Image-Guided Brain Surgery

26

Stephanie Schipmann-Miletić and Walter Stummer

Contents

S. Schipmann-Miletić (✉) · W. Stummer
Department of Neurosurgery, University Hospital Münster, Albert-Schweitzer-Campus 1, Building A1, 48149 Münster, Germany
e-mail: Stephanie.schipmann@ukmuenster.de

O. Schober et al. (eds.), *Molecular Imaging in Oncology*,
Recent Results in Cancer Research 216,
https://doi.org/10.1007/978-3-030-42618-7_26

26.1 Introduction

Over the past decades, brain tumor surgery has undergone large changes as medical technology and surgical experience have grown. A milestone was the introduction of the microscope and microsurgical techniques by Yasargil [129]. The paradigm of brain tumor surgery has shifted from removing tumor that is obvious to the human eye to resection of malignant cells beyond the scope of the microscope and visual inspection aided by technological innovations. In the treatment of gliomas, an independent factor for better outcome regarding overall survival (OS) and progression-free survival (PFS) that can be directly influenced by the neurosurgeon is the extent of resection (EOR) [7, 58, 65, 96, 115]. It is well known that gliomas infiltrate surrounding brain parenchyma in a manner that is not perceived by the human eye even with the aid of the surgical microscope and infiltrated brain often cannot be differentiated from normal brain tissue based on tactile features.

Therefore, several attempts have been undertaken during the last decades to develop techniques for better tumor visualization and identification, aiming at higher EOR. Among them are techniques such as neuronavigation, intraoperative ultrasound, intraoperative magnetic resonance imaging (iMRI), and, more recently, fluorescence-guided surgery (FGS). Especially the latter has taken the process of intraoperative tissue visualization to another level with the potential of true real-time imaging of tumor without any interruptions to surgery for identifying tumor. Recently, other highly precise techniques have been introduced and are being translated into clinical medicine, promising even more exact delineation of tumor tissue, such as confocal microscopy, Raman spectroscopy, or targeted fluorescence opening the frontiers to real-time intraoperative molecular and cellular imaging.

All these tools have improved the ability of the surgeon to identify tumor tissue and distinguish tumor from normal brain parenchyma. In this chapter, the different techniques with their applicability in brain tumor surgery and their benefits and limitations will be discussed.

26.2 Conventional Intraoperative Imaging

Conventional intraoperative imaging techniques like neuronavigation and ultrasound are the basis for most cases in brain tumor surgery and have been well integrated into the operative setting in neurosurgery.

26.2.1 Ultrasound

Intraoperative ultrasound provides an easy, cost-effective, and rapidly available method for localization of lesions prior to durotomy and residual tumor during tumor resection. It is a dynamic method that enables visualization of tumor borders and adjacent normal brain structures and anatomy [76]. It has been shown that use

of ultrasound has the potential to increase the grade of resection and thus improving outcome [93]. Ultrasound is more effective with cystic and heterogenous lesions with different echogenicities from the cortex. Some navigation systems use intraoperatively acquired data from the ultrasound to update navigational data to avoid the limitations of brain shift [67]. However, peritumoral edematous tissue also appears hyperechogenic and can be confused with brain tumor, potentially resulting in resections being carried into functional brain and thus potentially endangering the patient's neurological function [92] (Fig. 26.1a).

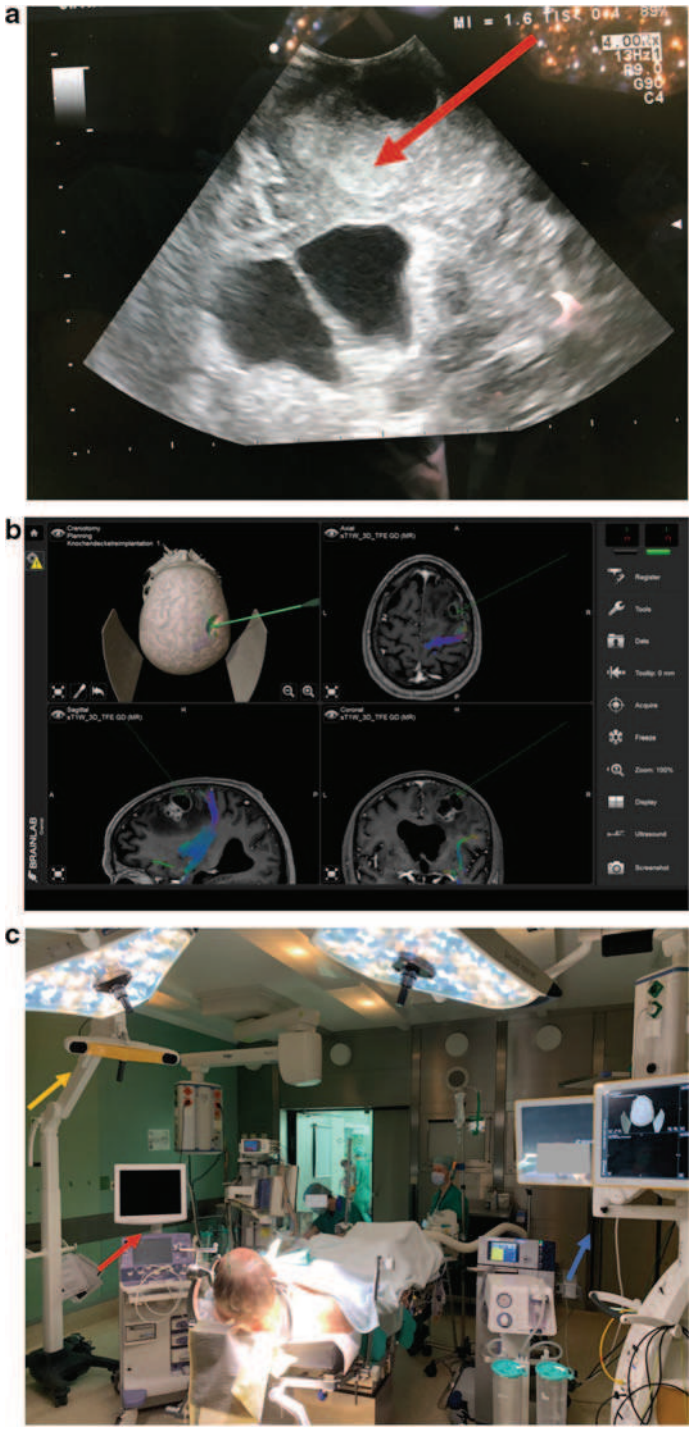
26.2.2 Neuronavigation

Neuronavigation has become a ubiquitously available and indispensable tool for the surgical treatment of brain tumors. Neuronavigation is based on three-dimensional preoperative radiological imaging data, which is merged with the patient`s anatomy by registration [104]. The navigation system consists of a reference arm attached to the head clamp that is fixed to the surgical table. Infrared cameras track the position of the probe relative to the fixed reference arm and preoperative imaging is shown on the screen with the real-time intraoperative position of the probe [76]. Imaging acquired preoperatively, such as CT, MRI, and PET, can be entered into the neuronavigation software and provides the neurosurgeon with intraoperative almost real-time localization and orientation. Anatomical information and data on the extent of the tumor and its relation to adjacent structures can be gained from the system, leading to higher surgical accuracy and precision for the resection of brain tumors [104].

Furthermore, functional data can be incorporated into the neuronavigation system. Functional MRI (fMRI) can help identifying localization of functional brain regions, e.g., for language and motor functions [138].

Diffusion tensor imaging (DTI) is based on the preferential diffusion of water in the direction of white matter tracts within the central nervous system [3, 76]. These data can be added to the navigation system to give detailed information on subcortical fiber tracts, e.g., the corticospinal tract, that could be at risk during tumor resection [25]. In addition, neuronavigation is very useful for planning the surgical approach and craniotomy [42].

However, there are distinct limitations using neuronavigation. A major issue is loss of accuracy due to intraoperative brain shift caused by positioning, application of mannitol, drainage of cerebral spinal fluid (CSF), and bulk tumor resection, as navigational systems rely on preoperative imaging. A further limitation is that most navigational systems display the information used during surgery on a screen outside the surgical field, forcing the surgeon to draw his attention away from the surgical field to a screen. However, modern neuronavigational systems enable injecting navigational information into the display within the operating microscope (Fig. 26.1b, c).


a
MI = 1.6
b
BRAINLAB
c

◀ **Fig. 26.1** Techniques for intraoperative imaging. **a** Ultrasound. The lesion can be seen transdural and can be clearly distinguished from normal brain structures (red arrow). **b** MRI: Patient with fiber tracking (pyramid tract) for intraoperative orientation and planning of surgical approach. **c** Setting in the operating room with simultaneous use of ultrasound (red arrow), neuronavigation with display (blue arrow) and camera (yellow arrow)

26.2.3 Intraoperative MRI (iMRI)

Intraoperative MRI (iMRI) has been introduced in the 1990s [124] and has since undergone several improvements. iMRI has the propensity for providing images during surgery and for updating the information on the navigation system to correct for changes in anatomy due to brain shift [41]. In addition, iMRI can be used to identify residual tumor and to improve the extent of tumor resection. A randomized controlled trail evaluating the benefit of iMRI in glioma surgery performed by Senft et al. demonstrated a gross total resection (GTR) rate of 96% in the iMRI group versus 68% in the control group, operated with conventional microsurgery [106]. Especially, in the treatment of low-grade gliomas (LGG) several studies demonstrated the potential of iMRI, with a 30–60% of return to surgery after initial resection to address residual tumor identified on iMRI [55, 107, 108].

Despite the increasing use of iMRI, there are disadvantages as this technology comes with high costs, is time-consuming, and prolongs the duration of surgery and anesthesia. iMRI cannot be used for patients with ferromagnetic implants. Furthermore, the frequent application of gadolinium might lead to extravasation in the resection cavity, impeding interpretation of imaging.

26.3 Fluorescence-Guided Brain Surgery

For neurosurgeons, the ability to differentiate abnormal from normal tissues is of utmost importance in order to perform safe and effective surgery. In this regard, fluorescence-guided surgery (FGS) has shown to be extremely helpful for visualization and delineation of pathological tissues. In principle, FGS is based on the administration of optical imaging agents to patients prior or during surgery that are selectively accumulated in tumor tissues. FGS was first described for neurosurgery by George E. Moore in 1947, who showed that glioma and meningioma cells could be better visualized by fluorescence after intravenous application of fluorescein [70]. This ability of real-time intraoperative detection of tumor tissue has further developed throughout recent years with the great advantage of intraoperative visualization of tumor tissue independent from neuronavigation, which is often affected by brain shift [28]. Besides fluorescein, two further agents have been introduced into the field of neurosurgery, 5-aminolevulinic acid (5-ALA) [119] and indocyanine green (ICG) [83].

26.3.1 5-Aminolevulinic Acid (5-ALA)

5-aminolevulinic acid (5-ALA) is a natural metabolite in the hemoglobin pathway. 5-ALA is metabolized into protoporphyrin IX (PpIX), a strongly fluorescent precursor of heme. Glioma cells selectively take up 5-ALA, convert this to PpIX, resulting in tumor cell fluorescence [14, 117]. Fluorescence can be visualized by coupling the surgical microscope to a xenon light source that is capable of switching between white and violet-blue light (wavelength: 370-440 nm) and adding an emission filter in order to visualize the red tumor fluorescence at a peak of 635 and 704 nm [117]. Besides the visualization of fluorescence, the filters enable sufficient background discrimination in order to perform major parts of surgery under conditions suitable for visualizing fluorescence. At present, all modern surgical microscopes offer adjuncts with the ability to visualize PpIX fluorescence. 5-ALA (brand name Gliolan® in the EU or Gleolan® in the US) is administered as an oral solution at a dose of 20 mg/kg body weight 3 h before induction of anesthesia. Plasma clearance is achieved within 2 h after administration [118]. Peak fluorescence can be expected after about 6–8 h, with fluorescence beginning to become visible after about 3 h [48, 117, 120]. Typically, high-grade gliomas show solid red fluorescence with a slightly pink fluorescence at the tumor margins, representing the tumor-infiltrating zone [113]. Several studies demonstrate toxicological safety. At most, 5-ALA leads to transient skin phototoxicity or temporarily elevated liver enzymes [114, 128].

5-ALA is the most widely studied fluorescent agent worldwide and has been approved as the only optical imaging agent by both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for real-time visualization of malignant tissue during glioma surgery. Next to its clinical application in malignant gliomas, several studies confirm benefit for resection in other primary and metastatic brain tumors, such as meningiomas, brain metastases, and pediatric brain tumors [19, 24, 38, 46, 71, 141].

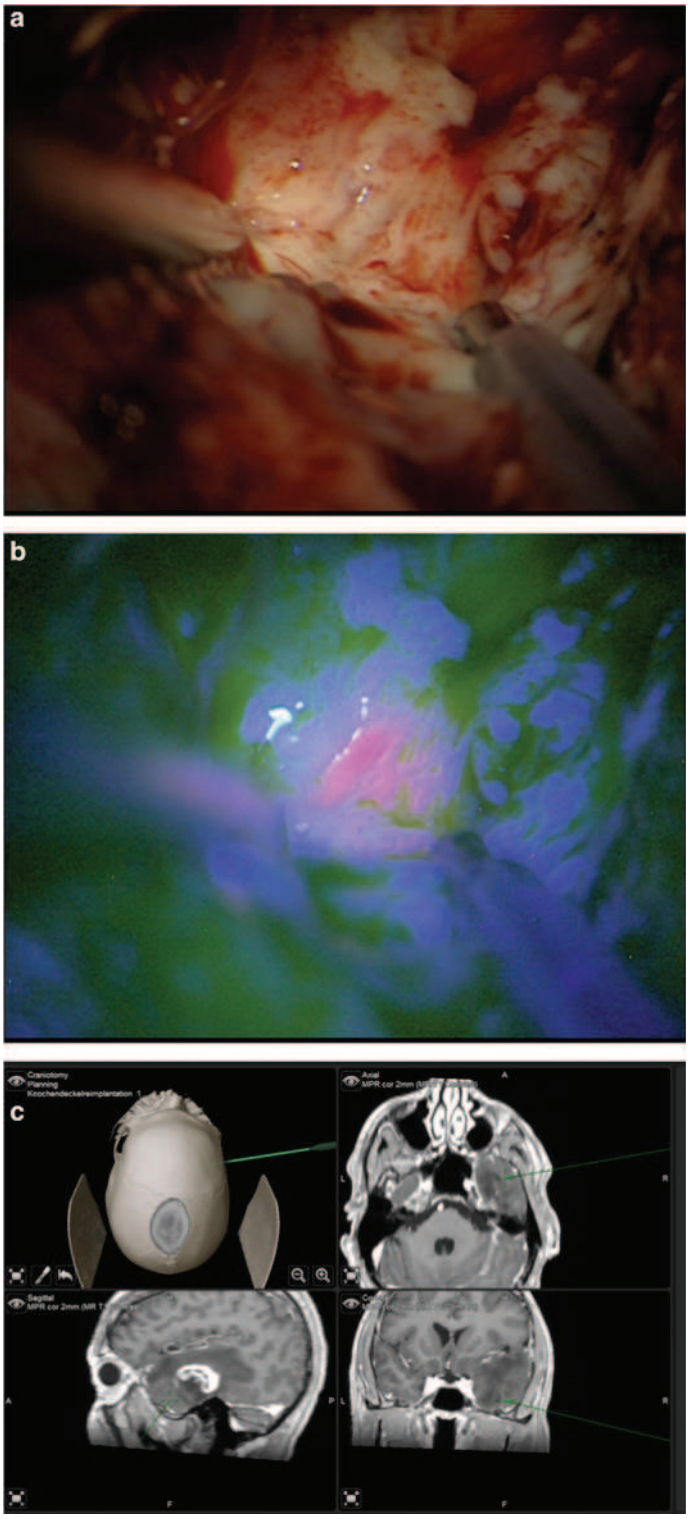
26.3.1.1 5-Aminolevulinic Acid in High-Grade Gliomas

High-grade gliomas (HGGs) are the most frequent primary malignant brain tumors in adults and are known to be highly infiltrative tumors [10]. Glioblastoma is characterized by a poor prognosis with median survival of 15 months and a 2-year survival rate of 17.4% [52, 78]. Typically, the solid tumor core is surrounded by normal brain with invading tumor cells and no histologically distinct border. Despite the fact that surgery is never curative, given the infiltrating nature of high-grade gliomas, the benefit of complete resection of contrast-enhancing tumor regarding overall survival has been shown in several studies, all supporting maximal safe resection [7, 58, 65, 96, 115]. Identification and complete resection of contrast-enhancing tumor only by the unenhanced visual impression or haptic information is almost impossible to achieve [75].

5-ALA can aid in the visualization of glioma tissue. The first clinical usage was reported in 1998 in a cohort of nine high-grade glioma patients, revealing a high sensitivity of 85% and specificity of 100% for detection of malignant tissue [119]. A phase III randomized controlled multicenter trial revealed that 5-ALA enables more complete resections of contrast-enhancing tumor (65% of patients assigned to the 5-ALA group compared with 36% of those assigned to the conventional surgery white light group, $p < 0.001$), leading to improved 6-month progression-free survival (41.0% in the 5-ALA group versus 21.1% in the white light group) [114]. Since this study, 5-ALA has been widely used in resection of high-grade glioma. The resection rates of 65% initially reported in that study have improved over the last years due to a gain in experience and the addition of modern intraoperative monitoring and brain mapping methodology, allowing safe resections even in eloquent regions. At present, resection rates between 80 and 100% are reported [13, 18]. Strong 5-ALA fluorescence shows a strong correlation with contrast enhancement on MRI, while marginal, weaker pink fluorescence often exceeds the contrast-enhancing margins, representing the infiltration zone [121] (Fig. 26.2). Strong correlations between histological grading, features of malignancy, a higher Ki-67/MIB-1 index, and the intensity of 5-ALA fluorescence were observed [37, 90].

26.3.1.2 5-Aminolevulinic Acid in Recurrent High-Grade Gliomas

In recurrent high-grade gliomas, several small non-randomized patient cohorts suggest that completeness of resection of contrast-enhancing tumor also results in better survival [82, 89, 122]. Therefore, 5-ALA-guided resection also seems to be an attractive approach for recurrent glioma, as corroborated by several studies. Mean rates of complete resection of 91% have been described using 5-ALA [32]. In addition, longer OS was associated with 5-ALA FGS in recurrent gliomas compared to patients undergoing repeat surgery without 5-ALA [32]. However, the utility of 5-ALA might be impacted by post-therapeutic tissue changes such as gliosis, necrosis, and vascular hyalinization [6, 17, 126], affecting specificity and sensitivity. Positive fluorescence was reported in the vast majority of recurrent high-grade gliomas and was regarded a good predictor for the presence of tumor [32, 45, 73, 133]. However, in contrast to the findings in primary glioma the absence of fluorescence is not essentially a reliable marker for absence of tumor [59]. In some cases, no active tumor tissue could be diagnosed despite visible fluorescence linked to reactive tissue changes or inflammation [45, 133]. In summary, the use of 5-ALA appears to be feasible in recurrent gliomas, keeping in mind possible false positive or negative fluorescence and the not yet understood impact of adjuvant tumor treatment on the degree of fluorescence.


a
b
c

◀ **Fig. 26.2** 5-ALA-guided resection of glioblastoma. **a** Tumor under white light, delineation between tumor and normal tissue is almost impossible. **b** Tumor under violet light, showing a clear and solid fluorescence in tumor tissue and a vague fluorescence at the border and infiltrating zone. **c** Screenshot from neuronavigation, demonstrating the area of the above-taken images. 5-ALA fluorescence goes beyond the borders of the contrast enhancement on MRI, enabling a higher extent of resection

26.3.1.3 5-Aminolevulinic Acid in Low-Grade Gliomas

There is clear evidence that more extensive resection of low-grade gliomas (LGG) results in improved outcomes [31, 94, 110]. Consequently, the aim of surgery is maximal safe tumor resection. A challenge in surgery of LGG is the fact that these tumors often show only slight differences in texture and consistency compared to normal brain. LGG often demonstrate histological heterogeneity with circumscribed areas of malignant transformation (anaplastic foci) [81]. Most LGG do not reveal visible 5-ALA fluorescence; however, several studies demonstrated correlations between 5-ALA fluorescence and patchy/faint contrast enhancement on preoperative MRI, areas with higher metabolic activity in PET and higher proliferation rate [26, 39, 140]. Consequently, the use of 5-ALA enables intraoperative identification of anaplastic foci, more precise histopathological diagnoses, reducing the risk of histopathological undergrading and enabling allocation of patients to proper adjuvant treatment. At present, the use of 5-ALA in suspected LGG is recommended in case of patchy or faint contrast enhancement on MRI and in case of ^{18}F-FET-PET hot spots with a standardized uptake value (SUV) greater than 1.9 [39] (Fig. 26.3).

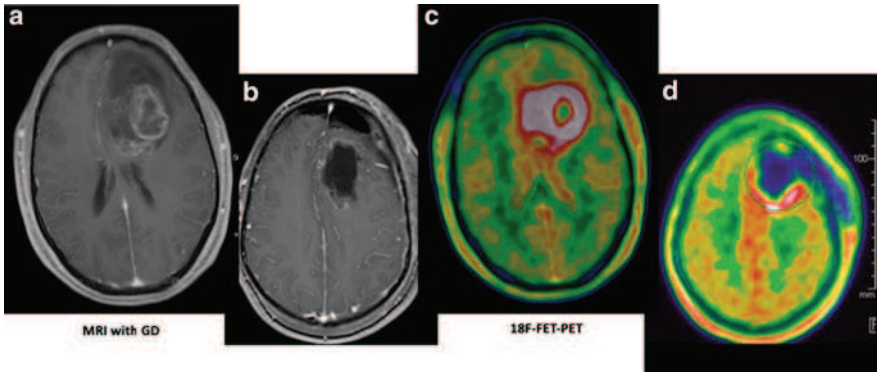


Fig. 26.3 **a** MRI T1+Gd before and after surgery (**a** and **b**) with corresponding F18-FET-PET imaging pre- and postoperatively (**c** and **d**). The enhanced metabolism of the tumor can be visualized using PET imaging. Images are reprinted with permission of the Department of Nuclear Medicine, University Hospital Münster, Germany

26.3.1.4 5-Aminolevulinic Acid in Other Brain Tumors

In addition to the wide use of 5-ALA for glioma surgery, several studies demonstrated the utility of 5-ALA as a surgical adjunct for resection of other brain tumors. In meningioma surgery, 5-ALA fluorescence can help to identify tumor infiltration of dura, brain, bone, and satellite lesions beyond the tumor bulk allowing to maximize resection [19, 69, 71, 134, 141] (Fig. 26.4).

Cerebral metastases have a high local recurrence rate even after assumed complete surgical resection, often due to residual tumor tissue [46]. In order to improve the degree of resection, some groups studied the use of 5-ALA for resection of cerebral metastases. Altogether, 5-ALA fluorescence has been observed in only half of all studied cerebral metastases [47, 131]. Studies suggested that 5-ALA does not allow reliable visualization of residual tumor after resection and so far, there are no known predictors for positive 5-ALA fluorescence in metastasis [46, 131].

Spinal cord ependymomas frequently show strong 5-ALA fluorescence and this technique was shown to be useful for differentiating tumor from normal tissue [38].

In the pediatric brain tumor population, promising results have been found regarding the use of 5-ALA for resection of astrocytomas, glioblastomas, ependymomas, and medulloblastomas [24, 91, 105, 116].

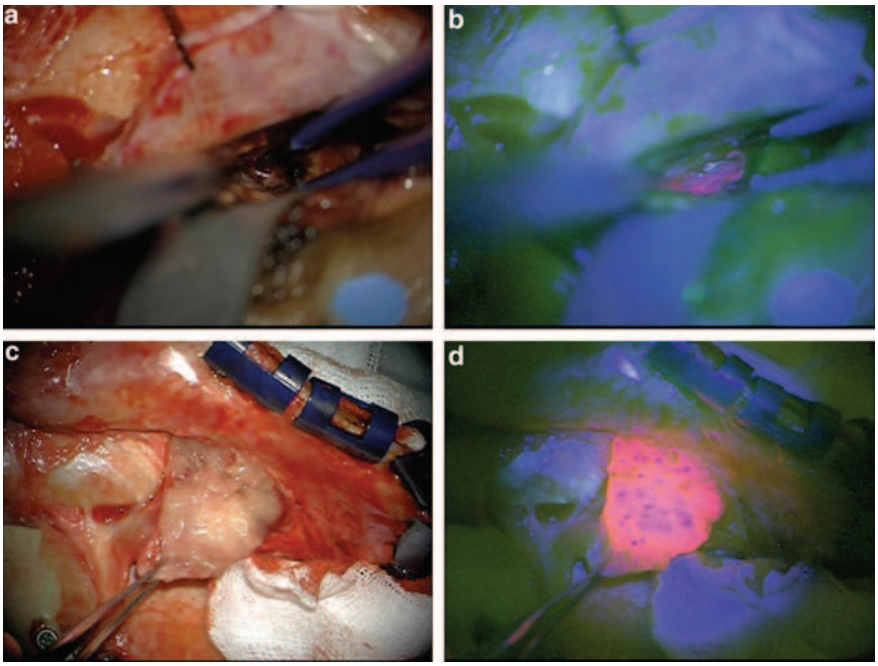


Fig. 26.4 Usage of 5-ALA in meningioma. After dural opening, 5-ALA-induced fluorescence of tumor tissue is visible (**a**, **c**: white light, **b**, **d**: violet light)

In cases of cerebral lymphoma, application of 5-ALA appears to help in obtaining representative biopsy samples as most lymphomas show positive 5-ALA fluorescence [142, 143].

In addition, 5-ALA fluorescence-guided surgery has been described in single cases for resection of hemangioblastoma [132], subependymomas [5], and germ cell tumors [127].

5-ALA appears to bear potential for the resection of different non-glial brain tumors in individual cases; however, only case reports are available so far, with the difficulty of drawing conclusions at this time. Prospective studies are warranted to evaluate the benefit and full value of 5-ALA-guided surgery in these tumor entities.

26.3.2 Fluorescein

Fluorescein sodium has been discovered almost 150 years ago and is nowadays mostly used in ophthalmology for detection of corneal abrasions and for retinal angiography [85, 98]. A possible utility for neurosurgery was first suggested by George E. Moore in 1947, showing that glioma and meningioma cells could be differentially visualized by fluorescence after intravenous application of fluorescein [70]. Fluorescein is considered as a robust, inexpensive, and safe fluorescent biomarker with characteristic yellow-green fluorescence. Its peak absorption spectrum occurs at 465–490 nm with emission peaks at 500–530 nm. Fluorescein fluorescence can also be discriminated under white light [57, 109]. After intravenous administration, fluorescein is distributed systemically through the bloodstream and extravasates into regions with increased vascular permeability, abnormal vasculature, and neovascularization [70]. Under normal circumstances, circulating fluorescein is excluded from normal brain tissue by the blood–brain barrier (BBB) [100]. In case of disruption of the BBB, for example, in tumors, fluorescein accumulates in the extracellular space of tumor tissue and can be visualized under yellow-filtered (560 nm) light [57].

A surgical microscope enabling visualization of fluorescein was introduced for resection of high-grade glioma [57]. Nowadays, a variety of fluorescent filters are available, e.g., the YELLOW 560 system (Carl Zeiss) or the FL560 System (Leica Microscopes).

Fluorescein can also be visualized as a yellow dye under the white light of surgical microscopes at high concentrations. However, lower concentrations of fluorescein can be used when using specialized microscopes equipped with appropriate emission filters. A dose of 3–5 mg/kg body weight is administered intravenously after induction of anesthesia [22, 29]. Fluorescein is eliminated renally and mostly free of side effects apart from leading to transient discoloration of skin and urine after administration. Single cases of anaphylactic shock have been reported [23].

26.3.2.1 Critical Points/Problems with the Usage of Fluorescein

Recent studies have confirmed fluorescein staining not to be tumor-cell-specific [22]. Additionally, fluorescein mainly marks areas with BBB breakdown, which are somewhat, but no strictly related to tumor tissue. Thus, fluorescein serves as an excellent marker of edema propagation [111, 112]. There is no real consensus on timing of administration of fluorescein before surgery and dosage in the current literature, although this is critical due to the fact that extravasation and distribution of this agent follow a certain time course regarding plasma fluorescein contents, perfusion of brain, and finally extravasation. After a half-life of 4 and ½ h, intravascular fluorescein will slowly subside, resulting in prolonged staining of normal perfused brain, while being extravasated and traveling with edema through peritumoral tissue, raising the danger of staining non-tumorous tissue [111]. Consequently, the timing of surgery is critical and duration of craniotomy and preparation before reaching the lesion have to be considered. A further aspect confounding applicability of fluorescein is the problem with surgical injury of normal brain tissue, which will lead to unselective extravasation of fluorescein from the bloodstream along the cut margins.

Consequently, further studies are required to evaluate fluorescein in the context of tumor histology, timing of administration, illumination, tissue perfusion, and edema. In summary, from the current point of view, the abovementioned confounders have to be considered using a fluorophore that does not have a specific tumor–fluorophore interaction and is rather a marker of BBB integrity.

26.3.2.2 Fluorescein-Guided Surgery for High-Grade Gliomas

With the success of 5-ALA-guided glioma resection regarding the improvement of the extent of resection, several studies have attempted to evaluate the use of the more inexpensive agent fluorescein regarding its usefulness in improving EOR in malignant gliomas [1, 11, 74, 88, 98]. The groups of Schebesch and Acerbi reported a gross total resection rate of 80% in their series of $n = 35$ and $n = 20$, respectively, high-grade glioma patients using YELLOW 560 filter [1, 100] (Fig. 26.5). Other studies reported GTR in up to 100% of cases using fluorescein, and additionally showing that intraoperative fluorescence correlated well with contrast enhancement on MRI scans [2, 22]. Sensitivity and specificity analyses after administration of low-dose fluorescein revealed a sensitivity of 94% and a specificity of 90% [1]. In 2011, a phase II trial (FLUOGLIO) started to evaluate the safety and efficacy of fluorescein-guided glioma surgery, showing that this approach is feasible, safe [2]. However, the current evidence for usage of fluorescein in high-grade gliomas is still limited to small cohort studies with inherent case selection issues. No further prospective randomized controlled trials are available that investigate the possible benefit of fluorescein in terms of survival and EOR.

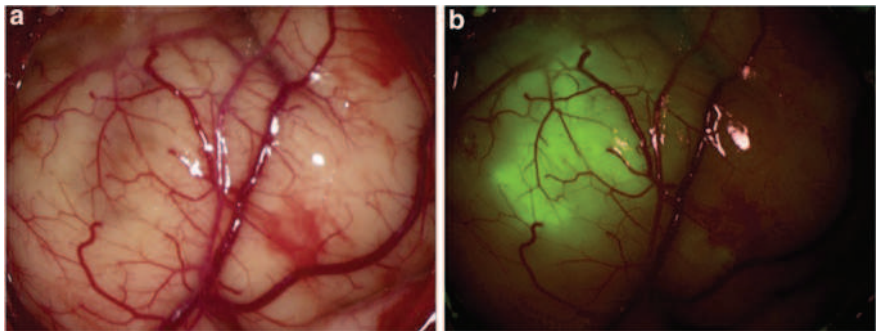


Fig. 26.5 Fluorescein-guided resection of malignant glioma. Intraoperative view of a malignant glioma under white light (**a**) and after application of fluorescein under YELLOW 560 filter (**b**) before corticotomy. Reprinted with permission [35]

Interestingly, the simultaneous usage of fluorescein with 5-ALA has been investigated for the resection of high-grade glioma. This concept results in better background discrimination from circulating fluorescein while retaining the selectivity of 5-ALA-induced porphyrins for discriminating tumor [123].

26.3.2.3 Fluorescein-Guided Surgery for Cerebral Metastases

GTR of cerebral metastases is known as an independent predictor of patient survival [44]. Consequently, intraoperative imaging that helps in increasing EOR is in focus to improve outcome. The benefit of fluorescein in improving extent of resection (EOR) has been shown for metastases, yielding 83–100% GTR. In comparison with traditional white light microscopy, GTR has been reported to be achieved in 54–74% [29, 35, 80].

26.3.2.4 Fluorescein-Guided Surgery for Other CNS Tumors

As in 5-ALA-guided surgery, more and more reports on fluorescein-guided resection of other CNS tumors are becoming available. However, so far, there are only studies with small patient numbers and without standardized protocols for the administration of fluorescein. Fluorescein has been described as helpful for resection of convexity meningioma, enabling visualization and differentiation of meningioma and its dural tail from normal brain and dura [16]. Furthermore, fluorescein might help to enhance contrast between normal brain structures and cranial nerves and meningiomas in skull base surgery [15]. Primary central nervous system (CNS) lymphomas are typically treated with radio- and chemotherapy. However, a surgical biopsy is usually required. Fluorescein has been shown to help visualizing lymphomas by delineating malignant tissue from normal brain [29, 99].

26.3.3 Indocyanine Green (ICG)

Indocyanine green (ICG) is a tricarbocyanine with fluorescence in the near-infrared range with a peak emission at 780 nm and excitation at 810 nm. ICG was approved by the Food and Drug Administration (FDA) in 1959, providing information of liver and cardiocirculatory functions, and later widely used for ophthalmologic applications [12, 87]. The group of Raabe et al. was the first to describe the use of ICG for visualization of blood flow in cerebral vessels exposed in the surgical microscope field, a technique now known as ICG videoangiography [83]. ICG videoangiography was originally used to provide augmentive information on cerebrovascular pathologies and especially used for vascular cases (aneurysms and arteriovenous malformations) [30, 84]. In addition, the utility of ICG to help understand the angioarchitecture of hypervascular tumors, e.g., in case of hemangioblastomas and to identify surrounding vessels has been described. This helps in understanding the angioarchitecture related to the tumor and hereby increasing the safety of the procedure. Following the intravenous administration of 0.2–0.5 mg ICG/kg, NIR light (range 700–850 nm) is used to excite the dye, and a NIR camera —integrated into the latest generations of surgical microscopes—captures the emission at 780–950 nm of the molecules flowing through the vessels.

The use of ICG is safe with an incidence of adverse reactions ranging from 0.05% for severe side effects such as hypotension, arrhythmia, anaphylactic shock to 0.2% for mild or moderate side effects such as nausea, skin eruption, and pruritus [83].

A growing body of research has led to the utilization of ICG in neuro-oncological surgery as well. One technique has been referred to as second-window ICG (SWIG), a procedure in which higher doses up to 5.0 mg/kg are administered to the patient up to 24 h in advance of surgery with intraoperative imaging [61]. Within the 24 h, ICG accumulates in the tumor tissue due to enhanced permeability within the tumor and selective retention effects [125]. It is assumed that ICG binds to serum albumin and can pass through the disrupted blood–brain barrier. ICG is retained possibly due to a lack of drainage [61, 125]. However, the exact mechanisms of retention are not entirely clear.

Unlike 5-ALA-PpIX and fluorescein, which emit fluorescence within the visible spectrum, ICG's excitation and emission are in the near-infrared (NIR) region of the spectrum [137]. These properties enable visualization of ICG fluorophore situated deeper in the tissue since longer wavelength excitation and emission light undergo less absorption than shorter wavelength [137]. Therefore, ICG permits visualization of tumors up to a depth of 2 cm and through the dura, facilitating planning of dural opening and corticectomy. On the other hand, NIR cameras are necessary and for the moment ICG fluorescence can only be visualized on the video screen and not directly within the cavity during surgery.

Studies revealed ICG fluorescence to be detectable in contrast-enhancing gliomas and have discussed ICG's value for the detection of tumor margins. Strong tumor-to-background fluorescence ratios were found and fluorescence appeared to correlate with the degree of contrast enhancement on preoperative MRI [61]. So far,

there are no studies demonstrating a benefit for extent of resection in glioma surgery. Furthermore, ICG is not tumor-specific, incorporating the risk of resection of normal false positive tissue.

In addition, some authors suggest the usage of second-window ICG in order to visualize residual tumor and the margins in surgery for metastases and meningioma [62, 63]. However, the benefit of this intraoperative tool warrants further investigation.

26.4 Novel Techniques

Despite several advantages and the broad use of wide-field fluorescence imaging techniques, there are some limitations regarding sensitivity for the detection of malignant cells. Improvements in FGS are ongoing and further techniques are being developed to improve brain tumor surgery. Most of these advanced technologies are in their fledgling stage and are presently subject to intensive research.

26.4.1 Tumor-Targeted Alkylphosphocholine Analogs for Intraoperative Visualization

A major aim in surgery of malignant brain tumors is complete resection of tumor cells while sparing healthy brain parenchyma. To this end, cancer-targeted alkylphosphocholine analogs (APCs) attached to fluorophores are being explored for intraoperative visualization of tumor cells. APC analogs are small synthetic phospholipid ether molecules that are taken up by tumor cells through overexpressed lipid rafts and undergo prolonged retention due to decreased catabolism [33, 139]. APC analogs were originally developed for PET imaging and targeted radiotherapy and appear to have a broad tumor-targeting potential [139]. Two fluorescent APCs (CLR1501-green fluorescence and CLR1502-near-infrared fluorescence) have been found to label tumor cells in a glioblastoma xenograft model with a high cancer cell selectivity [125]. Future step of this technology, which is translating into clinical use, is the development of a dual-labeled APC enabling NIR fluorescence with deep tissue penetration and PET imaging with the same agent. This offers the possibility of synergistic diagnostic detection of tumor cells and usage at multiple phases of tumor management with regard to tumor resection, staging, and possible localized radiotherapy. However, up to date, these are preclinical data and the benefit in the management of brain tumor patients has yet to be determined [56, 144].

26.4.2 Confocal Endomicroscopy

A major problem with wide-field fluorescence-guided surgery is the lack of high resolution and the subjective interpretation of fluorescence intensities, challenging delineation of normal brain tissue and tumor tissue especially in glioma surgery and at the tumor margins. Predictions of grade to predict tumor grade from preoperative imaging in glioma surgery are often uncertain [72]. Therefore, time-consuming intraoperative frozen sections are regularly performed. However, there are some shortcomings about frozen-section pathology as it can be non-diagnostic or misleading in some cases [130].

To overcome these limitations, confocal endomicroscopy has been recently introduced into the field of neurosurgery, a technique that allows standard neuropathological diagnostics as a real-time intraoperative technique [27, 66]. The system consists of a handheld probe that uses a single optical fiber for illumination and detection and displays high-resolution images with up to 1000-fold magnification to a movable LCD workstation [34]. Tissue contrast is achieved by administration of fluorescent dyes, e.g., fluorescein [27].

This "optical biopsy" facilitates surgeons to detect tumor remnants at resection margins with higher accuracy and in especially critical and eloquent regions were an aggressive maximal extent of resection is not possible and can be probed with confocal endomicroscopy for measurement of tumor cell densities before resection.

In cases of LGG, which often do not show visible PPIX fluorescence, intraoperative confocal endomicroscopy is able to visualize the presence of even small amounts of fluorescence in tumor cells [97]. Furthermore, visualization of tumor cells with confocal endomicroscopy has been shown for a variety of brain tumors, including meningiomas, hemangioblastomas, gliomas, and neurocytomas [95].

Confocal endomicroscopy only enables a small field of view of about 0.5 mm in diameter and therefore cannot be used to scan the whole tumor area, but can be utilized at the end of surgery to detect malignant tissue remnants. To interpret the images, however, knowledge of histopathology or the presence of a neuropathologist is required [4].

26.4.3 Raman Spectroscopy

All previously mentioned visualization techniques rely on labeling agents that have limitations regarding sensitivity or specificity for tumor detection. Recently, label-free techniques have emerged in the field of intraoperative tumor visualization that depends on the intrinsic biochemical properties of normal versus pathological tissue to provide image contrast [36, 43, 77].

Raman spectroscopy is one such technique that is based on the findings of C.V. Raman in 1928 [86]. The Raman Effect refers to the scattering of monochromatic light. Most photons in the visible spectrum are scattered elastically when interacting with tissue or a media. However, a small portion of photons absorbs energy or transfers energy from or to the object being imaged. The transfer in energy results in

inelastic scattering and is called the Raman Effect. The Raman Effect can be captured using a sensitive spectrometer. Raman spectroscopy is used to provide information on the chemical composition of different tissues, e.g., their lipid and protein ratios, providing a structural fingerprint. Investigations have shown that Raman spectroscopy is helpful in delineating normal brain parenchyma from tumor tissue and necrosis [43, 50, 51, 54]. Accuracies of up to 98% for discrimination in frozen sections have been described [43].

Recently, a Raman spectroscopy handheld probe system for in vivo intraoperative use has been developed [20]. Jermyn et al. were able to distinguish normal brain from high- and low-grade gliomas invaded brain with high accuracy [40]. The aim of this method is to analyze the molecular nature of the tissue prior to resection, with the aim of improving cancer targeting and patient safety [21] (Fig. 26.6).

26.4.4 BLZ-100 Fluorescence-Guided Brain Tumor Surgery

Targeted fluorescence imaging using fluorescent-labeled probes with tumor-specific molecular targets is the next obvious step toward a higher accuracy for discriminating tumor-infiltrated tissue from normal brain. The combination of the fluorophore with a tumor-specific peptide enhances specificity for detection of malignant cells. BLZ-100 (tozuleristide) is such a molecule that consists of a tumor-targeting peptide, chlorotoxin, coupled to the near-infrared fluorophore ICG [79]. Chlorotoxin, extracted from the venom of scorpions, specifically binds to gliomas and tumors of neuroectodermal origin [64]. BLZ-100 is administered intravenously 24 h prior to surgery. As mentioned above, ICG fluorescence can be visualized using a NIR camera. A high affinity of BLZ-100 toward human gliomas has been demonstrated [8]. However, further trials are needed to determine the utility of BLZ-100 in glioma surgery.

26.5 Combination of Different Techniques for Intraoperative Imaging

The combination of different imaging modalities, e.g., neuronavigation with MRI and PET with specific biomarkers and FGS, allows the generation of comprehensive information on tumor location, extent, anatomy, metabolism, and function [60, 68].

Newer techniques might help to add additional information on the chemical or molecular composition of the tissue. Using different modalities helps to overcome limitations of single techniques, e.g., brain shift, using synergistic effects and utilizing the benefits of several techniques [53, 102, 103].

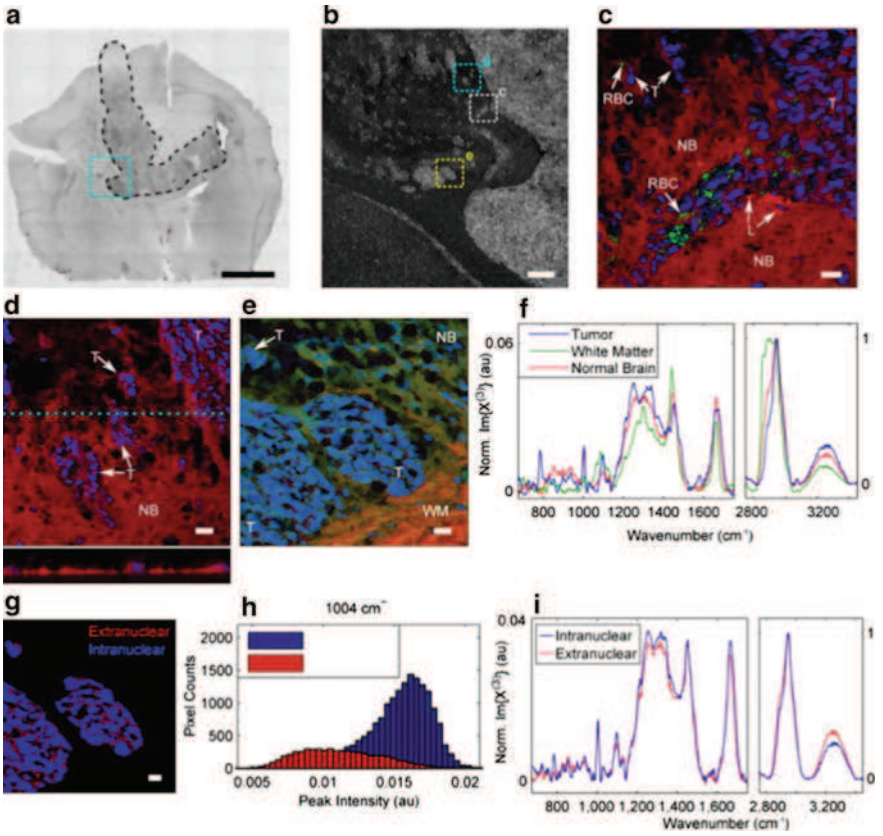


Fig. 26.6 Raman spectroscopy. **a** Brightfield image of xenograft glioblastoma in mouse brain outlining tumor hard boundary (black, dashed line), b, Cyan dashed box indicates region of interest (ROI). Scale bar, 2 mm. **b** Phase contrast micrograph of BCARS ROIs with boxes and associated subfigure labels. Scale bar, 200 μm. **c** Pseudocolor BCARS image of tumor and normal brain tissue highlighting nuclei (blue), lipid content (red), and red blood cells (green). **d** BCARS image and axial scan highlighting nuclei (blue) and lipid content (red). **e** BCARS image highlighting nuclei (blue), lipid content (red), and CH_3-stretch–CH_2-stretch (green). NB: normal brain; T: tumor cells; RBC: red blood cells; L: lipid bodies; WM: white matter. **f** Single-pixel spectra. **g** Spectrally segmented image of internuclear (blue) and extranuclear (red) tumoral spaces. **h** Histogram analysis of phenylalanine content. **i** Mean spectra from within tumor mass. **c**–**e** and **g**, Scale bars, 20 μm. Reproduced with permission [9]

26.6 Future Directions

All the developments discussed here aim at better intraoperative visualization in order to improve accuracy and extent of resection. However, current methods, which have been established in the clinical routine setting, face individual limitations. Consequently, further research is required to overcome these limitations. For

methods exploiting induced tissue fluorescence, quantitative as opposed to qualitative assessments require more scrutiny. At the moment, the decision of whether tissue is "fluorescent" relies on the subjective impression and assessment of the surgeon. Several attempts to measure and quantify fluorescence have been undertaken. One promising technology given quantitative information is fluorescence spectrography. Small handheld devices for intraoperative spectroscopy are available that enable determining the actual PpIX concentration in tumor tissue using 5-ALA FGS, even when no fluorescence is visible under the microscope [49, 121, 136]. Using this method, objective measurements of tissue fluorescence can be achieved. In addition, invisible PpIX accumulation, e.g., in patients with LGG, that would have gone unnoticed by the surgeon's visual perception alone can be detected using spectroscopy. A 100-fold increase of sensitivity of fluorescence detection in LGG using handheld spectroscopy has been shown [135].

Furthermore, more specific labeling of tumor cells is under investigation, e.g., targeted fluorescence imaging. Innovations of neurosurgical microscopes will improve the view of the surgical field using all the mentioned visualization tools, detecting additional optical features in tumor tissue [137].

Multimodality in the applied imaging techniques and development of new techniques will provide comprehensive information derived from different sources regarding biological, metabolic, anatomical, and functional properties of tissue (Table 26.1). Neurosurgeons are beginning to integrate imaging concepts into their daily routine for brain tumor surgery, aiming at better extent of resection while lowering the risk of removal of functional non-tumor tissue.

Table 26.1 Overview on the current techniques for intraoperative tumor visualization with their advantages and disadvantages, modified from [101]

Technique	Advantages	Disadvantages
5-ALA	• Selectively absorbed by tumor cells and is converted into fluorescent PPIX • Low toxicity, high safety • Intraoperative real-time feedback • Brain shift does not interfere with this technique • Full integration into the surgical microscope • View of the full surgical field • Use without interruption to the surgical workflow • Reliable correlation with preoperative contrast enhancement on MRI • Correlation with histopathology • Metabolic labeling	• Low background illumination, loss of normal optical information – Alternating between white light and fluorescence mode • Imaging surface tool, depth can limit visualization • Requires special microscope • Expensive • Bleaching effect • Time dependency • Subjective interpretation of fluorescence intensities

(continued)

Table 26.1 (continued)

Technique	Advantages	Disadvantages
Fluorescein	• Robust and inexpensive • Can be visualized by the naked eye (using higher concentrations) • Low toxicity, high safety • Intraoperative real-time feedback • Brain shift does not interfere with this technique • Full integration into the surgical microscope • View of the full surgical field • Use without interruption to the surgical workflow	• Not tumor-cell-specific – Marker of BBB breakdown • Unselective extravasation during surgery • Time dependency • Subjective interpretation of fluorescence intensities • Passive labeling
Indocyanine green (ICG)	• Excitation and emission in the near-infrared region – Enables visualization of fluorescence situated deeper in the tissue • Less scatter of emitted light • Low tissue autofluorescence • Low toxicity, high safety • Intraoperative real-time feedback • Brain shift does not interfere with this technique • Full integration into the surgical microscope • View of the full surgical field • Use without interruption to the surgical workflow	• Requires special cameras and instrumentation to visualize fluorescence • Not tumor-specific • Accumulates due to an enhanced permeability of the BBB • Time dependency • Subjective interpretation of fluorescence intensities • Passive labeling
Neuronavigation	• Ubiquitous • Several imaging modalities can be entered into the software, providing information on – Anatomy – Localization and orientation (almost real time) – Extent of tumor – Function (fMRI) – Fiber tracts (DTI) – Metabolic (PET) • Planning of surgical approach/craniotomy • Use without interruption to the surgical workflow	• Loss of accuracy due to intraoperative brain shift caused by positioning, application of mannitol, and drainage of cerebral spinal fluid (CSF) • Information used during surgery is displayed on a screen outside the surgical field—interruptions to surgical workflow

References

1. Acerbi F et al (2014) Is fluorescein-guided technique able to help in resection of high-grade gliomas? Neurosurg Focus 36:E5. https://doi.org/10.3171/2013.11.FOCUS13487
2. Acerbi F et al (2018) Fluorescein-guided surgery for resection of high-grade gliomas: a multicentric prospective phase II study (FLUOGLIO). Clin Cancer Res 24:52–61. https://doi.org/10.1158/1078-0432.ccr-17-1184
3. Basser PJ, Mattiello J, LeBihan D (1994) Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B 103:247–254
4. Belykh E et al (2016) Intraoperative fluorescence imaging for personalized brain tumor resection: current state and future directions. Front Surg 3:55. https://doi.org/10.3389/fsurg.2016.00055
5. Bernal Garcia LM, Cabezudo Artero JM, Marcelo Zamorano MB, Gilete Tejero I (2015) Fluorescence-guided resection with 5-aminolevulinic Acid of subependymomas of the fourth ventricle: report of 2 cases: technical case report. Neurosurgery 11(Suppl 2):E364–E371; discussion E371. https://doi.org/10.1227/neu.0000000000000682
6. Brandes AA, Tosoni A, Spagnolli F, Frezza G, Leonardi M, Calbucci F, Franceschi E (2008) Disease progression or pseudoprogression after concomitant radiochemotherapy treatment: pitfalls in neurooncology. Neuro Oncol 10:361–367. https://doi.org/10.1215/15228517-2008-008
7. Brown TJ et al (2016) Association of the extent of resection with survival in glioblastoma: a systematic review and meta-analysis. JAMA Oncol 2:1460–1469. https://doi.org/10.1001/jamaoncol.2016.1373
8. Butte PV et al (2014) Near-infrared imaging of brain tumors using the tumor paint BLZ-100 to achieve near-complete resection of brain tumors. Neurosurg Focus 36:E1. https://doi.org/10.3171/2013.11.focus13497
9. Camp CH Jr et al (2014) High-speed coherent Raman fingerprint imaging of biological tissues. Nat Photonics 8:627–634. https://doi.org/10.1038/nphoton.2014.145
10. Carlsson SK, Brothers SP, Wahlestedt C (2014) Emerging treatment strategies for glioblastoma multiforme. EMBO Mol Med 6:1359–1370. https://doi.org/10.15252/emmm.201302627
11. Chen B et al (2012) Gross total resection of glioma with the intraoperative fluorescence-guidance of fluorescein sodium. Int J Med Sci 9:708–714. https://doi.org/10.7150/ijms.4843
12. Cherrick GR, Stein SW, Leevy CM, Davidson CS (1960) Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 39:592–600. https://doi.org/10.1172/jci104072
13. Coburger J, Hagel V, Wirtz CR, Konig R (2015) Surgery for glioblastoma: impact of the combined use of 5-aminolevulinic acid and intraoperative MRI on extent of resection and survival. PLoS One 10:e0131872. https://doi.org/10.1371/journal.pone.0131872
14. Colditz MJ, Leyen K, Jeffree RL (2012) Aminolevulinic acid (ALA)-protoporphyrin IX fluorescence guided tumour resection. Part 2: theoretical, biochemical and practical aspects. J Clin Neurosci 19:1611–1616. https://doi.org/10.1016/j.jocn.2012.03.013
15. da Silva CE, da Silva JL, da Silva VD (2010) Use of sodium fluorescein in skull base tumors. Surg Neurol Int 1:70. https://doi.org/10.4103/2152-7806.72247
16. da Silva CE, da Silva VD, da Silva JL (2014) Convexity meningiomas enhanced by sodium fluorescein. Surg Neurol Int 5:3. https://doi.org/10.4103/2152-7806.124978
17. de Wit MC, de Bruin HG, Eijkenboom W, Sillevis Smitt PA, van den Bent MJ (2004) Immediate post-radiotherapy changes in malignant glioma can mimic tumor progression. Neurology 63:535–537
18. Della Puppa A et al (2013) 5-aminolevulinic acid (5-ALA) fluorescence guided surgery of high-grade gliomas in eloquent areas assisted by functional mapping. Our experience and review of the literature. Acta Neurochir (Wien) 155:965–972; discussion 972. https://doi.org/10.1007/s00701-013-1660-x

19. Della Puppa A et al. (2014) Predictive value of intraoperative 5-aminolevulinic acid-induced fluorescence for detecting bone invasion in meningioma surgery J Neurosurg 120:840–845 https://doi.org/10.3171/2013.12.jns131642
20. Desroches J et al (2015) Characterization of a Raman spectroscopy probe system for intraoperative brain tissue classification. Biomed Opt Express 6:2380–2397. https://doi.org/10.1364/BOE.6.002380
21. Desroches J et al (2018) A new method using Raman spectroscopy for in vivo targeted brain cancer tissue biopsy. Sci Rep 8:1792. 10.1038/s41598-018-20233-3
22. Diaz RJ et al (2015) Study of the biodistribution of fluorescein in glioma-infiltrated mouse brain and histopathological correlation of intraoperative findings in high-grade gliomas resected under fluorescein fluorescence guidance. J Neurosurg 122:1360–1369. https://doi.org/10.3171/2015.2.JNS132507
23. Dilek O, Ihsan A, Tulay H (2011) Anaphylactic reaction after fluorescein sodium administration during intracranial surgery. J Clin Neurosci 18:430–431. https://doi.org/10.1016/j.jocn.2010.06.012
24. Eicker S et al (2011) ALA-induced porphyrin accumulation in medulloblastoma and its use for fluorescence-guided surgery. Cent Eur Neurosurg 72:101–103. https://doi.org/10.1055/s-0030-1252010
25. Elhawary H et al (2011) Intraoperative real-time querying of white matter tracts during frameless stereotactic neuronavigation. Neurosurgery 68:506–516; discussion 516. https://doi.org/10.1227/neu.0b013e3182036282
26. Ewelt C et al (2011) Finding the anaplastic focus in diffuse gliomas: the value of Gd-DTPA enhanced MRI, FET-PET, and intraoperative, ALA-derived tissue fluorescence. Clin Neurol Neurosurg 113:541–547. https://doi.org/10.1016/j.clineuro.2011.03.008
27. Foersch S et al (2012) Confocal laser endomicroscopy for diagnosis and histomorphologic imaging of brain tumors in vivo. PLoS ONE 7:e41760. https://doi.org/10.1371/journal.pone.0041760
28. Hadjipanayis CG, Widhalm G, Stummer W (2015) What is the surgical benefit of utilizing 5-aminolevulinic acid for fluorescence-guided surgery of malignant gliomas? Neurosurgery 77:663–673. https://doi.org/10.1227/NEU.0000000000000929
29. Hamamcioglu MK, Akcakaya MO, Goker B, Kasimcan MO, Kiris T (2016) The use of the YELLOW 560 nm surgical microscope filter for sodium fluorescein-guided resection of brain tumors: our preliminary results in a series of 28 patients. Clin Neurol Neurosurg 143:39–45. https://doi.org/10.1016/j.clineuro.2016.02.006
30. Hanggi D, Etminan N, Steiger HJ (2010) The impact of microscope-integrated intraoperative near-infrared indocyanine green videoangiography on surgery of arteriovenous malformations and dural arteriovenous fistulae. Neurosurgery 67:1094–1103; discussion 1103–1094. https://doi.org/10.1227/neu.0b013e3181eb5049
31. Hervey-Jumper SL, Berger MS (2014) Role of surgical resection in low- and high-grade gliomas. Curr Treat Options Neurol 16:284. https://doi.org/10.1007/s11940-014-0284-7
32. Hickmann AK, Nadji-Ohl M, Hopf NJ (2015) Feasibility of fluorescence-guided resection of recurrent gliomas using five-aminolevulinic acid: retrospective analysis of surgical and neurological outcome in 58 patients. J Neurooncol 122:151–160. https://doi.org/10.1007/s11060-014-1694-9
33. Hilgard P, Klenner T, Stekar J, Unger C (1993) Alkylphosphocholines: a new class of membrane-active anticancer agents. Cancer Chemother Pharmacol 32:90–95
34. Hoffman A, Goetz M, Vieth M, Galle PR, Neurath MF, Kiesslich R (2006) Confocal laser endomicroscopy: technical status and current indications. Endoscopy 38:1275–1283. https://doi.org/10.1055/s-2006-944813
35. Hohne J, Hohenberger C, Proescholdt M, Riemenschneider MJ, Wendl C, Brawanski A, Schebesch KM (2017) Fluorescein sodium-guided resection of cerebral metastases-an update. Acta Neurochir (Wien) 159:363–367. https://doi.org/10.1007/s00701-016-3054-3

36. Hollon T, Lewis S, Freudiger CW, Sunney Xie X, Orringer DA (2016) Improving the accuracy of brain tumor surgery via Raman-based technology. Neurosurg Focus 40:E9. https://doi.org/10.3171/2015.12.FOCUS15557
37. Idoate MA, Diez Valle R, Echeveste J, Tejada S (2011) Pathological characterization of the glioblastoma border as shown during surgery using 5-aminolevulinic acid-induced fluorescence. Neuropathology 31:575–582. https://doi.org/10.1111/j.1440-1789.2011.01202.x
38. Inoue T, Endo T, Nagamatsu K, Watanabe M, Tominaga T (2013) 5-aminolevulinic acid fluorescence-guided resection of intramedullary ependymoma: report of 9 cases. Neurosurgery 72:ons159–168; discussion ons168. https://doi.org/10.1227/neu.0b013e31827bc7a3
39. Jaber M et al (2016) The value of 5-aminolevulinic acid in low-grade gliomas and high-grade gliomas lacking glioblastoma imaging features: an analysis based on fluorescence, magnetic resonance imaging, 18F-fluoroethyl tyrosine positron emission tomography, and tumor molecular factors. Neurosurgery 78:401–411; discussion 411. https://doi.org/10.1227/neu.0000000000001020
40. Jermyn M et al (2015) Intraoperative brain cancer detection with Raman spectroscopy in humans. Sci Transl Med 7:274ra219. https://doi.org/10.1126/scitranslmed.aaa2384
41. Jolesz FA (2011) Intraoperative imaging in neurosurgery: where will the future take us? Acta Neurochir Suppl 109:21–25. https://doi.org/10.1007/978-3-211-99651-5_4
42. Jung TY, Jung S, Kim IY, Park SJ, Kang SS, Kim SH, Lim SC (2006) Application of neuronavigation system to brain tumor surgery with clinical experience of 420 cases. Minim Invasive Neurosurg 49:210–215. https://doi.org/10.1055/s-2006-948305
43. Kalkanis SN et al (2014) Raman spectroscopy to distinguish grey matter, necrosis, and glioblastoma multiforme in frozen tissue sections. J Neurooncol 116:477–485. https://doi.org/10.1007/s11060-013-1326-9
44. Kalkanis SN, Linskey ME (2010) Evidence-based clinical practice parameter guidelines for the treatment of patients with metastatic brain tumors: introduction. J Neurooncol 96:7–10. https://doi.org/10.1007/s11060-009-0065-4
45. Kamp MA et al (2015) 5-ALA-induced fluorescence behavior of reactive tissue changes following glioblastoma treatment with radiation and chemotherapy. Acta Neurochir (Wien) 157:207–213; discussion 213–204. https://doi.org/10.1007/s00701-014-2313-4
46. Kamp MA et al (2016) 5-ALA fluorescence of cerebral metastases and its impact for the local-in-brain progression. Oncotarget 7:66776–66789. https://doi.org/10.18632/oncotarget.11488
47. Kamp MA, Grosser P, Felsberg J, Slotty PJ, Steiger HJ, Reifenberger G, Sabel M (2012) 5-aminolevulinic acid (5-ALA)-induced fluorescence in intracerebral metastases: a retrospective study. Acta Neurochir (Wien) 154:223–228; discussion 228. https://doi.org/10.1007/s00701-011-1200-5
48. Kaneko S, Suero Molina E, Ewelt C, Warneke N, Stummer W (2019) Fluorescence-based measurement of real-time kinetics of protoporphyrin IX after 5-aminolevulinic acid administration in human in situ malignant gliomas. Neurosurgery
49. Kim A, Khurana M, Moriyama Y, Wilson BC (2010) Quantification of in vivo fluorescence decoupled from the effects of tissue optical properties using fiber-optic spectroscopy measurements. J Biomed Opt 15:067006. https://doi.org/10.1117/1.3523616
50. Kirsch M, Schackert G, Salzer R, Krafft C (2010) Raman spectroscopic imaging for in vivo detection of cerebral brain metastases. Anal Bioanal Chem 398:1707–1713. https://doi.org/10.1007/s00216-010-4116-7
51. Kohler M, Machill S, Salzer R, Krafft C (2009) Characterization of lipid extracts from brain tissue and tumors using Raman spectroscopy and mass spectrometry. Anal Bioanal Chem 393:1513–1520. https://doi.org/10.1007/s00216-008-2592-9
52. Koshy M et al (2012) Improved survival time trends for glioblastoma using the SEER 17 population-based registries J Neurooncol 107:207–212 https://doi.org/10.1007/s11060-011-0738-7

53. Kracht LW et al (2004) Delineation of brain tumor extent with [11C]L-methionine positron emission tomography: local comparison with stereotactic histopathology. Clin Cancer Res 10:7163–7170. https://doi.org/10.1158/1078-0432.ccr-04-0262
54. Krafft C, Neudert L, Simat T, Salzer R (2005) Near infrared Raman spectra of human brain lipids. Spectrochim Acta A Mol Biomol Spectrosc 61:1529–1535. https://doi.org/10.1016/j.saa.2004.11.017
55. Kremer P et al (2006) Intraoperative MRI for interventional neurosurgical procedures and tumor resection control in children. Childs Nerv Syst 22:674–678. https://doi.org/10.1007/s00381-005-0030-2
56. Kuo JS, Zhang RR, Pinchuk AN, Clark PA, Weichert JP (2016) Creation of a dual-labeled cancer-targeting alkylphosphocholine analog for dual modality quantitative positron emission tomography and intraoperative tumor visualization. Neurosurgery 63:208
57. Kuroiwa T, Kajimoto Y, Ohta T (1998) Development of a fluorescein operative microscope for use during malignant glioma surgery: a technical note and preliminary report. Surg Neurol 50:41–48; discussion 48–49
58. Lacroix M et al (2001) A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg 95:190–198. https://doi.org/10.3171/jns.2001.95.2.0190
59. Lau D, Hervey-Jumper SL, Chang S, Molinaro AM, McDermott MW, Phillips JJ, Berger MS (2016) A prospective Phase II clinical trial of 5-aminolevulinic acid to assess the correlation of intraoperative fluorescence intensity and degree of histologic cellularity during resection of high-grade gliomas. J Neurosurg 124:1300–1309. https://doi.org/10.3171/2015.5.jns1577
60. Laukamp KR et al (2017) Multimodal imaging of patients with gliomas confirms (11) C-MET PET as a complementary marker to MRI for noninvasive tumor grading and intraindividual follow-up after therapy. Mol Imaging 16:1536012116687651. https://doi.org/10.1177/1536012116687651
61. Lee JY et al (2016) Intraoperative near-infrared optical imaging can localize gadolinium-enhancing gliomas during surgery. Neurosurgery 79:856–871. https://doi.org/10.1227/NEU.0000000000001450
62. Lee JYK, Pierce JT, Thawani JP, Zeh R, Nie S, Martinez-Lage M, Singhal S (2018) Near-infrared fluorescent image-guided surgery for intracranial meningioma. J Neurosurg 128:380–390. https://doi.org/10.3171/2016.10.JNS161636
63. Lee JYK, Pierce JT, Zeh R, Cho SS, Salinas R, Nie S, Singhal S (2017) Intraoperative near-infrared optical contrast can localize brain metastases. World Neurosurg 106:120–130. https://doi.org/10.1016/j.wneu.2017.06.128
64. Lyons SA, O'Neal J, Sontheimer H (2002) Chlorotoxin, a scorpion-derived peptide, specifically binds to gliomas and tumors of neuroectodermal origin. Glia 39:162–173. https://doi.org/10.1002/glia.10083
65. Marko NF, Weil RJ, Schroeder JL, Lang FF, Suki D, Sawaya RE (2014) Extent of resection of glioblastoma revisited: personalized survival modeling facilitates more accurate survival prediction and supports a maximum-safe-resection approach to surgery. J Clin Oncol 32:774–782. https://doi.org/10.1200/JCO.2013.51.8886
66. Martirosyan NL et al (2011) Use of in vivo near-infrared laser confocal endomicroscopy with indocyanine green to detect the boundary of infiltrative tumor. J Neurosurg 115:1131–1138. https://doi.org/10.3171/2011.8.JNS11559
67. Mercier L et al (2011) New prototype neuronavigation system based on preoperative imaging and intraoperative freehand ultrasound: system description and validation. Int J Comput Assist Radiol Surg 6:507–522. https://doi.org/10.1007/s11548-010-0535-3
68. Meyer GJ, Schober O, Hundeshagen H (1985) Uptake of 11C-L- and D-methionine in brain tumors. Eur J Nucl Med 10:373–376

69. Millesi M et al (2016) Analysis of the surgical benefits of 5-ALA-induced fluorescence in intracranial meningiomas: experience in 204 meningiomas. J Neurosurg 125:1408–1419. https://doi.org/10.3171/2015.12.jns151513
70. Moore GE (1947) Fluorescein as an agent in the differentiation of normal and malignant tissues. Science 106:130–131. https://doi.org/10.1126/science.106.2745.130-a
71. Morofuji Y, Matsuo T, Hayashi Y, Suyama K, Nagata I (2008) Usefulness of intraoperative photodynamic diagnosis using 5-aminolevulinic acid for meningiomas with cranial invasion: technical case report. Neurosurgery 62:102–103; discussion 103–104. https://doi.org/10.1227/01.neu.0000317378.22820.46
72. Muragaki Y et al (2008) Low-grade glioma on stereotactic biopsy: how often is the diagnosis accurate? Minim Invasive Neurosurg 51:275–279. https://doi.org/10.1055/s-0028-1082322
73. Nabavi A et al (2009) Five-aminolevulinic acid for fluorescence-guided resection of recurrent malignant gliomas: a phase II study. Neurosurgery 65:1070–1076; discussion 1076–1077. https://doi.org/10.1227/01.neu.0000360128.03597.c7
74. Neira JA et al (2017) Aggressive resection at the infiltrative margins of glioblastoma facilitated by intraoperative fluorescein guidance. J Neurosurg 127:111–122. https://doi.org/10.3171/2016.7.JNS16232
75. Orringer D et al. (2012a) Extent of resection in patients with glioblastoma: limiting factors, perception of resectability, and effect on survival. J Neurosurg 117:851–859. https://doi.org/10.3171/2012.8.jns12234
76. Orringer DA, Golby A, Jolesz F (2012b) Neuronavigation in the surgical management of brain tumors: current and future trends. Expert Rev Med Devices 9:491–500. https://doi.org/10.1586/erd.12.42
77. Orringer DA et al. (2017) Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy. Nat Biomed Eng 1. https://doi.org/10.1038/s41551-016-0027
78. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS (2018) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. Neuro Oncol 20:iv1–iv86. https://doi.org/10.1093/neuonc/noy131
79. Parrish-Novak J et al (2017) Nonclinical profile of BLZ-100, a tumor-targeting fluorescent imaging agent. Int J Toxicol 36:104–112 https://doi.org/10.1177/1091581817697685
80. Patchell RA et al (1998) Postoperative radiotherapy in the treatment of single metastases to the brain: a randomized trial. JAMA 280:1485–1489
81. Paulus W, Peiffer J (1989) Intratumoral histologic heterogeneity of gliomas. A quantitative study. Cancer 64:442–447
82. Perrini P et al (2017) Survival outcomes following repeat surgery for recurrent glioblastoma: a single-center retrospective analysis. J Neurooncol 131:585–591. https://doi.org/10.1007/s11060-016-2330-7
83. Raabe A, Beck J, Gerlach R, Zimmermann M, Seifert V (2003) Near-infrared indocyanine green video angiography: a new method for intraoperative assessment of vascular flow. Neurosurgery 52:132–139; discussion 139
84. Raabe A et al (2005) Prospective evaluation of surgical microscope-integrated intraoperative near-infrared indocyanine green videoangiography during aneurysm surgery. J Neurosurg 103:982–989. https://doi.org/10.3171/jns.2005.103.6.0982
85. Rabb MF, Burton TC, Schatz H, Yannuzzi LA (1978) Fluorescein angiography of the fundus: a schematic approach to interpretation. Surv Ophthalmol 22:387–403
86. Raman C (1928) A new type of secondary radiation. Nature 121:501–502
87. Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA (2016) Indocyanine green: historical context, current applications, and future considerations. Surg Innov 23:166–175. https://doi.org/10.1177/1553350615604053
88. Rey-Dios R, Hattab EM, Cohen-Gadol AA (2014) Use of intraoperative fluorescein sodium fluorescence to improve the accuracy of tissue diagnosis during stereotactic needle biopsy of

high-grade gliomas. Acta Neurochir (Wien) 156:1071–1075; discussion 1075. https://doi.org/10.1007/s00701-014-2097-6
89. Ringel F et al (2016) Clinical benefit from resection of recurrent glioblastomas: results of a multicenter study including 503 patients with recurrent glioblastomas undergoing surgical resection. Neuro Oncol 18:96–104. https://doi.org/10.1093/neuonc/nov145
90. Roberts DW et al (2011) Coregistered fluorescence-enhanced tumor resection of malignant glioma: relationships between delta-aminolevulinic acid-induced protoporphyrin IX fluorescence, magnetic resonance imaging enhancement, and neuropathological parameters. Clinical Artic J Neurosurg 114:595–603. https://doi.org/10.3171/2010.2.JNS091322
91. Ruge JR, Liu J (2009) Use of 5-aminolevulinic acid for visualization and resection of a benign pediatric brain tumor. J Neurosurg Pediatr 4:484–486. https://doi.org/10.3171/2009.6.peds08428
92. Rygh OM, Selbekk T, Torp SH, Lydersen S, Hernes TA, Unsgaard G (2008) Comparison of navigated 3D ultrasound findings with histopathology in subsequent phases of glioblastoma resection. Acta Neurochir (Wien) 150:1033–1041; discussion 1042. https://doi.org/10.1007/s00701-008-0017-3
93. Saether CA, Torsteinsen M, Torp SH, Sundstrom S, Unsgard G, Solheim O (2012) Did survival improve after the implementation of intraoperative neuronavigation and 3D ultrasound in glioblastoma surgery? a retrospective analysis of 192 primary operations. J Neurol Surg A Cent Eur Neurosurg 73:73–78. https://doi.org/10.1055/s-0031-1297247
94. Sanai N, Berger MS (2008) Glioma extent of resection and its impact on patient outcome. Neurosurgery 62:753–764; discussion 264–756. https://doi.org/10.1227/01.neu.0000318159.21731.cf
95. Sanai N et al. (2011a) Intraoperative confocal microscopy for brain tumors: a feasibility analysis in humans. Neurosurgery 68:282–290; discussion 290. https://doi.org/10.1227/neu.0b013e318212464e
96. Sanai N, Polley MY, McDermott MW, Parsa AT, Berger MS (2011b) An extent of resection threshold for newly diagnosed glioblastomas. J Neurosurg 115:3–8. https://doi.org/10.3171/2011.2.JNS10998; https://doi.org/10.3171/2011.7.JNS10238
97. Sanai N, Snyder LA, Honea NJ, Coons SW, Eschbacher JM, Smith KA, Spetzler RF (2011c) Intraoperative confocal microscopy in the visualization of 5-aminolevulinic acid fluorescence in low-grade gliomas. J Neurosurg 115:740–748. https://doi.org/10.3171/2011.6.jns11252
98. Schebesch KM, Brawanski A, Hohenberger C, Hohne J (2016) Fluorescein sodium-guided surgery of malignant brain tumors: history, current concepts, and future project. Turk Neurosurg 26:185–194. https://doi.org/10.5137/1019-5149.JTN.16952-16.0
99. Schebesch KM et al (2015) Fluorescein sodium-guided surgery in cerebral lymphoma. Clin Neurol Neurosurg 139:125–128. https://doi.org/10.1016/j.clineuro.2015.09.015
100. Schebesch KM et al (2013) Sodium fluorescein-guided resection under the YELLOW 560 nm surgical microscope filter in malignant brain tumor surgery–a feasibility study. Acta Neurochir (Wien) 155:693–699. https://doi.org/10.1007/s00701-013-1643-y
101. Schipmann S, Schwake M, Suero Molina E, Stummer W (2019) Markers for identifying and targeting glioblastoma cells during surgery. Acta Neurochir (Wien)
102. Schober O, Creutzig H, Meyer GJ, Becker H, Schwarzrock R, Dietz H, Hundeshagen H (1985a) 11C-methionine PET, IMP-SPECT, CT and MRI in brain tumors. Rofo 143:133–136. https://doi.org/10.1055/s-2008-1052777
103. Schober O, Meyer GJ, Stolke D, Hundeshagen H (1985b) Brain tumor imaging using C-11-labeled L-methionine and D-methionine. J Nucl Med 26:98–99
104. Schulz C, Waldeck S, Mauer UM (2012) Intraoperative image guidance in neurosurgery: development, current indications, and future trends. Radiol Res Pract 2012:197364. https://doi.org/10.1155/2012/197364

105. Schwake M et al (2014) Kinetics of porphyrin fluorescence accumulation in pediatric brain tumor cells incubated in 5-aminolevulinic acid. Acta Neurochir (Wien) 156:1077–1084. https://doi.org/10.1007/s00701-014-2096-7
106. Senft C, Bink A, Franz K, Vatter H, Gasser T, Seifert V (2011) Intraoperative MRI guidance and extent of resection in glioma surgery: a randomised, controlled trial. Lancet Oncol 12:997–1003. https://doi.org/10.1016/s1470-2045(11)70196-6
107. Senft C, Franz K, Ulrich CT, Bink A, Szelenyi A, Gasser T, Seifert V (2010) Low field intraoperative MRI-guided surgery of gliomas: a single center experience. Clin Neurol Neurosurg 112:237–243. https://doi.org/10.1016/j.clineuro.2009.12.003
108. Senft C, Seifert V, Hermann E, Franz K, Gasser T (2008) Usefulness of intraoperative ultra low-field magnetic resonance imaging in glioma surgery. Neurosurgery 63:257–266; discussion 266–257. https://doi.org/10.1227/01.neu.0000313624.77452.3c
109. Shinoda J, Yano H, Yoshimura S, Okumura A, Kaku Y, Iwama T, Sakai N (2003) Fluorescence-guided resection of glioblastoma multiforme by using high-dose fluorescein sodium. Technical note. J Neurosurg 99:597–603. https://doi.org/10.3171/jns.2003.99.3.0597
110. Smith JS et al (2008) Role of extent of resection in the long-term outcome of low-grade hemispheric gliomas. J Clin Oncol 26:1338–1345. https://doi.org/10.1200/JCO.2007.13.9337
111. Stummer W (2015) Poor man's fluorescence? Acta Neurochir (Wien) 157:1379–1381. https://doi.org/10.1007/s00701-015-2471-z
112. Stummer W, Gotz C, Hassan A, Heimann A, Kempski O (1993) Kinetics of Photofrin II in perifocal brain edema. Neurosurgery 33:1075–1081; discussion 1081–1072
113. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ (2000) Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. J Neurosurg 93:1003–1013. https://doi.org/10.3171/jns.2000.93.6.1003
114. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ, Group AL-GS (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. https://doi.org/10.1016/s1470-2045(06)70665-9
115. Stummer W et al (2008) Extent of resection and survival in glioblastoma multiforme: identification of and adjustment for bias. Neurosurgery 62:564–576; discussion 564–576. https://doi.org/10.1227/01.neu.0000317304.31579.17
116. Stummer W et al (2014a) Predicting the "usefulness" of 5-ALA-derived tumor fluorescence for fluorescence-guided resections in pediatric brain tumors: a European survey. Acta Neurochir (Wien) 156:2315–2324. https://doi.org/10.1007/s00701-014-2234-2
117. Stummer W, Stepp H, Moller G, Ehrhardt A, Leonhard M, Reulen HJ (1998a) Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue. Acta Neurochir (Wien) 140:995–1000
118. Stummer W, Stepp H, Wiestler OD, Pichlmeier U (2017) Randomized, prospective double-blinded study comparing 3 different doses of 5-aminolevulinic acid for fluorescence-guided resections of malignant gliomas. Neurosurgery 81:230–239. https://doi.org/10.1093/neuros/nyx074
119. Stummer W et al (1998b) Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. Neurosurgery 42:518–525; discussion 525–516
120. Stummer W, Suero Molina E (2017) Fluorescence imaging/agents in tumor resection. Neurosurg Clin N Am 28:569–583. https://doi.org/10.1016/j.nec.2017.05.009
121. Stummer W et al (2014b) 5-Aminolevulinic acid-derived tumor fluorescence: the diagnostic accuracy of visible fluorescence qualities as corroborated by spectrometry and histology and postoperative imaging. Neurosurgery 74:310–319; discussion 319–320. https://doi.org/10.1227/neu.0000000000000267

122. Suchorska B et al (2016) Complete resection of contrast-enhancing tumor volume is associated with improved survival in recurrent glioblastoma-results from the DIRECTOR trial. Neuro Oncol 18:549–556. https://doi.org/10.1093/neuonc/nov326
123. Suero Molina E, Wolfer J, Ewelt C, Ehrhardt A, Brokinkel B, Stummer W (2018) Dual-labeling with 5-aminolevulinic acid and fluorescein for fluorescence-guided resection of high-grade gliomas: technical note. J Neurosurg 128:399–405. https://doi.org/10.3171/2016.11.jns161072
124. Sutherland GR, Kaibara T, Louw D, Hoult DI, Tomanek B, Saunders J (1999) A mobile high-field magnetic resonance system for neurosurgery. J Neurosurg 91:804–813. https://doi.org/10.3171/jns.1999.91.5.0804
125. Swanson KI, Clark PA, Zhang RR, Kandela IK, Farhoud M, Weichert JP, Kuo JS (2015) Fluorescent cancer-selective alkylphosphocholine analogs for intraoperative glioma detection Neurosurgery 76:115–123; discussion 123–114. https://doi.org/10.1227/neu.0000000000000622
126. Taal W et al (2008) Incidence of early pseudo-progression in a cohort of malignant glioma patients treated with chemoirradiation with temozolomide. Cancer 113:405–410. https://doi.org/10.1002/cncr.23562
127. Takeda J et al. (2017) 5-ALA fluorescence-guided endoscopic surgery for mixed germ cell tumors. J Neurooncol 134:119–124. https://doi.org/10.1007/s11060-017-2494-9
128. Teixidor P et al (2016) Safety and efficacy of 5-aminolevulinic acid for high grade glioma in usual clinical practice: a prospective cohort study. PLoS One 11:e0149244. https://doi.org/10.1371/journal.pone.0149244
129. Tew JM (1999) M. Gazi Yasargil: neurosurgery's man of the century. Neurosurgery 45:1010–1014
130. Uematsu Y, Owai Y, Okita R, Tanaka Y, Itakura T (2007) The usefulness and problem of intraoperative rapid diagnosis in surgical neuropathology. Brain Tumor Pathol 24:47–52. https://doi.org/10.1007/s10014-007-0219-z
131. Utsuki S, Miyoshi N, Oka H, Miyajima Y, Shimizu S, Suzuki S, Fujii K (2007a) Fluorescence-guided resection of metastatic brain tumors using a 5-aminolevulinic acid-induced protoporphyrin IX: pathological study. Brain Tumor Pathol 24:53–55. https://doi.org/10.1007/s10014-007-0223-3
132. Utsuki S, Oka H, Kijima C, Miyajima Y, Hagiwara H, Fujii K (2011) Utility of intraoperative fluorescent diagnosis of residual hemangioblastoma using 5-aminolevulinic acid. Neurol India 59:612–615. https://doi.org/10.4103/0028-3886.84349
133. Utsuki S et al (2007b) Histological examination of false positive tissue resection using 5-aminolevulinic acid-induced fluorescence guidance. Neurol Med Chir (Tokyo) 47:210–213; discussion 213–214
134. Valdes PA et al (2014) 5-Aminolevulinic acid-induced protoporphyrin IX fluorescence in meningioma: qualitative and quantitative measurements in vivo. Neurosurgery 10(Suppl 1):74–82; discussion 82–73. https://doi.org/10.1227/neu.0000000000000117
135. Valdes PA, Jacobs V, Harris BT, Wilson BC, Leblond F, Paulsen KD, Roberts DW (2015) Quantitative fluorescence using 5-aminolevulinic acid-induced protoporphyrin IX biomarker as a surgical adjunct in low-grade glioma surgery. J Neurosurg 123:771–780. https://doi.org/10.3171/2014.12.jns14391
136. Valdes PA et al (2011) Quantitative fluorescence in intracranial tumor: implications for ALA-induced PpIX as an intraoperative biomarker. J Neurosurg 115:11–17. https://doi.org/10.3171/2011.2.JNS101451
137. Valdes PA, Roberts DW, Lu FK, Golby A (2016) Optical technologies for intraoperative neurosurgical guidance. Neurosurg Focus 40:E8. https://doi.org/10.3171/2015.12.FOCUS15550
138. Villanueva-Meyer JE, Mabray MC, Cha S (2017) Current clinical brain tumor imaging. Neurosurgery 81:397–415. https://doi.org/10.1093/neuros/nyx103

139. Weichert JP et al (2014) Alkylphosphocholine analogs for broad-spectrum cancer imaging and therapy. Sci Transl Med 6:240ra275. https://doi.org/10.1126/scitranslmed.3007646
140. Widhalm G et al (2013) 5-Aminolevulinic acid induced fluorescence is a powerful intraoperative marker for precise histopathological grading of gliomas with non-significant contrast-enhancement. PLoS One 8:e76988. https://doi.org/10.1371/journal.pone.0076988
141. Wilbers E, Hargus G, Wolfer J, Stummer W (2014) Usefulness of 5-ALA (Gliolan(R))-derived PPX fluorescence for demonstrating the extent of infiltration in atypical meningiomas. Acta Neurochir (Wien) 156:1853–1854. https://doi.org/10.1007/s00701-014-2148-z
142. Yamamoto T et al (2015) Photodynamic diagnosis using 5-aminolevulinic acid in 41 biopsies for primary central nervous system lymphoma. Photochem Photobiol 91:1452–1457. https://doi.org/10.1111/php.12510
143. Yun J, Iwamoto FM, Sonabend AM (2016) Primary central nervous system lymphoma: a critical review of the role of surgery for resection. Arch Cancer Res 4. https://doi.org/10.21767/2254-6081.100071
144. Zhang RR, Swanson KI, Hall LT, Weichert JP, Kuo JS (2016) Diapeutic cancer-targeting alkylphosphocholine analogs may advance management of brain malignancies. CNS Oncol 5:223–231. https://doi.org/10.2217/cns-2016-0017