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Optical Surgical Navigation for Precision in Tumor Resections

Stefan Harmsen¹, Nutte Teraphongphom², Michael F. Tweedle³, James P. Babilion^{4,5,6}, and Eben L. Rosenthal^{7,8}

¹Department of Pediatrics, Stanford University, Stanford, CA, USA

²Department of Otolaryngology—Head and Neck Surgery, Stanford University, Stanford, CA, USA

³Department of Radiology, The Wright Center for Innovation in Biomedical Imaging, The Ohio State University College of Medicine, Columbus, OH, USA

⁴Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA

⁵Department of Radiology, Case Western Reserve University, Cleveland, OH, USA

⁶National Foundation for Cancer Research Center for Molecular Imaging, Case Western Reserve University, Cleveland, OH, USA

⁷Department of Otolaryngology—Head and Neck Surgery and Radiology, Stanford University, Stanford, CA, USA

⁸Ann and John Doerr Medical Director, Stanford Cancer Center, Stanford, CA, USA

Abstract

Optical imaging methods have significant potential as effective intraoperative tools to visualize tissues, cells, and biochemical events aimed at objective assessment of the tumor margin and guiding the surgeon to adequately resect the tumor while sparing critical tissues. The wide variety of approaches to guide resection, the range of parameters that they detect, and the interdisciplinary nature involving biology, chemistry, engineering, and medicine suggested that there was a need for an organization that could review, discuss, refine, and help prioritize methods to optimize patient care and pharmaceutical and instrument development. To address these issues, the World Molecular Imaging Society created the Optical Surgical Navigation (OSN) interest group to bring together scientists, engineers, and surgeons to develop the field to benefit patients. Here, we provide an overview of approaches currently under clinical investigation for optical surgical navigation and offer our perspective on upcoming strategies.

Keywords

Optical navigation; Surgery; Image guidance; Probes; Oncology

Correspondence to: Eben Rosenthal; elr@stanford.edu.

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Compliance with Ethical Standards

Conflict of Interests

S.H., N.T., M.F.T., and E.L.R. declare no conflicts of interests. J.P.B. has an interest in Akrotome Imaging Inc., a company developing probes in this space, and consults for Vergent Biosciences and LightPoint Medical Ltd.

Introduction

Traditionally, oncological surgeons rely on palpation, visual inspection, and experience—all of which are subjective determinants of tumor location. While these determinants enable bulk tumor assessment, they do not offer the sensitivity to adequately identify tumor margins. This results in high positive margin rates correlating with locoregional recurrence and poor patient outcomes [1, 2]. By contrast, optical image-guided surgery has been shown to reduce the positive margin rate and improve patient outcomes by offering an objective and sensitive assessment of the surgical margin status by enhancing tumor visualization in intraoperative scenarios [3, 4]. In fact, several optical imaging modalities have been translated to the clinic for this purpose [5, 6], and this is the focus of the interest group and of this overview. Strategies for optical surgical navigation constitute both label-free and contrast-enhanced approaches and include markers of diseased or normal tissue. The wide variety of optical methods for guided resection and range of parameters that they detect suggested that there was a need for an organization that could review, discuss, refine, and help prioritize these methods to optimize patient care. For this reason, we formed an interest group under the umbrella of the World Molecular Imaging Society, called Optical Surgical Navigation (OSN), and through this forum, we discuss improvements in imaging agents and hardware that can be used to advance the nascent technique of surgical imaging. The novel nature of these agents and tools has posed new regulatory processes that require OSN cooperation with federal agencies to explore. Here, we provide a summary of contrast-enhanced approaches that are applied clinically or are currently under clinical investigation and offer our perspectives on upcoming modalities for optical surgical navigation. Ideally, navigation would be low cost, highly selective for malignancies, non-toxic, and display a high tumor to background ratio. Furthermore, the regulatory path and use case should be designed to result in early successes with a limited number of patients hopefully showing clear patient benefits.

State of the Art

Non-Targeted Optical Imaging

Conventional, non-targeted fluorescent dyes have been used in intraoperative procedures for decades albeit for different applications than tumor margin detection. Methylene blue is used for sentinel node mapping but was recently shown to enable tumor margin detection during breast cancer surgery [7]. 5-Aminolevulinic acid (5-ALA) is a non-fluorescent precursor that is metabolized to the fluorescent protoporphyrin IX (PpIX) in neoplastic tissues [4] and is used for image-guided resection of high-grade gliomas. Compared to normal white light visualization, 5-ALA-induced PpIX fluorescence-guided glioma resection significantly increased complete resection rate improving functional outcome and progression-free survival [4]. Indocyanine green (ICG), which emits in the near-infrared (NIR) region, is the most widely applied fluorophore in oncologic image-guided surgery [8]. While initially used primarily for sentinel node mapping, ICG has been shown to enable visualization of margins in a variety of tumors including hepatocellular carcinomas [9] and extrahepatic hepatocellular metastases [10], colorectal cancer (CRC)-associated liver metastases [11],

breast cancers [12], and lung cancers [13], however, with low sensitivity [13]. High-dose ICG at 24 h before surgery has been studied in several cancers including brain and lung with improved results [14]. A clinical trial for intraoperative detection of solid tumor and residual disease using ICG is currently under way (NCT02280954).

Ligand-Based Tumor Targeting

Ligand-based approaches target tumor markers that are overexpressed within the tumor mass relative to adjacent healthy tissues. One of the earliest examples was folate-FITC (EC17) to guide debulking of folate receptor α -positive ovarian cancer to improve intraoperative staging and cytoreductive therapy by highlighting the true tumor extent compared to white light visualization alone [15]. OTL38—a folate analog conjugated to a NIR fluorescent dye (IR783)—has higher sensitivity and brightness relative to EC17 [16] and enabled detection of lesions up to 1 cm beneath the tissue surface [17]. A phase 2 clinical trial for intraoperative imaging of ovarian cancer with OTL38 was recently completed (NCT02317705).

While peptides such as cyclic RGD [18], bombesin [19], and octreotide [20] are commonly used as vectors for the delivery of radiotracers for visualization of tumors using positron emission tomography (PET) or single-photon emission computed tomography (SPECT), bombesin (BBN) is the first peptide co-labeled with a fluorescent dye for use in optical surgical navigation in glioblastoma patients (NCT02910804). BLZ100 (chlorotoxin-ICG) is another non-cleavable fluorescent peptide-based agent undergoing clinical evaluation (phase 1) for intraoperative margin visualization in glioma (NCT02234297) and sarcoma patients (NCT02464332). Although no dose limiting toxicity or adverse events have been observed and tumor-selective fluorescence imaging has been achieved in situ [21], histological correlatives have not been demonstrated to suggest the viability of this approach in humans. In a first-in-human pilot study, GE-137, a 26-amino acid cyclic peptide specific for c-MET labeled with a proprietary dye, was safe and enabled detection of polyps missed by other techniques [22]. In contrast to intravenous administration, first-in-human clinical validation of a topically applied, fluorescently labeled peptide, *ASY-FITC, visualized high-grade dysplasia and esophageal adenocarcinoma in Barrett's esophagus patients (NCT01391208) [23].

Antibodies have been widely studied for delivery of fluorophores to tumors, because they are produced under conditions suitable for human use and have a well-established safety profile but do present possible intellectual property issues, among others. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) targeting antibodies have been conjugated to IRDye800CW to image head and neck cancers in the operating room and in pathology (NCT01987375). To mitigate hypersensitivity reactions seen with cetuximab, the fully human EGFR-targeting antibody panitumumab-IRDye800CW is being evaluated for safety in head and neck cancer patients as well (NCT01998273) [24–26]. In preclinical studies, panitumumab-IRDye800CW was shown to have 8-fold greater EGFR-binding affinity resulting in a 2-fold increase in relative tumor fluorescence compared to cetuximab. A clinical trial evaluating the VEGF targeting bioconjugate bevacizumab-IRDye800CW (NCT01508572) was recently completed for tumor margin assessment in

breast cancer where it was shown to be safe for breast cancer guidance and confirmed tumor and tumor margin uptake [27]. A first-in-class dual-modality antibody-based probe ^{111}In -labeled girentuximab-IRDye800CW is currently being evaluated for tumor margin detection in clear cell renal cell carcinoma (ccRCC) (NCT02497599). This carbonic anhydrase IX (CAIX) targeting agent is uniquely aimed at combined radio-guided and NIR fluorescence-guided surgery.

Limitations of antibodies include their size (i.e., ~150 kDa; ~10 nm) which limits their use in low-grade gliomas, because they cannot cross the blood-brain barrier. Affibodies are small (~7 kDa), highly stable combinatorial engineered protein molecules with favorable pharmacokinetics. Compared to EGFR targeting antibody cetuximab, an EGFR-targeting affibody conjugated to IRDye800CW—ABY-029—achieved significantly higher accumulation at the margin in low-grade gliomas, preclinically [28]. A clinical trial aimed at determining if microdoses of ABY-029 lead to detectable signals in sampled tissues in patients with recurrent glioma has recently been initiated (NCT02901925).

Substrate-Based Probes for Activatable Contrast Enhancement

Substrate-based probes generally consist of a fluorophore, an enzymatically cleavable linker, and a quencher. In this way, the uncleaved construct remains in a quenched “off-state” until it becomes cleaved enzymatically, which significantly increases the fluorescence of the probe. The most prominent substrate-based probes in clinical trials are protease-activatable probes, because increased activity of proteases is a phenotype that is shared between many different types of cancer [29]. AVB-620 is a ratiometric activatable cell-penetrating peptide (RACPP) that upon cleavage produces a fluorescent signal. In a phase 1b clinical trial (NCT02391194), AVB-620 produced no adverse events and achieved intraoperative discrimination of tumor tissue to adjacent healthy tissues (www.avelasbio.com). A phase 2 clinical trial in breast cancer is expected to commence early 2017. Another activatable probe that recently entered phase 2 clinical trials (NCT02438358, NCT02584244) is LUM015. LUM015 consists of a commercially available quencher (QSY21) and Cy5 fluorophore, which are linked via a PEGylated GGRK peptide substrate for cathepsin K, L, and S [30]. In phase 1 (NCT01626066), LUM015 was shown to be well tolerated without showing probe-associated adverse effects. Further, in 15 patients of which 12 had soft tissue sarcomas (STS) and 3 ductal breast cancer, the probe demonstrated tumor-specific fluorescence. Of note, the clinical trial was not limited to the evaluation of the activatable probe alone but uniquely evaluated the combined performance of LUM015 with Lumicell’s handheld imaging device LUM 2.6 as well.

Nanoparticles

Nanoparticles have a large surface area to volume ratio that increases with decreasing diameter. For a nanoparticle with the size of an antibody thus enables more than 1500 surface modifications compared to about 10 substitutions for a similarly sized antibody before it loses its biological activity (cetuximab-IRDye800CW has a dye/protein ratio of 1.8 [31]). Thus, nanoparticles can be functionalized with multiple different imaging modalities (i.e., fluorescence, PET) and targeting agents. In fact, the first inorganic nanoparticle tested in humans constituted a dual-modal optical PET imaging, RGD-targeted silica nanoparticle

probe of ~6–7 nm in diameter [32]. The C-dots were the first in their class to be approved as a “drug” for clinical use (IND#110375) and were shown to be safe in humans [32]. These ultrasmall Cy5-embedded silica nanoparticles enabled whole-body tumor detection using PET and should enable intraoperative fluorescence-based tumor margin detection. While these ultrasmall silica nanoparticles, which are on the order of the glomerular filtration size cutoff, demonstrate bulk renal clearance, a typical disadvantage of nanoparticle probes (>10 nm) is uptake and long-term sequestration in the organs of the mononuclear phagocyte system (MPS) such as the liver and spleen, which limits their bioavailability [33].

Instrumentation

In recent years, many different probes have been developed and evaluated in the clinic for optical surgical navigation. However, just as many (intraoperative) fluorescence camera systems were developed and used to clinically evaluate these probes. Table 1 provides an overview of the fluorescence camera systems that were used to evaluate fluorescent probes. Recently, DSouza et al. [36] provided a comprehensive overview of current preclinical and clinical NIRF camera systems, their specifications, and performance characteristics [36] (with the exception of the FLARE™ [37], Mini-FLARE™ [38] system, Quest’s Artemis system, Lumicell’s LUM 2.6 imaging device, and Intuitive Surgical’s da Vinci system). While differences in costs and ergonomical features (e.g., working distance, mount type, etc.) were noted, more importantly, a 200-fold difference in sensitivity (at 800 nm) was observed among clinically applied systems [36]. Further, there were also significant differences among the excitation sources and wavelengths; some of which were suboptimal for the fluorescent probe used. The review by Dsouza et al. illustrates that the choice of fluorescent camera system (and how it is operated) may impact the results of clinical trials in terms of sensitivity and, potentially, compromise efficacy and/or outcome. Obviously, the fluorescent probe should be matched with the most sensitive camera system that best fits its optical properties. However, such a system may not be the obvious choice due to poor ergonomics, ease of use for surgeons, or high costs. Furthermore, it is unrealistic to test each individual fluorescent probe on all available commercial systems; therefore, it is generally considered important for the industry to characterize devices in a way that would provide quantified sensitivity data based on phantoms generated from clinical dyes to facilitate informed decision-making.

Future Perspectives and Outlook

Integration of photodynamic therapy (PDT), in which a photosensitizer produces reactive oxygen species to kill tumor cells upon light irradiation, could be applied after gross total resection and before closing the resection cavity to achieve supra-maximum cytoreduction. In a preliminary study where fluorescence-guided glioblastoma surgery was combined with PDT, a significant survival benefit was reported (52.8 weeks in the treatment group versus 24.6 weeks in the control group ($p < 0.01$)) [39]. Such a *theranostic* approach, where an agent provides intraoperative image guidance and therapy, can be a valuable treatment option for other cancers such as GI tract, head and neck, and skin cancers. A longer wavelength photoimmunotherapeutic (PIT) agent, RM1929 (cetuximab-IRDye700DX), is being explored to achieve better tissue penetration to treat head and neck cancers with PDT

(NCT02422979). It is foreseeable that future studies will be directed at the potential of combining delivery vectors with NIRF agents (e.g., IRDye800CW) and PDT agents (e.g., IRDye700DX) to enable intraoperative fluorescent-guided surgery and PDT, respectively, to achieve supra-maximal cytoreduction and improve survival.

Another important future direction is the improvement of patient outcome in terms of functional outcomes. For instance, while radical prostatectomy in patients with localized prostate cancer is often curative with low mortality, the morbidity that includes erectile dysfunction and incontinence associated with this operation because of nerve trauma is significant and has a major impact on the patients' quality of life [40]. To reduce morbidity associated with nerve damage, small molecule dyes and peptide-based dye conjugates have been developed and evaluated preclinically to highlight peripheral nerves during surgery [41, 42]. Combinations of "nerve paints" with "tumor paints" and potentially other tissue paints (e.g., lymphatic, vasculature) will enable intraoperative multiplexed visualization to accentuate critical structures collectively or individually in the surgical bed. Further, in patients with low-risk localized prostate cancer, it was shown that compared to active surveillance, disease progression was significantly less in patients treated with vascular-targeted PDT using padeliporfin [43]. In this phase 3 trial (NCT01310894), vascular-targeted photodynamic therapy was shown to be safe and an effective treatment for low-risk, localized prostate cancer, which might allow more men to consider a tissue-preserving approach and defer or avoid radical therapy and associated morbidities [43].

While several FDA 510(k)-cleared commercial systems already enable wide-field, two-channel imaging [36], multiplexed tissue visualization will require more than two-channel visualization and will require advancements in intraoperative imaging system and software design that enable combinations of wide-field, low-resolution systems with high-resolution, small field of view devices. Such advances would serve to link OSN with point-of-care pathology enabling localization and cellular/molecular characterization of cancers in the OR. In addition, advances in chemistry and instrumentation may extend the excitation and emission beyond the first NIR window (700–900 nm) into the so-called NIR-2 optical window (1000–1700 nm) to offer a broader window for imaging. Recently, a small molecule dye for NIR-2 imaging was reported that enabled imaging of structures at centimeter depths with higher resolution relative to first NIR window due to reduced tissue scattering in the NIR-2 window [44]. Combinations of instruments that image over a range of scales are being developed. One such a device is the dual-axis confocal (DAC) microscope coupled to fiber-based microendoscopes and wide-field systems. The handheld DAC microscope allows the surgeon to perform optical biopsies of suspected lesions or survey the resection bed during tumor resection to accelerate decision-making and improve precision [45].

Conclusion

Currently, clinical safety and feasibility studies are being performed with single-wavelength and ratiometric optical agents. While it is expected that such approaches will contribute to more precise cancer resection to improve patient functional and oncologic outcomes, to date there is no approved agent on the market in the USA, which makes the path to approval unclear. Given the lower return on investment for diagnostic agents compared to therapeutic

agents, clinical trials will require careful design to show patient benefit without an expansive number of patients or long-term endpoints. Further, while data has shown improved disease-free survival without a survival advantage in a multiinstitutional trial, which earned 5-ALA its approval in multiple countries, 5-ALA has yet to be approved in the US [4]. The OSN interest group is committed to an active dialog with regulatory agencies to identify an approach that will benefit our patients in a safe and effective manner.

While we are currently witnessing the clinical translation of the first wave of imaging agents and instrumentation to guide the surgeon in accomplishing more adequate tumor resections, tremendous opportunities lie ahead as evidenced by the breadth of optical imaging approaches currently being developed and evaluated preclinically. However, to maintain this pace, it is critical to introduce standardization and fully disclose parameters and specifications of the instrumentation used to enable comparison of the performance of novel probes or imaging systems relative to the current state of the art. Our forum has the unique opportunity to set these standards and work with the relevant organizations and authorities to implement them.

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Table 1

Probe and imaging systems

Probe	Indication	Camera system	Reference/Trial
5-ALA	Glioma	OPMI Neuro/NC4 system (Carl Zeiss)	[4]
Methylene blue	Breast cancer	Mini-FLARE™	[7]
ICG	Hepatocellular carcinoma	PDE (Hamamatsu)	[9]
	CRC liver metastases	Mini-FLARE™	[11]
	Breast cancer	FloCam (BioVision)	[12]
	Lung cancer	Artemis (Quest Medical Imaging) Iridium (VisionSense) SpectroPen	[13, 34]
EC17	Ovarian cancer	Custom-built multispectral system	[15]
OTL38	Ovarian cancer	Artemis (Quest Medical Imaging)	[17]
[⁶⁸ Ga]BBN-IRDye800CW	Glioblastoma	ND	NCT02910804
BLZ-100	Glioma, sarcoma	ND	NCT02234297;02464332
Cetuximab-IRDye800CW	Head and neck cancer	Luna Imaging System (Novadaq)	[35];NCT01987375
	Glioma	Pinpoint (Novadaq) /	NCT02855086
	Pancreatic Cancer	Explorer Air (SurgVision)	NCT02736578
Panitumumab-IRDye800CW	Head and neck cancer	Explorer Air (SurgVision)/ Pinpoint (Novadaq)	NCT02415881
Bevacizumab-IRDye800CW	Breast Cancer	Explorer Air (SurgVision)	[27];NCT01508572/02583568
	Pancreatic Cancer	ND	NCT02743975
[¹¹¹ In]girentuximab-IRDye800CW	Renal cell carcinoma	ND	NCT02497599
MDX1201-A488	Prostate Cancer	ND	NCT02048150
ABY-029	Recurrent glioma	ND	NCT02901925
AVB-620	Breast cancer	ND	NCT02391194
LUM015	Breast Cancer, STS	LUM 2.6	[30]; NCT01626066
C-dots	Metastatic melanoma	Artemis (Quest Medical Imaging)	[32]

ND: Not disclosed