the detection, staging, and treatment monitoring of various cancers [45], including, but not limited to prostate, thyroid, and breast cancer and melanoma [47-48] as well as for clinical grading for benign diseases, such as inflammatory bowel disease [49-50]. Other clinical applications include real-time measurement of blood flow and tissue temperature. Combined measurements of flow and hemoglobin concentration can be used to assess oxygen consumption and tissue metabolism. These factors are important for determining tumor aggressiveness, tissue healing, and viability [51], as well as for prognostication of several ophthalmic diseases.

Photoacoustic imaging is also an ideal for surgical navigation and biopsy guidance. Several groups have performed phantom studies to assess the feasibility of using acoustic-resolution photoacoustic imaging to accurately locate and identify features of interest, such as blood vessels, nerves, and tendons. These phantom studies mimicked endonasal endoscopic neurosurgeries near the internal carotid arteries [52], the administration of local nerve blocks without causing intraneural damage [53], and teleoperated surgeries using the da Vinci robot [54]. In these approaches, an optical fiber (attached or adjacent to the surgical tool) was used to deliver light into the region of interest while a standard ultrasound probe was placed distally. Both ultrasound and photoacoustic images were

reconstructed with either conventional delay-and-sum beamforming or the short lag spatial coherence technique.

Some preclinical works utilized a probe consisting of optical fibers integrated on top of a medical transducer [55], or a device combining ultrasound, photoacoustic and fluorescence imaging [56]. These photoacoustic devices were used for assessing the viability of different tissues and to images cancer in the lymph nodes. Animal experiments have shown the ability of these devices to accurately guide surgery and biopsies to relevant disease foci and to monitor the local delivery of photoacoustic agents.

Other works have investigated a virtual intraoperative surgical photoacoustic microscope [57] used for demonstrating guided needle insertion and retraction. While the resolution achieved here is superior the small field-of-view and penetration depth might limit its clinical use.

Like other imaging modalities, photoacoustics provides limited molecular information in the absence of targeted contrast agents. Consequently, fluorophores are often dually used as photoacoustic agents, of which biocompatible, highly-absorbing NIR dyes are preferred. While only a handful of dyes are currently approved for clinical use, many have been tested preclinically and are expected to receive clinical approval over the next few years. The majority of these exogenous contrast agents are used for tumor delineation. For example, trastuzumab labeled with Black Hole Quencher 3 or fluorescein has been tested by both photoacoustic and fluorescence imaging to assess HER2 overexpression in breast cancer diagnosis, margin assessment, and surgical guidance [58]. Another is the gastrin-releasing peptide receptor targeted photoacoustic agent for prostate cancer imaging [59]. *In vivo* results of both works have shown high resolution and penetration depth, and the ability to provide tomographic views of cancer lesions, demonstrating the advantages of photoacoustics over fluorescence. Finally, Tummers *et al.* have recently addressed the operative management of pancreatic ductal adenocarcinoma (PDAC) by using EGFR-specific cetuximab-IRDye800 as a targeted dual fluorescence and photoacoustics agent [60]. In a first-in-human study in a small cohort of patients undergoing surgical resection for pancreatic cancer. Fluorescence imaging and photoacoustic imaging successfully delineated the tumor region compared to its surroundings during surgery.

In conclusion, photoacoustics has immense potential for surgical guidance because it is portable, can provide real-time imaging at high resolution and penetration depth uses both endogenous and

exogenous contrast agents, and uses nonionizing radiation. Multiple systems and contrast agents have begun to emerge in recent years, and much current work is focused on *ex vivo* and phantom studies to characterize these systems.

Another emerging medical imaging technique that could prove valuable in surgical navigation is thermoacoustic imaging (also called radio-frequency (RF)-acoustics) [61]. Thermoacoustics is mechanistically very similar to photoacoustics, but far-infrared light to ultra-high frequency radio waves are used for excitation instead of NIR light. Since tissue is mostly transparent at these wavelengths and scattering is minimal, a few-fold increase in imaging depth is usually achieved compared to photoacoustics. However, the low tissue absorption, particularly in the longer wavelength regime, results in low endogenous contrast, which explains why this modality has received little attention thus far. However, thermoacoustic contrast stems from differences in dielectric properties, which have been harnessed in developing some interesting exogenous contrast agents [62]. The most common agents are magnetic or superparamagnetic nanoparticles (such as Fe_3O_4), some of which are already FDA approved for clinical use. Another interesting class of agents includes polar molecules like sucrose or ionic solutions like saline [63], which can be encapsulated into nanostructures and functionalized to target various molecular targets.

Nuclear imaging modalities

Nuclear imaging modalities, single photon emission computed tomography (SPECT) and positron emission tomography [64], are non-invasive whole-body imaging technologies, valued for their sensitivity and limitless depth of penetration. 2D scintigraphy and 3D SPECT employ nuclides like Technetium-99m (^{99m}Tc, $t_{1/2}$ 6 h) and Indium-111 (^{111}In, $t_{1/2}$ 2.8 days), that emit gamma photons of varying energies, which are then detected by gamma cameras. The sensitivity of SPECT (10^{-10}-10^{-11} mol/L) is further surpassed by PET ($10^{-11}10^{-12}$ mol/L), which additionally yields quantitative information [3]. PET isotopes like Fluorine-18 (^{18}F, $t_{1/2}$ 1.8 h) and Iodine-124 (^{124}I, $t_{1/2}$ 100.3 h) decay by emitting positrons (beta particulate emissions) that travel short distances before colliding with electrons in the surrounding tissues. This results in the production and coincident detection of two gamma rays (of 511 KeV), 180 degrees apart.

Pre-operative nuclear imaging has a strong record in providing surgeons with diagnostic information, therapy follow-up as well as providing a road map to plan the most optimal surgical

approach towards resection. For example, intravenously administered ^{18}Fluorodeoxyglucose (^{18}F-FDG), the most widely used clinical PET tracer enables measurement of glucose transport and glycolysis, which are up-regulated across a range of malignancies. Moreover, scintigraphy and SPECT imaging are performed routinely prior to intra-operative assessment of radiotracer localization using radio-guided surgery (RGS). For intraoperative localization of gamma emitting tracers in real-time, first conceived in 1949, RGS typically uses a hand-held gamma radio-detection probe in conjunction with SPECT isotopes that are injected directly into or next to suspected lesions prior to surgery (Fig. 1h). RGS has become standard-of-care in a defined set of surgical procedures. These include SLN biopsy (SLNB) in breast, head and neck cancer and malignant melanoma and radioguided occult (impalpable) lesion localization (ROLL) in breast cancer, providing surgeons with valuable real-time information regarding the location of SLN for further pathological evaluation and extent of disease respectively. ^{99m}Tc in various colloidal forms (e.g. ^{99m}Tc-sulfur colloid) has been most commonly used in the clinic for SLNB [65], favored for its wide availability and the relative low absorbed dose associated with its use. Meta-analysis of numerous clinical trials have shown that RGS used in conjunction with optical dyes leads to higher rates of SLN detection (>91%) vs when blue dyes used on their own (~83%) and also reduces false negatives [66].

Another interesting application of RGS is the use of ^{125}I-seeds to mark small, impalpable lesions, prior to surgery in patients with breast cancer and as an alternative to wire-guided localization. Intraoperative, gamma tracing of these ^{125}I-seeds allow the surgeon to precisely localize and excise the lesions. Advantageously, this approach can be used in conjunction with injection of colloidal ^{99m}Tc for SLN identification due to the unique gamma energy signatures of both isotopes [67]. The use of ^{125}I-seeds provides a more comfortable alternative to patients in comparison to the wire-guided approach and reduces conflicts between radiologist and surgeons.

The lack of image documentation in RGS with gamma probes however, fueled the development of hand-held and portable small gamma cameras (Fig. 1i), such as the Sentinella S102® system. These can cover larger fields of view (FOV), can increase surgical accuracy and are increasingly being included in the RGS workflow. Vidal-Sicart *et al.* reported the higher detection of SLN in patients with melanoma, breast and gynecologic cancers with a portable γ-camera used in conjunction with a hand-held γ-probe (95%) versus when the latter was used on its own (75%)

[68]. The advent of freehand SPECT systems such as the declipse®SPECT that enable 3D visualization of radioactivity overlayed on a real-time video of the surgical field, provide virtual or augmented reality information with the potential to further improve surgical navigation [69].

Though RGS using PET tracers is not as well established, it has been evaluated in the clinic to guide resection, assess lymph node metastasis in a range of malignancies, and locoregional nodal status for initial staging of breast cancer [64, 70]. Most of these studies have used ^{18}F-FDG, typically administered a few hours prior to surgery. A dedicated PET high-energy gamma probe, designed specifically to detect the 511 keV photons, has been evaluated in numerous clinical studies. However, a recent study by Orsaria *et al.* concluded that hand-held PET gamma probe-guided surgery with ^{18}F-FDG performed poorly in evaluating axillary lymph nodes due to high background gamma levels from local and distant parts of the body [64]. PET tracers have also been evaluated with a hand-held positron detection probe, although the short range of beta emissions (typically 2-3 mm in soft tissue for ^{18}F-labeled agents) precludes the detection of deep-seated lesions, detection of beta emissions provides greater specificity (higher TBRs) in the localization of superficial tumor deposits. However, their use is limited by a small FOV, low resolution, and long acquisition times. In 2013, Singh *et al.* reported a prototype of a handheld beta-imaging intraoperative probe to allow visual mapping of beta emissions. This was evaluated *ex vivo* on rabbit tumor tissue, and was reported to have high spatial resolution and sensitivity, but is yet to be evaluated *in vivo* and in the clinic [71]. Ongoing hardware and software development for these PET-based RGS technologies is expected to enhance spatial resolution and improve disease detection. The success of these studies, however, has been limited not only by current probe design, but also by limitations in the commonly used ^{18}F-FDG tracer itself, including its inability to distinguish inflammation from malignant processes, high accumulation in the urinary tract due to excretion, and high background in glucose-avid tissues such as the brain and heart. Additionally, the ability of the hand-held probes to detect lesions is variable and dependent on the ^{18}F-FDG avidity of the tumor. In this regard, other radiotracers can overcome limitations of ^{18}F-FDG. Advantageously, numerous small-molecule and immunoPET tracers are in preclinical development in addition to the many that are already FDA approved.

An interesting feature of positron-emitting PET tracers is that they generate Cerenkov luminescence, produced when a charged β-particle traverses a dielectric medium at a speed greater

than the velocity of light in the same medium. The feasibility of clinical Cerenkov imaging was firstly demonstrated by Thorek et al. for ^{18}F-FDG and Spinelli et al. using 131-Iodine [72-73]. Because Cerenkov is intrinsic to the PET tracer, it circumvents the need for an external excitation light source, avoids the depth limitations of excitation light, and uses compact imaging equipment. Although in its infancy, preclinical and early ex vivo clinical studies have demonstrated Cerenkov's utility in the identification of tumor (margins) and metastatic lymph nodes [74] or are currently underway (NCT02666079). Dependent on the detection of low levels of blue-weighted light, Cerenkov is susceptible to absorption and scatter and therefore lacks sensitivity and resolution, making it more suitable for visualization of superficial structures.

RGS could benefit significantly from a multimodal approach, combining for example the detection radioisotopes with fluorescence imaging. The in-depth and sensitive detection capabilities of emitted gamma-rays could synergize well with the more superficial, but higher resolution fluorescent signals. This concept was first illustrated using a self-assembled complex of ^{99m}Tc-albumin radiocolloids and ICG for SLN biopsies [75-76] and is also being applied to the radical resection of renal cell carcinoma with a dual-labeled (^{111}In and IRdye800CW) Girentuximab monoclonal antibody [77] (NCT02497599). Several groups are working to generate true hybrid detection modalities for intra-operative use, e.g. combining fluorescence and gamma tracing [78].

Deep learning

One technology that could fundamentally change how diagnostic images are analyzed and read over the coming years is deep learning. Deep learning is a type of machine learning that has the potential to not only improve diagnostic accuracy but significantly reduce the workload and backlog faced by physicians and radiologists. A particularly effective deep learning model for image classification is the convolutional neural network (CNN), a type of artificial neural network, which is inspired by the hierarchical connectivity of neurons in the brain [79-81]. Previously, most machine learning algorithms require images to undergo significant pre-processing prior to analysis and classification. For example, a radiologist or surgeon would first need to contour, or segment, organs or lesions of interest in an image by hand and annotate objects of interest based on shape, texture, or other distinguishing characteristics. By contrast, a CNN can directly use an unannotated raw image as an input and classify the image (for example, for the presence or absence of a lesion)

based on comparison with a large dataset of pre-classified images [79-81]. Deep learning for computer-aided diagnosis has been demonstrated on images taken in the pre-operative setting: for example, to detect lung nodules in chest X-rays [82-84], differentiate malignant from benign lung lesions on CT [85], detect masses and microcalcifications in mammography [86-89], and segment brain tumors in MRI imaging [90]. CNN models have also demonstrated utility in minor interventional procedures, such as in determining whether a peripherally inserted central catheter (PICC) has been positioned correctly based on chest X-rays [91]. However, as these algorithms become more efficient and processing times rapidly decrease, the prospect of using deep learning to guide the surgeon intraoperatively in real-time is already on the horizon. For example, a recent study trained a CNN model to detect colorectal polyps in colonoscopy images[92]. The training and validation sets contained a total of 8641 images: half contained polyps of all sizes and morphologies, and half contained no polyps. The classification had an accuracy of 91% and an area under the curve (AUC) of 0.96. Because their CNN model can process 170 images per second, it has the potential to identify polyps in real-time from the live video stream during the colonoscopy procedure. In another study, a CNN model was trained on 7894 confocal laser microendoscopy (CLE) [21] images from 116 video sequences of the oral cavity (both benign and cancerous regions) in 12 patients with oral squamous cell carcinoma, and was found to have an accuracy of 88.3% and an AUC of 0.96 [93]. By subdividing the images into smaller patches, the investigators were able to reduce the complexity of the computation, which can enable faster processing with the eventual goal of a CLE system that can accurately identify high-risk regions in real-time for immediate biopsy and histologic evaluation. Similar deep learning technologies could be envisioned for real-time lesion identification in more complex surgical procedures. Furthermore, CNN models have been used that can recognize and potentially even track the surgical instrument being used (grasper, hook, scissors) during laparoscopic procedures as well as the type of surgical action (blunt dissection, cutting, suturing) being carried out [94-96]. One can foresee the use of such algorithms for training assistive or semi-autonomous robots in picking up the appropriate surgical instrument and correctly performing a set of surgical tasks. In addition, deep learning algorithms that can analyze video in real-time could be combined with augmented reality surgical navigation systems and used in robot-assisted surgeries to alert or even resist the surgeon when an impending action could compromise critical blood vessels or anatomic structures.

Conclusion and Future Perspectives

Over the last few decades, a wide variety of new technologies have emerged for intraoperative guidance that mostly relies on molecular imaging principles. Because of their higher sensitivity, higher resolution, and real-time capabilities, they have the potential to overcome the current limitations of state-of-the-art modalities such as CT, MRI and US. The ease of use and current availability of tracers, either already clinically approved or close to clinical translation, places fluorescence and radio-guided approaches as the most advanced. Nevertheless, although we can be highly enthusiastic about the first results, large multicentric clinical trials still need to demonstrate their impact on surgical outcome and patient survival. The respective shortcomings of optical and nuclear approaches can be overcome with further advancements towards hybrid modalities, e.g. nuclear/fluorescence or photoacoustics/fluorescence, or with other experimental technologies. However, the latter still need to prove their utility in the clinic and the specific use-cases for which they can be of added-value remain to be identified. In the future, it can be expected that deep learning approaches will be used more frequently with intraoperative imaging to automatize detection of tissues of interest and further improve signal-to-noise ratio and superior recognition of structures, thus supporting the surgeon tremendously.

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Author contributions

All authors wrote, reviewed and approved the manuscript.

References

1. Prince AC, McGee AS, Siegel H, Rosenthal EL, Behnke NK, Warram JM (2017) Evaluation of fluorescence-guided surgery agents in a murine model of soft tissue fibrosarcoma. J Surg Oncol.
2. Rana M, Zapf A, Kuehle M, Gellrich NC, Eckardt AM (2012) Clinical evaluation of an autofluorescence diagnostic device for oral cancer detection: a prospective randomized diagnostic study. Eur J Cancer Prev 21:460-466.

3. James ML, Gambhir SS (2012) A molecular imaging primer: modalities, imaging agents, and applications. Physiol Rev 92:897-965.
4. Weissleder R, Ntziachristos V (2003) Shedding light onto live molecular targets. Nature medicine 9:123-128.
5. Scheuer W, van Dam GM, Dobosz M, Schwaiger M, Ntziachristos V (2012) Drug-based optical agents: infiltrating clinics at lower risk. Sci Transl Med 4:134ps111.
6. Koch M, Ntziachristos V (2016) Advancing Surgical Vision with Fluorescence Imaging. Annu Rev Med 67:153-164.
7. AV DS, Lin H, Henderson ER, Samkoe KS, Pogue BW (2016) Review of fluorescence guided surgery systems: identification of key performance capabilities beyond indocyanine green imaging. J Biomed Opt 21:80901.
8. Harmsen S, Teraphongphom N, Tweedle MF, Basilion JP, Rosenthal EL (2017) Optical Surgical Navigation for Precision in Tumor Resections. Mol Imaging Biol 19:357-362.
9. Mondal SB, Gao S, Zhu N, et al. (2015) Binocular Goggle Augmented Imaging and Navigation System provides real-time fluorescence image guidance for tumor resection and sentinel lymph node mapping. Sci Rep 5:12117.
10. Ringhausen E, Wang T, Pitts J, Sarder P, Akers WJ (2016) Evaluation of Dynamic Optical Projection of Acquired Luminescence for Sentinel Lymph Node Biopsy in Large Animals. Technol Cancer Res Treat 15:787-795.
11. Xu M, Wang LV (2006) Photoacoustic imaging in biomedicine. Review of scientific instruments 77:041101.
12. Hong G, Lee JC, Robinson JT, et al. (2012) Multifunctional in vivo vascular imaging using near-infrared II fluorescence. Nat Med 18:1841-1846.
13. Schaafsma BE, Mieog JS, Hutteman M, et al. (2011) The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol 104:323-332.
14. Tummers QR, Hoogstins CE, Peters AA, et al. (2015) The Value of Intraoperative Near-Infrared Fluorescence Imaging Based on Enhanced Permeability and Retention of Indocyanine Green: Feasibility and False-Positives in Ovarian Cancer. PLoS One 10:e0129766.
15. Stummer W, Pichlmeier U, Meinel T, et al. (2006) Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol 7:392-401.
16. Grossman HB, Stenzl A, Fradet Y, et al. (2012) Long-term decrease in bladder cancer recurrence with hexaminolevulinate enabled fluorescence cystoscopy. J Urol 188:58-62.
17. Boonstra MC, Prakash J, Van De Velde CJ, et al. (2015) Stromal Targets for Fluorescent-Guided Oncologic Surgery. Front Oncol 5:254.
18. van Dam GM, Themelis G, Crane LM, et al. (2011) Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results. Nat Med 17:1315-1319.
19. Nagaya T, Nakamura YA, Choyke PL, Kobayashi H (2017) Fluorescence-Guided Surgery. Frontiers in oncology 7:314.
20. Rosenthal EL, Moore LS, Tipirneni K, et al. (2017) Sensitivity and Specificity of Cetuximab-IRDye800CW to Identify Regional Metastatic Disease in Head and Neck Cancer. Clin Cancer Res 23:4744-4752.
21. Rosenthal EL, Warram JM, de Boer E, et al. (2015) Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. Clin Cancer Res 21:3658-3666.
22. Harlaar NJ, Koller M, de Jongh SJ, et al. (2016) Molecular fluorescence-guided surgery of peritoneal carcinomatosis of colorectal origin: a single-centre feasibility study. The Lancet Gastroenterology & Hepatology 1:283-290.

23. Lamberts LE, Koch M, de Jong JS, et al. (2016) Tumor-Specific Uptake of Fluorescent Bevacizumab-IRDye800CW Microdosing in Patients with Primary Breast Cancer: A Phase I Feasibility Study. Clin Cancer Res.
24. Warram JM, de Boer E, van Dam GM, et al. (2016) Fluorescence imaging to localize head and neck squamous cell carcinoma for enhanced pathological assessment. J Pathol Clin Res 2:104-112.
25. Elliott JT, Dsouza AV, Marra K, Pogue BW, Roberts DW, Paulsen KD (2016) Microdose fluorescence imaging of ABY-029 on an operating microscope adapted by custom illumination and imaging modules. Biomed Opt Express 7:3280-3288.
26. Debie P, Vanhoeij M, Poortmans N, et al. (2017) Improved Debulking of Peritoneal Tumor Implants by Near-Infrared Fluorescent Nanobody Image Guidance in an Experimental Mouse Model. Mol Imaging Biol.
27. Handgraaf HJM, Boonstra MC, Prevoo H, et al. (2017) Real-time near-infrared fluorescence imaging using cRGD-ZW800-1 for intraoperative visualization of multiple cancer types. Oncotarget 8:21054-21066.
28. Christensen A, Juhl K, Persson M, et al. (2017) uPAR-targeted optical near-infrared (NIR) fluorescence imaging and PET for image-guided surgery in head and neck cancer: proof-of-concept in orthotopic xenograft model. Oncotarget 8:15407-15419.
29. Fidel J, Kennedy KC, Dernell WS, et al. (2015) Preclinical Validation of the Utility of BLZ-100 in Providing Fluorescence Contrast for Imaging Spontaneous Solid Tumors. Cancer Res 75:4283-4291.
30. Burggraaf J, Kamerling IM, Gordon PB, et al. (2015) Detection of colorectal polyps in humans using an intravenously administered fluorescent peptide targeted against c-Met. Nat Med 21:955-961.
31. Sturm MB, Joshi BP, Lu S, et al. (2013) Targeted imaging of esophageal neoplasia with a fluorescently labeled peptide: first-in-human results. Science translational medicine 5:184ra161.
32. Whitley MJ, Cardona DM, Lazarides AL, et al. (2016) A mouse-human phase 1 co-clinical trial of a protease-activated fluorescent probe for imaging cancer. Sci Transl Med 8:320ra324.
33. Barth CW, Gibbs SL (2017) Direct Administration of Nerve-Specific Contrast to Improve Nerve Sparing Radical Prostatectomy. Theranostics 7:573-593.
34. KleinJan GH, Buckle T, van Willigen DM, et al. (2014) Fluorescent lectins for local in vivo visualization of peripheral nerves. Molecules 19:9876-9892.
35. Garai E, Sensarn S, Zavaleta CL, et al. (2015) A real-time clinical endoscopic system for intraluminal, multiplexed imaging of surface-enhanced Raman scattering nanoparticles. PLoS One 10:e0123185.
36. Jokerst JV, Pohling C, Gambhir SS (2013) Molecular imaging with surface-enhanced Raman spectroscopy nanoparticle reporters. MRS Bull 38.
37. Moore LS, Rosenthal EL, Chung TK, et al. (2017) Characterizing the Utility and Limitations of Repurposing an Open-Field Optical Imaging Device for Fluorescence-Guided Surgery in Head and Neck Cancer Patients. J Nucl Med 58:246-251.
38. Zavaleta CL, Kircher MF, Gambhir SS (2011) Raman's "effect" on molecular imaging. J Nucl Med 52:1839-1844.
39. Kang S, Wang Y, Reder NP, Liu JT (2016) Multiplexed Molecular Imaging of Biomarker-Targeted SERS Nanoparticles on Fresh Tissue Specimens with Channel-Compressed Spectrometry. PLoS One 11:e0163473.
40. Vendrell M, Maiti KK, Dhaliwal K, Chang YT (2013) Surface-enhanced Raman scattering in cancer detection and imaging. Trends in biotechnology 31:249-257.
41. Thakor AS, Luong R, Paulmurugan R, et al. (2011) The fate and toxicity of Raman-active silica-gold nanoparticles in mice. Sci Transl Med 3:79ra33.
42. Rogalla S, Contag CH (2015) Early Cancer Detection at the Epithelial Surface. Cancer J 21:179-187.

43. Harmsen S, Huang R, Wall MA, et al. (2015) Surface-enhanced resonance Raman scattering nanostars for high-precision cancer imaging. Sci Transl Med 7:271ra277.
44. Taruttis A, Ntziachristos V (2015) Advances in real-time multispectral optoacoustic imaging and its applications. Nature Photonics 9:219.
45. Ntziachristos V, Razansky D (2010) Molecular imaging by means of multispectral optoacoustic tomography (MSOT). Chemical reviews 110:2783-2794.
46. Tzoumas S, Nunes A, Olefir I, et al. (2016) Eigenspectra optoacoustic tomography achieves quantitative blood oxygenation imaging deep in tissues. Nature communications 7:ncomms12121.
47. Valluru KS, Willmann JK (2016) Clinical photoacoustic imaging of cancer. Ultrasonography 35:267.
48. Diot G, Metz S, Noske A, et al. (2017) Multispectral Optoacoustic Tomography (MSOT) of Human Breast Cancer. Clinical Cancer Research 23:6912-6922.
49. Knieling F, Neufert C, Hartmann A, et al. (2017) Multispectral Optoacoustic Tomography for Assessment of Crohn's Disease Activity. N Engl J Med 376:1292-1294.
50. Knieling F, Neufert C, Hartmann A, et al. (2017) Multispectral optoacoustic tomography for assessment of Crohn's disease activity. New England Journal of Medicine 376:1292-1294.
51. van den Berg P, Daoudi K, Steenbergen W (2015) Review of photoacoustic flow imaging: its current state and its promises. Photoacoustics 3:89-99.
52. Bell MAL, Ostrowski AK, Li K, Kazanzides P, Boctor EM (2015) Localization of transcranial targets for photoacoustic-guided endonasal surgeries. Photoacoustics 3:78-87.
53. Mari JM, Xia W, West SJ, Desjardins AE (2015) Interventional multispectral photoacoustic imaging with a clinical ultrasound probe for discriminating nerves and tendons: an ex vivo pilot study. Journal of biomedical optics 20:110503-110503.
54. Gandhi N, Allard M, Kim S, Kazanzides P, Bell MAL (2017) Photoacoustic-based approach to surgical guidance performed with and without a da Vinci robot. Journal of Biomedical Optics 22:121606.
55. Dima A, Gateau J, Claussen J, Wilhelm D, Ntziachristos V (2013) Optoacoustic imaging of blood perfusion: techniques for intraoperative tissue viability assessment. Journal of biophotonics 6:485-492.
56. Kang J, Chang JH, Kim SM, et al. (2017) Real-time sentinel lymph node biopsy guidance using combined ultrasound, photoacoustic, fluorescence imaging: in vivo proof-of-principle and validation with nodal obstruction. Scientific Reports 7:45008.
57. Lee C, Lee D, Zhou Q, Kim J, Kim C (2015) Real-time Near-infrared Virtual Intraoperative Surgical Photoacoustic Microscopy. Photoacoustics 3:100-106.
58. Maeda A, Bu J, Chen J, Zheng G, DaCosta RS (2015) Dual in vivo photoacoustic and fluorescence imaging of HER2 expression in breast tumors for diagnosis, margin assessment, and surgical guidance. Molecular imaging 14:7290.2014. 00043.
59. Levi J, Sathirachinda A, Gambhir SS (2014) A high-affinity, high-stability photoacoustic agent for imaging gastrin-releasing peptide receptor in prostate cancer. Clinical Cancer Research 20:3721-3729.
60. Tummers WS MS, Teraphongphom N, Gomez A, Steinberg I, Huland DM , Hong S, Kothapalli SR, Hasan A, Ertsey R, Bonsing BA, Vahrmeijer AL, Swijnenburg RJ, Longacre TA, Fisher G, Gambhir SS, Poultsides GA, Rosenthal EL (2018) Intraoperative Pancreatic Cancer Detection Using Multimodality Molecular Imaging. Annals of surgical oncology.
61. Kruger RA, Kiser WL, Miller KD, et al. (2000) Thermoacoustic CT: Imaging principles [abstract].
62. Wu D, Huang L, Jiang MS, Jiang H (2014) Contrast Agents for Photoacoustic and Thermoacoustic Imaging: A Review. Int J Mol Sci 15:23616-23639.
63. Ogunlade O, Beard P (2015) Exogenous contrast agents for thermoacoustic imaging: an investigation into the underlying sources of contrast. Medical physics 42:170-181.
64. Orsaria P, Chiaravalloti A, Fiorentini A, et al. (2017) PET Probe-Guided Surgery in Patients with Breast Cancer: Proposal for a Methodological Approach. In vivo 31:101-110.

65. Povoski SP, Neff RL, Mojzisik CM, et al. (2009) A comprehensive overview of radioguided surgery using gamma detection probe technology. World journal of surgical oncology 7:11.
66. Kim T, Giuliano AE, Lyman GH (2006) Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: a metaanalysis. Cancer 106:4-16.
67. Pouw B, van der Ploeg IM, Muller SH, et al. (2015) Simultaneous use of an (125)I-seed to guide tumour excision and (99m)Tc-nanocolloid for sentinel node biopsy in non-palpable breast-conserving surgery. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology 41:71-78.
68. Vidal-Sicart S, Paredes P, Zanon G, et al. (2010) Added value of intraoperative real-time imaging in searches for difficult-to-locate sentinel nodes. J Nucl Med 51:1219-1225.
69. KleinJan GH, Karakullukcu B, Klop WMC, Engelen T, van den Berg NS, van Leeuwen FWB (2017) Introducing navigation during melanoma-related sentinel lymph node procedures in the head-and-neck region. EJNMMI research 7:65.
70. Strong VE, Humm J, Russo P, et al. (2008) A novel method to localize antibody-targeted cancer deposits intraoperatively using handheld PET beta and gamma probes. Surgical endoscopy 22:386-391.
71. Singh B, Stack BC, Jr., Thacker S, et al. (2013) A hand-held beta imaging probe for FDG. Annals of nuclear medicine 27:203-208.
72. Thorek DL, Riedl CC, Grimm J (2014) Clinical Cerenkov luminescence imaging of (18)F-FDG. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 55:95-98.
73. Spinelli AE, Ferdeghini M, Cavedon C, et al. (2013) First human Cerenkography. Journal of biomedical optics 18:20502.
74. Grootendorst MR, Cariati M, Pinder SE, et al. (2017) Intraoperative Assessment of Tumor Resection Margins in Breast-Conserving Surgery Using (18)F-FDG Cerenkov Luminescence Imaging: A First-in-Human Feasibility Study. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 58:891-898.
75. Buckle T, van Leeuwen AC, Chin PT, et al. (2010) A self-assembled multimodal complex for combined pre- and intraoperative imaging of the sentinel lymph node. Nanotechnology 21:355101.
76. van der Poel HG, Buckle T, Brouwer OR, Valdes Olmos RA, van Leeuwen FW (2011) Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. European urology 60:826-833.
77. Hekman MC, Boerman OC, de Weijert M, et al. (2016) Targeted Dual-Modality Imaging in Renal Cell Carcinoma: An Ex Vivo Kidney Perfusion Study. Clinical cancer research : an official journal of the American Association for Cancer Research 22:4634-4642.
78. Bugby SL, Lees JE, Perkins AC (2017) Hybrid intraoperative imaging techniques in radioguided surgery: present clinical applications and future outlook. Clinical and translational imaging 5:323-341.
79. LeCun Y, Bengio Y, Hinton G (2015) Deep learning. Nature 521:436-444.
80. Suzuki K (2017) Overview of deep learning in medical imaging. Radiological physics and technology 10:257-273.
81. Shen D, Wu G, Suk H-I (2017) Deep learning in medical image analysis. Annual Review of Biomedical Engineering.
82. Lin J-S, Lo S-C, Hasegawa A, Freedman MT, Mun SK (1996) Reduction of false positives in lung nodule detection using a two-level neural classification. IEEE Transactions on Medical Imaging 15:206-217.
83. Lo S-C, Lou S-L, Lin J-S, Freedman MT, Chien MV, Mun SK (1995) Artificial convolution neural network techniques and applications for lung nodule detection. IEEE Transactions on Medical Imaging 14:711-718.

84. Lo S-CB, Chan H-P, Lin J-S, Li H, Freedman MT, Mun SK (1995) Artificial convolution neural network for medical image pattern recognition. Neural networks 8:1201-1214.
85. Suzuki K, Li F, Sone S, Doi K (2005) Computer-aided diagnostic scheme for distinction between benign and malignant nodules in thoracic low-dose CT by use of massive training artificial neural network. IEEE Transactions on Medical Imaging 24:1138-1150.
86. Lo S-CB, Li H, Wang Y, Kinnard L, Freedman MT (2002) A multiple circular path convolution neural network system for detection of mammographic masses. IEEE transactions on medical imaging 21:150-158.
87. Sahiner B, Chan H-P, Petrick N, et al. (1996) Classification of mass and normal breast tissue: a convolution neural network classifier with spatial domain and texture images. IEEE transactions on Medical Imaging 15:598-610.
88. Zhang W, Giger ML, Nishikawa RM, Schmidt RA (1996) An improved shift-invariant artificial neural network for computerized detection of clustered microcalcifications in digital mammograms. Medical Physics 23:595-601.
89. Zhang W, Giger ML, Wu Y, Nishikawa RM, Schmidt RA (1994) Computerized detection of clustered microcalcifications in digital mammograms using a shift-invariant artificial neural network. Medical Physics 21:517-524.
90. Pereira S, Pinto A, Alves V, Silva CA (2016) Brain tumor segmentation using convolutional neural networks in MRI images. IEEE transactions on medical imaging 35:1240-1251.
91. Lee H, Mansouri M, Tajmir S, Lev MH, Do S (2017) A Deep-Learning System for Fully-Automated Peripherally Inserted Central Catheter (PICC) Tip Detection. Journal of digital imaging:1-10.
92. Karnes WE, Alkayali T, Mittal M, et al. (2017) Su1642 Automated Polyp Detection Using Deep Learning: Leveling the Field. Gastrointestinal Endoscopy 85:AB376-AB377.
93. Aubreville M, Knipfer C, Oetter N, et al. (2017) Automatic Classification of Cancerous Tissue in Laserendomicroscopy Images of the Oral Cavity using Deep Learning. arXiv preprint arXiv:170301622.
94. Choi B, Jo K, Choi S, Choi J (2017) Surgical-tools detection based on Convolutional Neural Network in laparoscopic robot-assisted surgery [abstract]. 1756-1759P.
95. Petscharnig S, Schöffmann K (2017) Learning laparoscopic video shot classification for gynecological surgery. Multimedia Tools and Applications:1-19.
96. Pakhomov D, Premachandran V, Allan M, Azizian M, Navab N (2017) Deep Residual Learning for Instrument Segmentation in Robotic Surgery. arXiv preprint arXiv:170308580.

Figures

Figure 1: Principle and representative images obtained with distinct intraoperative imaging approaches: fluorescence imaging (a-e), photoacoustic imaging (d-e), Raman-spectrometry (f-g) and radio-guided surgery (h-i). Of note, the light source and detector for optical and photoacoustic imaging approaches are most often integrated in a single device. (b) **Fluorescence imaging** can be used to assess tumor margin in situ or (c) ex vivo on excised specimens (reproduced with permission from [21] and [23]) (e) **Raman imaging** can be displayed in pure spectra demonstrating the presence of Raman particles or displayed as a map either within a phantom tube mimicking the colon or directly inside the white-light camera view. (g) Combined **Photoacoustic** and ultrasonography can highlight molecular contrast overlaid on the anatomical view to guide

surgery or biopsy. Contrast is either endogenous (Hemoglobin) or based on absorbing or fluorescent agents. A triple modality hand held probe is used to probe Melanoma metastasis in a Rabbit's lymph node. (h) **Radioguided surgery** can be performed using hand-held gamma or beta probes to detect the presence of SPECT or PET based radiotracer for localization of sentinel lymph nodes and metastasis. (i) The use of hand-held probes can be supplemented with intra-operative gamma cameras as reported by Vidal-Sicart et al. [68]: pre-operative SPECT-CT of patient with malignant melanoma injected with 99mTc-nanocolloid and an intraoperative portable gamma camera being used (i, top panel, L to R). Gamma camera images of the surgical field before and after resection (i, bottom panel, L to R).

Figure

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