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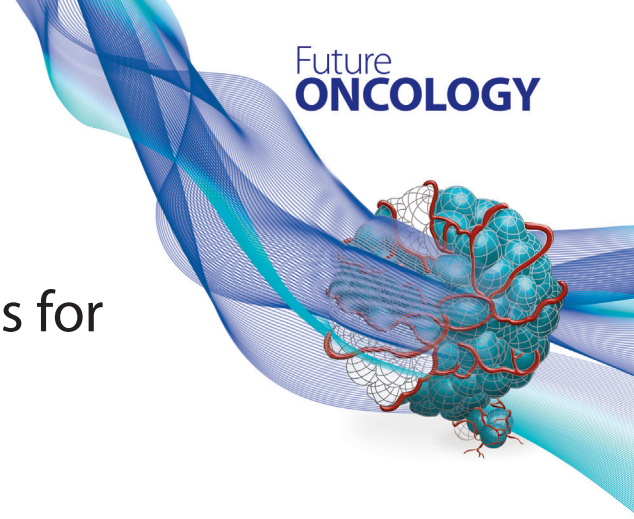

Intraoperative imaging techniques for glioma surgery

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Gliomas are CNS neoplasms that infiltrate the surrounding brain parenchyma, complicating their treatment. Tools that increase extent of resection while preventing neurological deficit are essential to improve prognosis of patients diagnosed with gliomas. Tools such as intraoperative MRI, ultrasound and fluorescence-guided microsurgery have been used in the surgical resection of CNS gliomas with the goal of maximizing extent of resection to improve patient outcomes. In addition, emerging experimental techniques, for example, optical coherence tomography and Raman spectroscopy are promising techniques which could 1 day add to the increasing armamentarium used in the surgical resection of CNS gliomas. Here, we present the potential advantages and limitations of these imaging techniques for the purposes of identifying gliomas in the operating room.

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KEYWORDS
• fluorescence-guided resection • intraoperative MRI • intraoperative ultrasound • optical coherence tomography • Raman spectroscopy

Gliomas are CNS neoplasms that infiltrate the surrounding brain parenchyma. This is a characteristic that complicates treatment of intrinsic brain tumors and is shared by low-grade (LGG, WHO grade II) and high-grade gliomas (HGGs, WHO grades III and IV). An increasing amount of evidence suggests that extent of resection is an important determinant of the outcome of patients diagnosed with LGGs and HGGs [1–6]. The determination of the tumor border using preoperative imaging techniques or intraoperative observations has proven to be challenging due to the heterogeneity of these tumor and because of the presence of cells infiltrating far away from contrast-enhancing regions seen in preoperative MRI studies; which are also invisible during microsurgical resection of gliomas. Hence, the development of techniques and tools to enhance the extent of surgical resection in patients with LGG and HGG is extremely important. Tools such as intraoperative MRI (iMRI), ultrasound (US), fluorescence-guided microsurgery, optical coherence tomography (OCT) and Raman spectroscopy (RS) are now part of the increasing armamentarium used in the surgical resection of gliomas with the goal of maximizing extent of resection to improve patient outcomes. Since the advent of these technologies, neurosurgeons have sought to implement them as part of the limited number of tools available to treat these difficult and devastating neoplasms. They have also been the subject of study of a multitude of investigators attempting to determine which is the best adjunct during surgical resection leading to survival and quality of life benefit.

Intraoperative US

The use of intraoperative US (IOUS) was introduced into the neurosurgical operating room in the 1950s initially as a lesioning device. When real-time ultrasonographic imaging capabilities were

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developed, the US became an important tool in the neurosurgical operating room [7–9]. Because of the great evolution in image quality and US equipment, IOUS has been used to treat a wide variety of intracranial tumors **(Figure 1)** [10–13]. IOUS is more available than other intraoperative imaging modalities because it is an inexpensive tool existent in places with limited resources. The utility of IOUS has been studied in glioma surgery. Woydt *et al*. studied whether IOUS was able to determine the presence of tumor tissue after surgical resection. This was correlated with histopathological analysis of tissue samples obtained after the surgeon had obtained what was determined to be a gross-total resection [14]. In a study including 38 patients with HGG and 9 with LGG, the authors concluded that if IOUS found a rim of more than 3 mm of hyperechogenicity, this was likely to be residual tumor, whereas if the rim of hyperechogenicity was smaller than 3 mm, the finding was not specific for the presence of residual tumor [14]. Other authors have found that the specificity and sensitivity were low before and after resection of the tumor, which can be caused by US artifact [15,16]. In a study of 156 patients that underwent surgery for HGG, Solheim *et al*. found that the effectiveness of 3D IOUS during surgical resection of gliomas is significantly affected by the quality of US images obtained. Additionally, the value of this imaging modality is decreased by the presence of hemorrhage and edema in the tumor and in the surrounding tissues [12]. However, IOUS provides real-time imaging of the neoplasm in an attempt to bypass the shift of tissues that occurs throughout surgery [13]. Other groups have evaluated the utility of a navigable 3D IOUS system in the resection of HGG. In

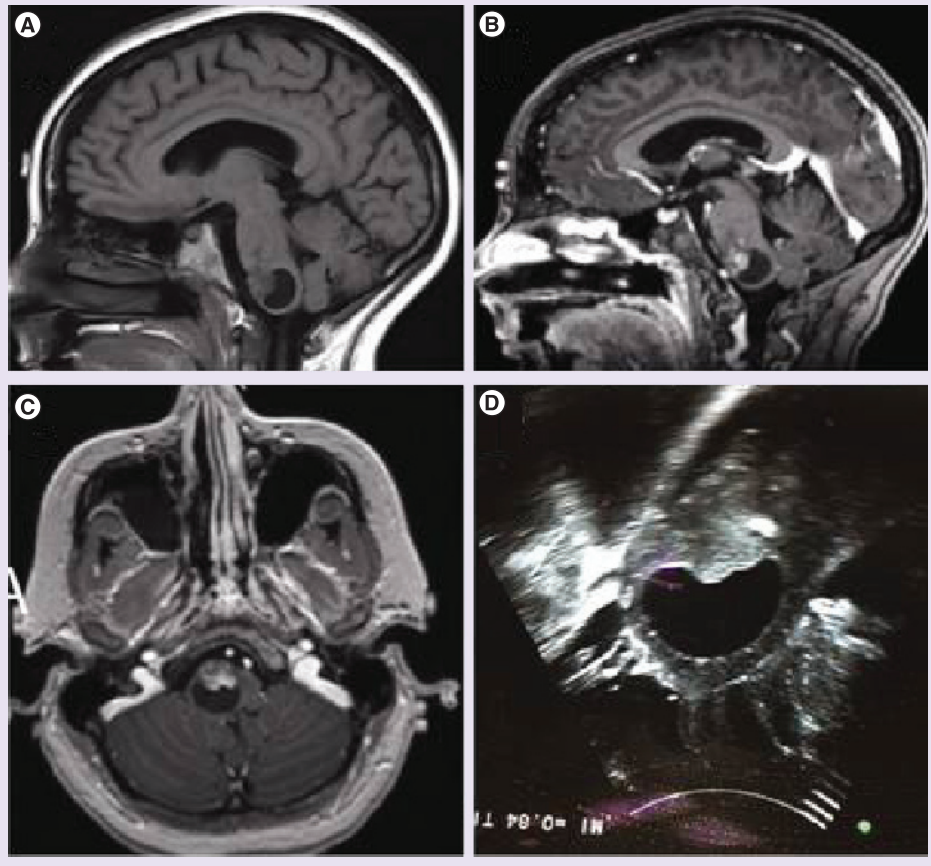

Figure 1. MRI scan and intraoperative ultrasound of a patient with a brainstem pilocytic astrocytoma. **(A)** Sagittal T1 without gadolinium; **(B)** Sagittal T1 with gadolinium; **(C)** axial T1 with gadolinium; **(D)** intraoperative ultrasound.

Table 1. Summary of significant studies evaluating different intraoperative imaging modalities for glioma surgery.

Study (year)	Type of study	Intraoperative imaging modality	Patient population	Conclusion	Ref.
Intraoperative ultrasound					
Moiyadi *et al.* (2015)	Prospective	Navigable US	88 gliomas, 32 GBM	Use of navigable US improved progression-free survival and overall survival	[27]
Saether *et al.* (2012)	Restrospective	IOUS	192 patients, GBM	Survival improved after implementation of IOUS	[28]
Jakola *et al.* (2011)	Retrospective	IOUS	63 high-grade glioma patients and 25 low-grade glioma patients	Use of IOUS preserves quality of life	[29]
Intraoperative MRI					
Senft *et al.* (2011)	Prospective, randomized controlled trial	iMRI	29 patients in iMRI group and 29 patients in conventional surgery group	More patients in the iMRI group had gross-total resection	[36]
Kubben *et al.* (2014)	Prospective randomized controlled trial (interim analysis)	Ultralow field iMRI vs neuronavigation	14 patients	No difference in extent of resection and survival	[38]
Coburger *et al.* (2015)	Retrospective	High-field iMRI	199 patients	Progression-free and overall survival in GBM patients undergoing surgery with iMRI have improved since it was initially introduced	[37]
Coburger *et al.* (2015)	Prospective	iMRI + 5-ALA	33 patients iMRI + 5-ALA, 144 controls iMRI alone	Significant increase in extent of resection in the iMRI + 5-ALA group compared with iMRI alone	[41]
Fluorescence-guided microsurgery					
Yamada *et al.* (2015)	Prospective	5-ALA + iMRI	97 patients, WHO III and IV gliomas	Improved identification of tumor tissue beyond contrast enhancement	[53]
Díez Valle *et al.* (2011)	Prospective	5-ALA	36 patients, GBM	Enhanced resection of contrast-enhancing tissue	[50]
Roder *et al.* (2014)	Retrospective	5-ALA vs iMRI	117 patients, GBM	iMRI is superior to 5-ALA guided surgery	[52]
Schucht *et al.* (2014)	Prospective	5-ALA + motor mapping	67 patients, GBM	Gross-total resection of enhancing tumor tissue in 73% of patients in spite of close proximity to corticospinal tract	[63]
Koc *et al.* (2008)	Prospective	Fluorescein	47 patients with fluorescein-guided surgery and 33 patients without FGM	Fluorescein use increased gross-total resection from 55% (no FGM) to 83% (FGM)	[58]
Neira *et al.* (2016)	Prospective	Fluorescein	32 patients, GBM	Fluorescein is a good marker of tumor tissue in contrast-enhancing and noncontrast enhancing regions	[59]

5-ALA: 5-aminolevulinic acid; FGM: Fluorescence-guided microsurgery; GBM: Glioblastoma; iMRI: Intraoperative MRI; IOUS: Intraoperative ultrasound; US: Ultrasound.

a series of 90 patients with intracranial tumors (of which 51 were HGG and 17 were LGG), Moiyadi *et al.* found that the use of the IOUS improved resection in 59% of the patients operated and in 21%, the US showed residual tumor that was not resected due to the vicinity with eloquent tissue. The rate of gross-total resection was found to be 88% when this was evaluated in the group of tumors that were considered resectable. They also discuss that IOUS was helpful in identifying nearby vascular structures using Doppler angiography [17].

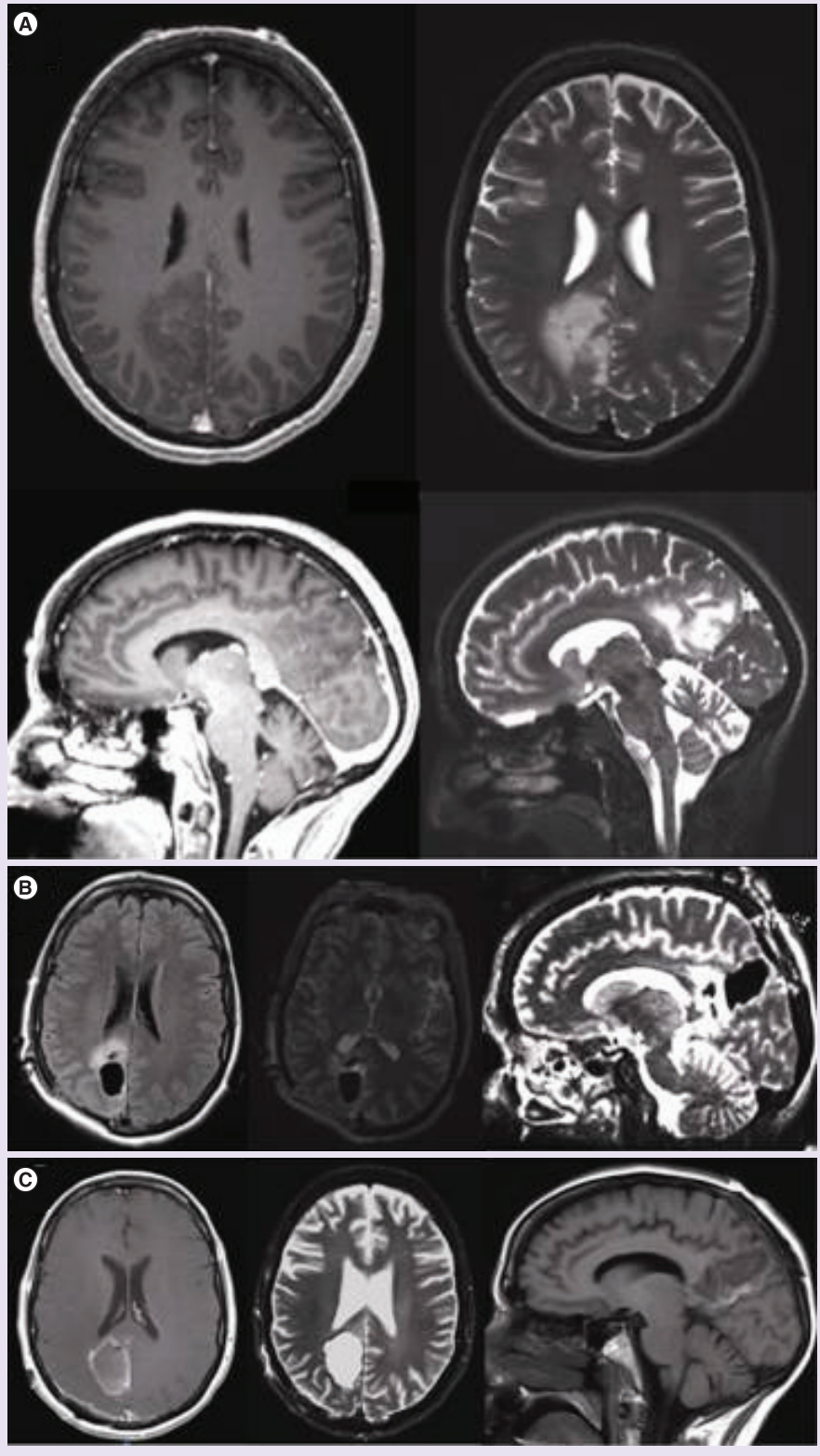

Figure 2. Pre and postoperative images after resection of a low grade glioma using intraoperative MRI.

Table 2. Summary of studies evaluating experimental intraoperative imaging modalities for glioma surgery.

Study (year)	Type of study	Imaging modality	Study population	Conclusion	Ref.
Raman imaging					
Ji *et al.* (2013)	*Ex vivo* mice *In vivo* mice *Ex vivo* human	Stimulated Raman scattering microscopy	Frozen sections in 12 mice *In vivo* imaging in 16 mice *Ex vivo* human surgical specimen in 1 patient	Strong correlation when compared with H&E microscopy for detection of glioma infiltration (κ = 0.98)	[65]
Jermyn *et al.* (2015)	*In vivo* human	Raman spectroscopy	17 patients with grade II–IV glioma	Raman-based probe differentiates normal brain cancer with 93% sensitivity and 91% specificity	[64]
Optical coherence tomography					
Bizheva *et al.* (2005)	*Ex vivo* human (formalin fixed)	OCT	Formalin-fixed tissues from 3 patients (meningioma, paraganglioma)	First OCT study to study histology features in human brain cancer	[72]
Bohringer *et al.* (2006)	*Ex vivo* mouse *Ex vivo* human (formalin fixed)	OCT	Formalin-fixed tissues from 1 mouse model and 1 human biopsy specimen	First OCT study to extract quantitative attenuation data in *ex vivo* mouse and human brain specimens	[68]
Böhringer *et al.* (2009)	*In vivo* human	OCT	9 patients with high-grade glioma (grade III, IV)	First OCT study to extract attenuation data from *in vivo* human brain cancer	[71]
Kut *et al.* (2015)	*In vivo* mice *Ex vivo* human (fresh surgical specimens)	OCT; optical property mapping	5 *in vivo* mice 32 glioma patients (grade II-IV)	First systematic study with established diagnostic threshold for cancer vs noncancer. Blinded validation study showed high sensitivity/specificity (92/100% for high-grade, 100/80% for low-grade glioma)	[76]

H&E: Hematoxylin and eosin; OCT: Optical coherence tomography.

In LGG surgery, IOUS has been useful in the identification of tumor and residual tissue. Le Roux, presented a series of 33 patients with LGG who underwent surgical resection aided with IOUS where it was possible to identify residual tissue and the authors concluded that this tool may enhance extent of resection of LGG [18]. Similarly, another group presented a series of 35 patients with parenchymal lesions, 11 of which were LGG and 22 were HGG [19]. The conclusions of this study were similar to the ones obtained by Le Roux, showing that IOUS was helpful in identifying intraparenchymal lesions and identifying its borders to enhance resection [19]. In a study of 32 patients with intra-axial tumors (15 of which were gliomas) where extent of resection has been evaluated in postoperative imaging studies, it was reported that a rim of 5 mm of hyperechoic tissue was predictive of residual tumor in 100%, but there was a negative predictive value of 83% when correlated with postoperative MRI [20]. Several other studies have addressed the rate of gross total resection (GTR) in glioma patients. The reported range of GTR ranges from 57 to 95% in the studies reviewed [12,17,20–25]. In a comprehensive analysis of a large number of studies, a meta-analysis by Mahboob *et al.* demonstrated that IOUS-guided resection of gliomas is a valuable tool by increasing extent of resection [26]. Ultimately, some studies indicate that the active use of IOUS results in increase in survival time and progression-free survival (PFS) in glioblastoma (GBM) patients [27,28]. In a study evaluating the quality of life of GBM patients, IOUS proved to improve quality of life by reducing the incidence of postoperative deficits [29].

A different modality employing US contrast agents, contrast-enhanced US (CEUS) has also been used in the resection of gliomas. US contrast agents are materials that have different echogenicity and provide better quality images of the tissue and definition of tumor borders. This IOUS modality can also be coupled to a navigable US machine. In a study of ten patients that underwent surgery for resection of GBM, Prada

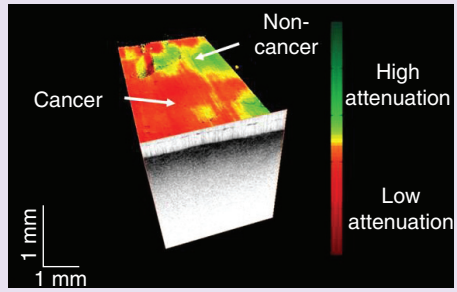

Figure 3. OCT image detecting cancer from non-cancer brain tissue. OCT image detecting cancer from non-cancer brain tissue. Optical Coherence Tomography can provide direct visual cues todistinguish glioma (red) from non-tumor (green). Here, a volumetric OCT dataset is shown with an overlaid color-coded optical property map which shows brain tumor versus non-tumor. The optical property map can be acquired, processed and displayed in real-time and without the need of any contrast agents for imaging.
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et al. showed that the use of CEUS is feasible and has the potential to locate residual tumor [30]. In a study of 50 patients with a variety of brain tumors including gliomas, metastases and meningiomas, other studies have also shown feasibility and usefulness of CEUS with good delineation of residual tumors particularly useful for GBM. Although CEUS is able to define tumor borders in gliomas, there are significant technical variables that may present as an obstacle for implementation of CEUS due to the extensive experience required for its use [31,32].

IOUS is a widely available and inexpensive tool that is helpful in the resection of HGG and LGG, among other intraparenchymal brain lesions. Further studies and advances in technology will enhance the usefulness of IOUS in resection of CNS tumors. The results of significant studies are summarized in **Table 1**. However, it is extremely important to mention that IOUS has limitations, which are mainly user dependent. User-dependent limitations occur due to the knowledge, skill and practice that the neurosurgeon has using this intraoperative imaging modality. Therefore, the usefulness of this imaging modality is highly dependent on the comfort and experience of the operator. Other limitations include but are not limited to image quality, difficulties with edema and hemorrhage within tumors and absence of functional data.

Intraoperative MRI

iMRI was initially developed in 1997. In their study, Black *et al*. used iMRI to treat a wide range of intracranial lesions **(Figure 2)** [33]. Since then it has been used as part of the neurosurgical armamentarium to determine presence of residual tumor and also to circumvent the presence of shift after the dura has been opened and tumor has been resected when neuronavigation is being used. Since its introduction to the neurosurgical operating room, MRI machines have evolved together with image quality [34]. This intraoperative imaging modality has improved extent of resection and, in turn, patient outcomes by providing information regarding residual tumor in the operative bed obtained during the surgery in the treatment of HGG as well as LGG [35]. Additionally, neuronavigation can be updated during surgery with the information obtained from iMRI, bypassing the shift after cerebrospinal fluid drainage, dural opening and/or tumor removal.

In the only randomized controlled trial evaluating the benefit or iMRI in glioma surgery, Senft *et al*. provided the only level I evidence to date to support iMRI use in glioma surgery. In a series of 58 patients randomized to iMRI-aided resection or conventional neuronavigation, show that a higher percentage of the patients included in the iMRI arm had GTR than the group in which navigation was performed. Additionally, the rate of new neurological deficit was similar between both groups and extent of resection correlated with better survival, but being in the iMRI group did not result in improved survival [36]. In a recent series of 170 patients treated with high-field iMRI, Coburger *et al*. assessed rate of GTR, overall survival, PFS and incidence of new neurological deficits after surgery [37]. These parameters were compared with previous reports and the authors concluded that GTR and overall survival were better, whereas complication rate was lower than previously reported [37]. In a randomized controlled trial designed to compare the role of iMRI versus standard neuronavigation, it was found that there was no benefit in extent of resection or in survival for patients who had iMRI-guided surgery [38]. However, this study only included 14 patients and also utilized ultralow field MRI [38]. In a systematic review of multiple

studies addressing the use of iMRI for surgical GBM treatment, Kubben *et al*. concluded that the use of iMRI during GBM surgery improves extent of resection (EOR) as well as PFS. However, some of the drawbacks of this analysis of existing studies include the heterogeneity of populations and criteria to determine EOR, the different characteristics of MRI equipment used in the studies, among others. In a meta-analysis performed by Eljamel *et al*., patients with HGG undergoing iMRI had a GTR rate of 70% [39]. The authors analyzed the utility of IOUS, IMRI and fluorescence-guided surgery. All these modalities showed comparable rates of GTR in meta-analyses performed for each imaging technique, with slightly higher GTR rate for the fluorescein-guided resections [39].

Studies on LGGs are not as prevalent. In the case of LGG surgery, iMRI has an accuracy to detect residual tumor of 83%. iMRI performed better at detecting residual tumor tissue than IOUS. These findings were confirmed histologically [40].

Studies that combine iMRI with 5-aminolevulinic acid (5-ALA) fluorescence-guided surgery have shown an increased EOR as compared with iMRI alone, but there was no benefit in survival. [41,42]. Some of the limitations of iMRI include the high cost of the equipment as well as the need for special infrastructure [43]. Additionally, MRI does require that the surgery be stopped while image acquisition occurs, extending the surgical time. Moreover, the identification of tumor margins is defined by their imaging characteristics resulting in radiological GTR, while there is residual neoplastic tissue that cannot be identified by this imaging modality. The correlation between the presence of contrast enhancement in iMRI and the presence of histopathological diagnosis of neoplastic tissue is not perfect. Although contrast enhancement correlates with tumor presence, the absence of

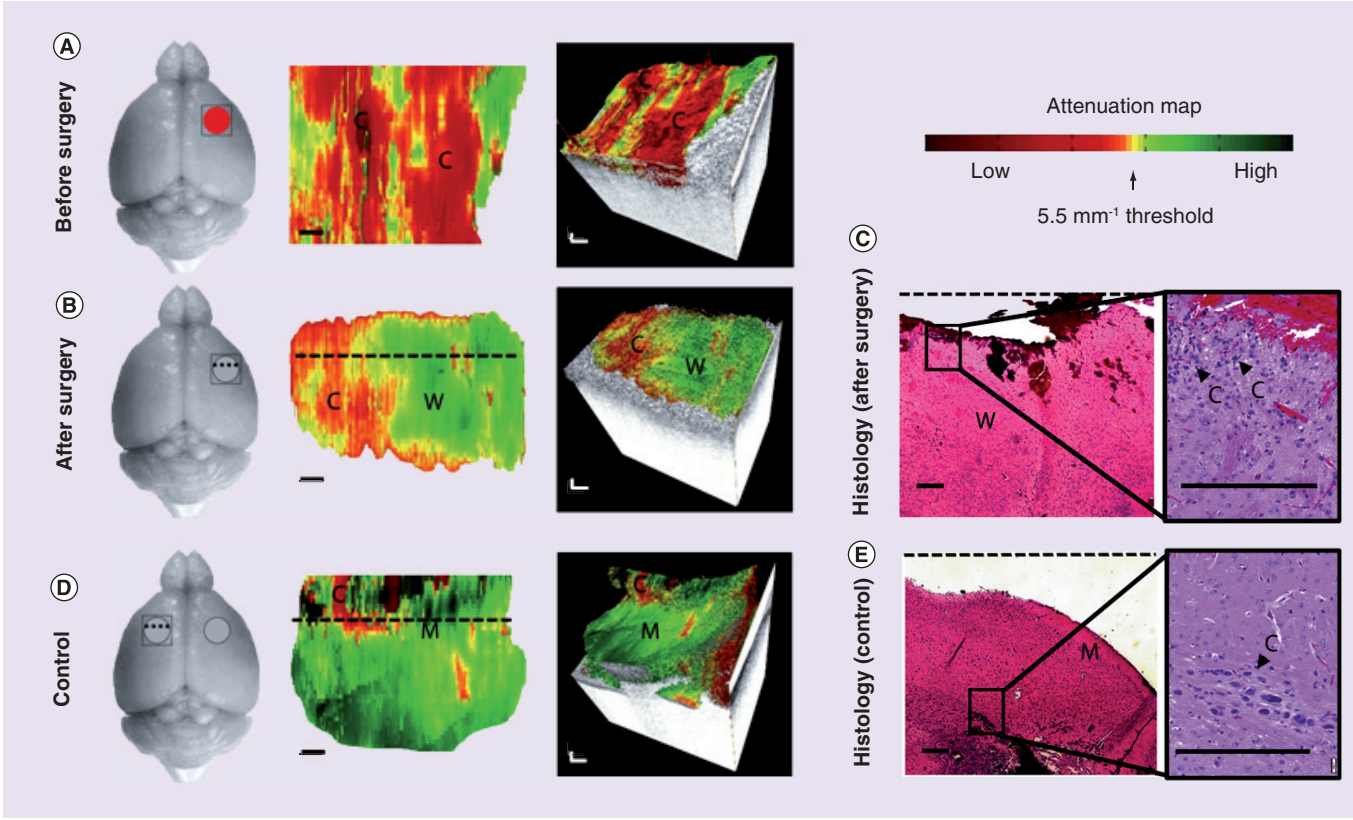

Figure 4. *In vivo* OCT imaging and optical property mapping in mice implanted with human glioma before **(A)** and after **(B)** surgery, and at normal brain surface **(C)**. OCT results are confirmed with corresponding histology as shown in **(D)**, which are 2D cross-sectional histological sections perpendicular to the optical attenuation map along the dotted lines.
Scale bars: 1 mm.
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Table 3. Comparison of different technologies in surgical guidance of brain cancer.

Feature	Ultrasound	iMRI	5-ALA fluorescence	Raman	OCT
Resolution	0.3 mm^3	3–20 mm^3	0.001 mm^2	0.00000025 mm^2	0.004 mm^3
FOV	12,500 mm^3	Whole brain	75–2000 mm^2	0.1225 mm^3	8–16 mm^3
Continuous guidance?	Yes	No	Yes	Yes	Yes
3D?	Yes	Yes	No	No	Yes
Numerical data?	No	No	Yes	Yes	Yes
Sensitivity (during and/or post-resection) (%)	26–87	N/A	47 (visual) 84 (spectrometry)	N/A (accuracy 99–100)	92–100
Specificity (during and/or post-resection) (%)	42–88	N/A	100 (visual) 92 (spectrometry)	N/A	80–100

Various technological advances have attempted to increase the surgeon's ability to identify cancer tissue. Each modality has different strengths and limitations in terms of resolution, FOV, sensitivity/specificity and the ability to provide quantitative and 3D continuous imaging guidance. Modified and reprinted with permission from Science Translational Medicine [76].
FOV: Field of view; OCT: Optical coherence tomography.

contrast enhancement does not correlate with tumor absence [44]. The results of significant studies are summarized in **Table 1**. It is conceivable that by combining multiple intraoperative techniques to identify tumor tissue the extent of resection may be improved further. This is ultimately important if the increase in EOR is accompanied with a survival benefit and the incidence of postoperative neurological deficits decreases.

Fluorescence-guided resection of gliomas

FGM to treat intrinsic brain tumors is based on the ability to label tumoral tissue with fluorescent agents such as protoporphyrin IX (PpIX) and fluorescein. PpIX-induced fluorescence has been used to guide surgical resection of gliomas after administration of its precursor 5-ALA. 5-ALA accumulates in tumor cells and hijacks the heme synthesis pathway to produce PpIX. PpIX accumulates in cancer cells through the activity and transported into cancer and normal cells by oligopeptide transporter 1 or 2 (PEPT1 or PEPT2) [45] and can be excited with blue–violet light generating the emission of red light [46]. 5-ALA has been approved for patient use in Europe, however, it has not been approved by the US FDA, except for centers with research protocols in place.

A significant amount of attention has been directed toward FGM, mainly toward 5-ALA technique, due to the simplicity of the technique. It has been tested in a randomized controlled trial that included 322 patients [47], where 65% of patients in the 5-ALA group received GTR compared with 36% of patients in the control group. Additionally, 41% of patients in the 5-ALA group had 6-month PFS compared with 21% in the control group. There were no statistically significant differences in survival between 5-ALA and control groups. Since then, multiple studies show the benefit of ALA-FGM in the treatment of HGG in increasing EOR, and combination with other tools such as intraoperative mapping techniques allows for safer resections [48–53]. In a systematic review by Zhao *et al.*, ALA-FGM is better than conventional neuronavigation in the treatment of HGG by improving EOR and improving survival [54].

In 1999, Kuroiwa *et al.* developed a surgical microscope equipped to detect fluorescein fluorescence and they used it to help in HGG resection [55]. Fluorescein was administered systemically and it accumulated in regions with faulty blood–brain barrier. After resection, they observed that areas of high fluorescence correlated with areas with high tumor cell density histologically. Availability of microscopes equipped with fluorescein filters has allowed the implementation of this technique [55–57]. Also, the low cost of use of fluorescein makes it a more cost-effective tool when compared with 5-ALA [39]. Since then, in 2008 Koc *et al.* executed a study evaluating the influence of fluorescein-FGM in the treatment of HGG [58]. They found that GTR was greatly improved with the use of fluorescein. Recently, Neira *et al.* demonstrated in a study of 32 patients that fluorescence was present in all patients after fluorescein administration. They also were able to achieve GTR in 93% of patients with an average resected volume of 99.7% [59]. Currently, fluorescein is being evaluated as an intraoperative brain tumor marker in a clinical trial, however it is not currently approved for brain tumor surgery by the FDA (clinical trial NCT02691923).

In a meta-analysis comparing different intraoperative imaging techniques in the resection of HGG, the patients included in the ALA-FGM group, there was a 61.9% of patients with no residual contrast enhancing tissue in postoperative imaging, whereas 84.4% of patients included in the fluorescein-FGM did not have residual contrast-enhancing tissue [39]. The rate of GTR in this meta-analysis in the IOUS and iMRI were 73.6 and 70%, respectively. The authors also determined that the cost of ALA-FGM was of approximately $1400, the cost of fluorescein-FGM was $300 and IOUS and iMRI were $330 and $1800, respectively. Additionally the specificity and sensitivity of ALA-FGM and fluorescein-FGM were found to be much better than those for IOUS and iMRI. Improvement in these techniques may allow for increased sensitivity in the detection of tumor tissue. The EOR achieved with ALA-FGM can be greatly improved by the use of PpIX-spectroscopy given that this technique is three-times more sensitive than the commercial microscopes equipped with fluorescence filters [60].

Although the use of FGM techniques improves EOR, the ultimate proof is the improvement in survival. In a meta-analysis, ALA-FGM and fluorescein-FGM showed an estimated survival improvement of 4 months after ALA-FGM [49] and approximately 2.1 weeks after fluorescein-FGM [58]. In this study, GTR did have a significant effect on improvement of survival and fluorescein-FGM patients had 83% GTR versus 55% of patients who did not have fluorescein-FGM.

Limitations of fluorescein-FGM include skin photosensitivity, hypotension and the limitation of its use in color blind surgeons [61,62]. Photosensitivity can be prevented through light protection after 5-ALA administration and hypotension can be avoided by identifying patients with cardiac disease. Fluorescein-FGM can be hampered by hypersensitivity [60]. Other potential pitfalls of this technique include the inadvertent extension of the resection to eloquent regions. This can be avoided by combining FGM with intraoperative mapping during glioma resection [63]. The results of significant studies are summarized in **Table 1**. Ultimately, by combining multiple technologies, with the combination tailored to the individual patient's characteristics, we may see improvement in outcomes and results in glioma resection. For example, in a tumor nearby eloquent structures, ALA or fluorescein-FGM in conjunction with intraoperative monitoring may be the most appropriate option.

Experimental technique: RS

RS is another relatively new experimental imaging technology which has been introduced into the operating room. Although it has not yet received FDA approval, it has shown promising pilot results in detecting tumor cells *in vivo* in mice and in humans. RS is a technique used to identify vibrational, rotational and other low-frequency modes. As a result, it can characterize tissues by observing the molecular fingerprint for tumor versus nontumor. The intraoperative feasibility of this technology was first tested by a Canadian group in 2015 with 17 patients [64], and the results are promising for spot detection of brain cancer during surgical resection. Nevertheless, limitations for RS include a small field of view at 0.00000025 mm^2 area to 1 mm^3 volume/spot. Furthermore, it has a slow scanning rate at 1 s/spot, and therefore cannot be used to scan the entire resection cavity within the surgical timeframe [64,65] **(Table 2)**.

Experimental technique: OCT

OCT is a label-free, real-time imaging technique which can be used to obtain volumetric images of biological tissues at a resolution equivalent to a low-powered microscope (e.g., around two- to fourfold magnification). OCT can be envisioned as an optical analog of US imaging, since both techniques acquire cross-sectional images of the tissues by collecting 'reflected' light or sound waves. Unlike US, however, OCT uses a near-infrared light source (instead of sound waves), and does not use any matching medium, for example, gels as used in US imaging. In addition, OCT is capable of non-contact imaging and generally acquires images at several centimeters above the tissue surface, which minimizes the risks of infection. Since its introduction about two to three decades ago, OCT has evolved to become a powerful medical imaging technique with the unique ability to visualize the cross-sectional structures of human tissues noninvasively, at high speed, and with micron-level resolution [66]. OCT was first used in ophthalmological applications to detect retina pathologies. Since then, it has become successful in multiple clinical specialties [66–70], with FDA approval for ophthalmic, gastrointestinal and intravascular applications.

Recent groups have made important contributions to the study of human brain cancer using OCT [67,69–74]. For example, Boppart *et al.* performed the first OCT study in imaging *ex vivo* human brain cancer in 2008, where the research group has imaged a melanoma tumor mass which had metastasized to the cerebral cortex of the patient [67]. In 2005, Bizheva *et al.* obtained the cross-sectional OCT images of formalin-fixed human brain cancer tissues such as meningioma and ganglioglioma [72]. In 2013, Assayag *et al.* performed the first *en face* OCT studies in human brain cancer specimens [74]. Finally, Bohringer *et al.* reported the results of several pilot studies which characterized the optical attenuation characteristics of human primary brain cancers *ex vivo* and *in vivo* [68,71,75] **(Table 2)**.

A study in Science Translational Medicine (published in June 2015) built upon previous studies and investigated the potential of OCT for label-free imaging of human brain cancer in a systematic study which compares brain cancer with noncancer tissues using freshly resected *ex vivo* human tissues, and *in vivo* murine model implanted with human gliomas **(Figure 3)** [76]. In addition, this study proposes a method to quantitatively analyze and display OCT imaging results with high sensitivity and specificity. Most importantly, a color-coded optical property map is generated in real time which provides direct visual cues in detecting brain cancer.

To enable real-time OCT imaging, the 2015 study utilized a home-built, swept-source OCT imaging system which operates at a central wavelength of 1300 nm and at a 3 dB spectral bandwidth of approximately 110–130 nm. On average, the laser source outputs about 15 mW to the brain tissue sample through the OCT handheld imaging probe. Using a high-speed data acquisition card and Graphics Processing Unit (GPU)-based signal processing, Swept Source (SS)-OCT images can be acquired, processed, displayed and stored in real time at a speed of up to 220,000 A-lines/s, or up to 220 frames/s (fps) assuming that each frame consists of approximately 1000 A-lines and approximately 2000 pixels per A-line. This translates to a total of 1.2–2.4 s for each 8–16 mm^3 volumetric tissue block [76].

For most systemic tumors, the optical attenuation is generally higher in cancer (compared with noncancer) due to a higher cell density and nuclear-to-cytoplasmic ratio. In the human brain, however, brain cancer actually has a lower attenuation when comparing with the surrounding noncancer white matter. To fully understand why brain cancer actually has a lower attenuation than noncancer white matter, we need to first understand the biological and physical properties which determine the optical attenuation for a tissue sample. In brain cancer, optical attenuation is governed by several important factors. First of all, it is well known that noncancer white matter in the brain has a high attenuation due to its rich myelin content [68,74,76,77]. When brain cancer proliferates, it will ultimately induce the breakdown of myelin in white matter to facilitate infiltration into the surrounding normal brain [78–80]. Noncancer gray matter, on the other hand, does not contain myelin. As a result, brain cancer is found to have a lower overall attenuation when compared with noncancer white matter, but a higher overall attenuation when compared with noncancer gray matter [76].

Given our understanding of the aforementioned methodologies, the 2015 study investigated the potential of OCT in imaging brain cancer via a systematic study with 32 consented patients (15 patients with HGG, 12 patients with LGG and 5 control patients with noncancer brain lesions) and over 4600 optical attenuation data points [76]. First, a set of optical attenuation parameters were established to analyze a training set (with 16 patients) to establish a diagnostic attenuation threshold (for detection of brain cancer vs noncancer); then the final 16 patients were entered into a double-blinded study to compute the OCT detection [76]. In this study, an independent and double-blinded study was conducted to determine the sensitivity and specificity associated with the optimal attenuation threshold (at 5.5 mm^{-1}). The OCT detection sensitivity was found to be 92% and specificity was determined to be at 100% for HGG patients (n = 7) [76]. Using the same diagnostic attenuation threshold, the sensitivity was found to be 100% and specificity was found to be 80% for LGG patients (n = 9) [76]. This was also confirmed with *in vivo* mouse models implanted with human GBM **(Figure 4)**. Thus, OCT represents an experimental imaging technology which has been successfully applied in clinical settings, for example, ophthalmology, gastrointestinal and intravascular applications, and has demonstrated early promise in differentiating cancer from noncancer tissue based on *ex vivo* human and *in vivo* animal data.

Conclusion

To summarize, maximizing the extent of brain cancer resection can prolong survival and delay recurrence. It is important to recognize that different technologies have different levels of resolution, sensitivity and specificity in detecting tumor tissue (Table 3). However, it is challenging to distinguish cancer from noncancer tissues intraoperatively, especially at the infiltrative margins. Thus, neurosurgeons are faced with the challenge of maximizing the removal of brain cancer while preserving surrounding normal brain (especially eloquent areas, e.g., motor, speech and sensory areas). This review described the use of both conventional (i.e., US, MRI and fluorescence-guided resections) as well as experimental imaging techniques (i.e., Raman and OCT) in imaging glioma surgery. Specifically, it provides details on the methodology, results and promise of different methodologies used to differentiate tumor from noncancer tissue in real time. Many of these technologies have been integrated into the neurosurgical operating room and other newer technologies have demonstrated effectiveness improving detection of tumor tissue. We believe that by combining some of these techniques, better results in terms of extent of resection, neurologic outcomes and survival of patients with gliomas will be achieved. Large-scale clinical trials are needed to truly assess the utility of the different intraoperative modalities in glioma surgery. As technology is incorporated into the neurosurgical operating room, more information and guidelines for its use will

EXECUTIVE SUMMARY

Intraoperative ultrasound

- Intraoperative ultrasound (IOUS) is a widely available and inexpensive tool.
- IOUS can differentiate normal tissue from neoplastic tissue improving resection in a large percentage of patients.
- Limitations of IOUS include user-dependent limitations, image quality, presence of edema and hemorrhage within tumors and absence of functional data.

Intraoperative MRI

- Intraoperative MRI (iMRI) can bypass the effects of brain shift and improve the accuracy of neuronavigation.
- iMRI can detect residual tissue accurately and improve the extent of resection.
- Limitations include its low prevalence throughout neurosurgical centers and cost of implementation.

Fluorescence-guided microsurgery

- Fluorescence-guided microsurgery is an inexpensive technique that can be combined with iMRI and IOUS to improve resection of gliomas.
- Fluorescent agents used are not currently US FDA approved for their use in glioma surgery in the US.
- Limitations include availability of microscopes with the necessary optics for implementation, skin photosensitivity, hypotension and the limitation of its use in color blind surgeons.

Raman spectroscopy

- New experimental imaging technology with promising pilot results.
- Raman spectroscopy characterizes tissue based on molecular fingerprint for tumor versus nontumor.

Optical coherence tomography

- FDA approved with successful clinical applications in ophthalmology, gastroenterology and cardiology.
- Experimental technique used to visualize brain tissues noninvasively and at micron-level resolution.
- Optical coherence tomography characterizes brain tissues based on optical attenuation differences in tumor versus nontumor.
- Color-coded optical property map can be generated in real time to provide direct visual cues in detecting brain cancer.
- *Ex vivo* human and *in vivo* animal studies show promise; additional *in vivo* clinical studies are needed to evaluate feasibility of this approach.

emerge. This will result in standardized use and maximization of the utility to provide optimal surgical treatment for brain cancer patients. Unfortunately, many of the studies reviewed are retrospective in nature and the data obtained from them have the inherent limitations of retrospective studies, highlighting the need for large randomized clinical trials to understand the usefulness of the different intraoperative imaging modalities in the care of glioma patients.

Future perspective

With the evolution of more sophisticated technologies and the potential of implementation in the neurosurgical operating room, the sensitivity and accuracy to detect glioma tissue during surgery will increase significantly. Through technological advances, the intraoperative technologies discussed in this manuscript could potentially be merged and used seamlessly in the operating room to spatially differentiate cancer from non-cancer within the resection cavity and to allow seamless and continuous surgical guidance in the operating room. Future large-scale clinical trials are required to obtain better evidence and identify better ways to employ intraoperative imaging in the operating room. With additional clinical studies and technological advances, we are confident that intraoperative imaging techniques will become a very important part of the surgical technique for brain tumor resection, which will ultimately result in cleaner resection margins for glioma patients and thus prolonged overall survival and delayed recurrence for patients.

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