Optical technologies for intraoperative neurosurgical guidance

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Biomedical optics is a broadly interdisciplinary field at the interface of optical engineering, biophysics, computer science, medicine, biology, and chemistry, helping us understand light–tissue interactions to create applications with diagnostic and therapeutic value in medicine. Implementation of biomedical optics tools and principles has had a notable scientific and clinical resurgence in recent years in the neurosurgical community. This is in great part due to work in fluorescence-guided surgery of brain tumors leading to reports of significant improvement in maximizing the rates of gross-total resection. Multiple additional optical technologies have been implemented clinically, including diffuse reflectance spectroscopy and imaging, optical coherence tomography, Raman spectroscopy and imaging, and advanced quantitative methods, including quantitative fluorescence and lifetime imaging. Here we present a clinically relevant and technologically informed overview and discussion of some of the major clinical implementations of optical technologies as intraoperative guidance tools in neurosurgery.

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KEY WORDS fluorescence-guided surgery; fluorescence lifetime; Raman spectroscopy and imaging; diffuse reflectance spectroscopy and imaging; laser speckle contrast imaging; optical coherence tomography; biomedical optics; image-guided surgery

BIO MEDICAL optics is a broadly interdisciplinary field at the interface between optical engineering, biophysics, computer science, medicine, biology, and chemistry, helping us understand light–tissue interactions to create applications with diagnostic and therapeutic value in medicine. Implementation of optical tools and principles in the neurosurgical operating theater dates back to the early part of the 20th century, when Moore used fluorescein sodium as a fluorescent biomarker for intraoperative identification of gliomas.⁹² Applications of biomedical optics have had a notable scientific and clinical resurgence in recent years in the neurosurgical community. The most well-known example is the use of fluorescence imaging for surgical guidance in brain tumor resection, culminating in a Phase III clinical trial by Stummer and colleagues using 5-aminolevulinic acid–induced protoporphyrin IX (ALA-PpIX) fluorescence for glioblastoma multiforme (GBM) resection. This major study subsequently prompted a collection of work worldwide utilizing this technology clinically to help improve the extent of resection.

In addition to this initial implementation of fluorescence technology and biomarker use in major trials to date, multiple additional optical tools or technologies are beginning to enter the clinical arena, including diffuse reflectance spectroscopy and imaging, optical coherence tomography (OCT), Raman spectroscopy, and quantitative methods, including quantitative fluorescence, lifetime imaging, and beyond (Tables 1 and 2). Here we present a clinically relevant and technologically informed overview of some of the major clinical implementations of optical technologies as intraoperative guidance tools in neurosurgery.

Fluorescence

Wide-Field Imaging

Exogenous Fluorescent Markers: ALA-PpIX, Fluorescein, and Beyond

Fluorescence-guided surgery dates back to 1948 when Moore et al.⁹² used fluorescein sodium in 46 patients with

ABBREVIATIONS ALA-PpIX = 5-aminolevulinic acid–induced protoporphyrin IX; BBB = blood-brain barrier; CARS = coherent anti-Stokes Raman scattering; CBF = cerebral blood flow; CCD = charge-coupled device; GBM = glioblastoma multiforme; GTR = gross-total resection; ICG = indocyanine green; LSCI = laser speckle contrast imaging; NIR = near-infrared; OCT = optical coherence tomography; SRS = stimulated Raman scattering; λ_{em} = emission wavelength; λ_{ex} = excitation wavelength.

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TABLE 1. Optical technologies in neurosurgery

Technology & Contrast		Mechanism	Contrast Agent	Tools	Biomolecules
Fluorescence					
Steady state	Tissue is interrogated with light at a λ_{exc} leading to absorption with subsequent emission at a longer λ_{em} based on the intrinsic excitation-emission properties of a fluorescent molecule	Fluorophore, endo or exo, e.g., PpIX, fluorescein, NAD(P)H	Spect, micro, WF	Endo, exo	
Time resolved	Tissue is interrogated with light at a λ_{exc} leading to absorption with subsequent emission at a longer λ_{em} based on the intrinsic excitation-emission properties of a fluorescent molecule, with measurement of the average time electrons spend in an excited state prior to fluorescence emission	Fluorophore, endo or exo, e.g., PpIX, fluorescein, NAD(P)H	Spect, micro	Endo	
Reflectance					
Reflectance	Tissue is interrogated with light at a predefined λ with reflected light at corresponding λ , undergoing differential levels of absorption and scattering based on tissue physiology to create maps such as blood volume, blood oxygenation, tissue attenuation, and tissue scattering	Endo chromophores, e.g., oxyhemoglobin, deoxy-hemoglobin, scattering	Spect, micro, WF	Endo	
Raman					
Classical	Tissue is interrogated with light where a small amount of this incident light at a specific λ is scattered at a different λ as a function of the vibrational energies of molecular bonds in tissues	Molecular bonds, e.g., DNA, lipids, proteins	Spect, micro	Endo	
CARS					
SRS					
SERS					

Endo = endogenous; exo = exogenous; micro = microscopy; SERS = surface-enhanced Raman scattering; spect = spectroscopy; WF = wide-field imaging; λ = wavelength; λ_{em} = emission wavelength; λ_{exc} = excitation wavelength.

brain tumors as a means for tumor tissue visualization. In 1982 Murray reported the use of fluorescein sodium as a surgical adjunct in 23 patients to distinguish between neoplastic versus necrotic tissue.⁹⁵ Murray concluded that his results were in agreement with the work by Moore et al., and that use of fluorescein might prove beneficial in surgical guidance for tumor resection. It was not until the end of the 20th century, when Walter Stummer presented a preliminary, groundbreaking study using ALA-PpIX fluorescence in patients with GBM, that fluorescence re-emerged as a tentatively powerful intraoperative surgical adjunct in neurosurgery. Stummer was familiar with the work in the field of photodynamic therapy, where exogenous administration of ALA was noted to preferentially accumulate PpIX in malignant tissues.^{48,134} Stummer and colleagues performed an initial preliminary study involving 9 patients with gliomas. The authors reported on the utility of ALA-PpIX fluorescence for the detection and removal of residual tumor tissue in 7 of 9 patients, noting no residual in situ fluorescence, no postoperative MRI contrast enhancement, and 85% sensitivity, 100% specificity, and 90% accuracy.¹³⁴

Briefly, patients are administered an oral dose of ALA

2–3 hours prior to the induction of anesthesia, leading to overproduction and accumulation of PpIX in tumor tissue, specifically in high-grade gliomas. ALA is a precursor in the heme biosynthetic pathway, and its exogenous administration bypasses a rate-limiting step (i.e., synthesis of ALA by ALA synthase) and leads to the overproduction of PpIX.²⁰ Violet light (excitation wavelength [λ_{exc}] = 405 nm) illuminates tissue to excite PpIX at its main excitation absorption peak, and PpIX emits a red-pink fluorescence (emission wavelength [λ_{em}] = 620–710 nm).¹⁵¹ Multiple mechanisms and actuators have been posited as contributors to the selective accumulation of PpIX in tumors, including enzymatic modifications (e.g., ferrochelatase, porphobilinogen deaminase), transporter regulation, varying cellular states like hypoxia and acidic environments, increased cellular growth, glucose levels, metabolic and structural changes.^{6,20,39,50,94,101,103,138,151,153,159,160}

Subsequently, Stummer et al. performed a larger study involving 52 patients with high-grade gliomas,¹³¹ which garnered clinical excitement by demonstrating 63% gross-total resection (GTR) on postoperative contrast-enhanced imaging¹³¹ (Fig. 1). The authors then carried out a landmark Phase III randomized control trial involving 270 patients

TABLE 2. Optical instrumentation in neurosurgery

Instrumentation	FOV	Resolution	Intraop Implementation	Technology
Spect	~mm	~mm to sub-mm	Contact handheld probes	Fluor intensity, fluor lifetime, diffuse reflectance, Raman
Micro	~ μm	~ μm	Contact handheld probes, noncontact imaging systems	Fluor intensity, fluor lifetime, OCT, Raman
WF	~cm	~mm	Microscopes, noncontact imaging systems	Fluor, diffuse reflectance, LSCI, OCT

Fluor = fluorescence; FOV = field of view; intraop = intraoperative.

FIG. 1. Intraoperative paired white light and fluorescence mode images during multiple stages of PpIX fluorescence-guided resection of a GBM, showing strong visible PpIX fluorescence at the beginning (A and B), middle (C and D), and near end (E and F) of surgery, and no visible fluorescence at the end (G and H) (heads up display contour lines in green). Heads up display corresponding to segmented contrast-enhancing tumor demonstrates strong PpIX fluorescent tissue outside (F) the segmented region near the end of the resection and no visible PpIX fluorescent tissue at the end (H). The patient underwent fluorescence-guided resection under a protocol approved by the institutional review board at Dartmouth-Hitchcock Medical Center.

in 17 centers, who were randomized to a group undergoing ALA-PpIX fluorescence-guided surgery and a group undergoing conventional white light-guided surgery. The trial results were notable for GTR of contrast-enhancing tumor on postoperative imaging in 65% of patients undergoing ALA-PpIX fluorescence guidance compared with 36% of patients in the white light group ($p < 0.001$), and progression-free survival of 41% versus 21.1% ($p < 0.003$) for fluorescence versus white light groups, respectively.¹³² In a subsequent reanalysis of the data to look at morbidity, no statistically significant difference in long-term morbidity was found between the fluorescence and white light groups.^{102,133,136,138}

ALA-PpIX use for GBM has become widespread in locations where it has met clinical approval, such as in countries in the European Union. Numerous studies have investigated the relationship of ALA-PpIX fluorescence and tumor location, histopathological correlates, and relationship with other intraoperative aids (e.g., intraoperative MRI, brain deformation modeling, functional mapping).^{26,46,49,51,115,116,122,127,144–146,148,149,152–154,156,164} Studies have mostly noted the utility of ALA-PpIX in high-grade gliomas, specifically in GBM.^{134,150} Additional work not powered for survival analysis noted beneficial diagnostic value in other tumor subtypes including meningiomas and metastases. Recent technological advances have provided exciting preliminary evidence regarding the utility of ALA-PpIX in additional tumor histologies previously thought to not be amenable to ALA-PpIX fluorescence-guided surgery, i.e., low-grade gliomas. The clinical value of ALA-PpIX fluorescence imaging with state of the art tools rests on the premise that it provides the surgeon with a tool for 1) immediate intraoperative feedback regarding the presence or absence of tumor in real-time mode; 2) reliable correlation between imaging features (i.e., positive fluorescence with high positive predictive value for

tumor) and histopathology; and 3) ease of integration into the neurosurgical workflow.

Numerous exogenous agents have been used clinically in neurosurgery, including fluorescein sodium, indocyanine green (ICG), mTHPC, Photofrin, and PpIX.^{22–25,48,58,68,93,111,112,132,169,174} Recent work notes renewed interest in fluorescein sodium for surgical guidance in resection of brain tumors,^{23,75,79,112,124} including gliomas,^{8,70} metastasis,⁹⁸ and meningiomas,²⁷ with reports noting varying specificities and sensitivities. Fluorescein sodium is administered intravenously, and accumulation follows the principles of the enhanced retention and permeability effect and is dependent on a compromised blood-brain barrier (BBB),⁷⁹ such that wherever there is blood or a disrupted barrier, fluorescein will accumulate in tissue, making it a potentially highly nonspecific biomarker. Once it accumulates, fluorescein sodium displays a green fluorescence that can be visualized either by the naked eye or via multiple different technological adaptations to current systems (Fig. 2). In a recent commentary, Stummer¹²⁹ discusses the limitations and care required in interpreting results using fluorescein sodium as a surgical adjunct in neurosurgery. Fluorescein has the potential to be a highly nonspecific biomarker that will accumulate in any areas with BBB disruption or blood leakage, and as such it will accumulate irrespective of tumor cell presence, e.g., in normal neuraxis regions without a BBB, such as choroid plexus, or in abnormal tissues such as edema, necrosis, leaking vessels, and bleeding vessels without hemostasis intraoperatively. A main concern in using this compound is that, given its limited specificity, surgeons would risk removal of viable, normal tissue and, given sensitivity concerns, would risk leaving behind tumor tissue.

A wealth of information is accumulating in the literature on the diagnostic capabilities of the 2 most commonly used intraoperative fluorescence imaging agents, ALA-

FIG. 2. A and B: Intraoperative images of fluorescein sodium in human gliomas showing intraoperative green fluorescence in the tumor bed with their corresponding T1-weighted contrast-enhanced MR images (C and D). Green fluorescence in the tumor bed (arrows) corresponds to tumor tissue but nonspecific diffuse fluorescence is also visible at the dural flap. Reproduced with permission from Acerbi et al: *Neurosurg Focus* 36(2):E5, 2014.

PpIX and fluorescein. Currently, the most numerous and thorough studies have been on the diagnostic capabilities of ALA-PpIX (e.g., accuracy, sensitivity, specificity, negative predictive value, and positive predictive value) using both visible and quantitative tools (see *Quantitative Versus Qualitative Fluorescence*); meanwhile, in comparison, the data on fluorescein are more limited and the results and conclusion pertaining to this imaging agent should be interpreted as such.^{2,22,42,92,95,99,112,116,131,135,148,150,156,167} Tables 3 and 4 provide a general overview of key studies using PpIX and fluorescein, respectively, and the associated derived diagnostic capabilities of these agents.

ICG has seen limited use in the resection of tumors, enabling evaluation of tumoral and peritumoral blood flow and vasculature, yet detailed diagnostic studies have not accrued in similar volume to that of ALA-PpIX and fluorescein.^{29,61,63,78,107–109,165,166} Unlike PpIX and fluorescein, which both emit fluorescence in the visible spectrum (i.e., visible spectrum 400–720 nm), ICG has the advantage of excitation and emission in the near-infrared (NIR) region of the spectrum. ICG has a major excitation peak at approximately $\lambda_{ex} = 800$ nm and a main emission peak at approximately $\lambda_{em} = 830$ –840 nm.^{114,151,171} The NIR properties of ICG enables visualization of fluorophore situated deeper in the tissue given that longer wavelength excitation and emission light undergoes less absorption than shorter wavelength, visible light, but requires special cameras and instrumentation to visualize the emitted fluorescence (i.e., cannot be visualized with the naked eye).

These initial studies as well as the majority of subsequent clinical work using fluorescence guidance have employed wide-field imaging approaches (i.e., imaging via a surgical microscope of macroscopic structures and

field of views in the order of centimeters) (Tables 1 and 2). Wide-field fluorescence imaging has the advantage of improved field of view with immediate visual feedback to the surgeon, which has exploited such fluorophores as PpIX,^{32,33,96,102,116,127,130–133,136,148,153–156} ICG,^{29,44,63,85} and fluorescein.^{1,22,23,65,71,74–76,92,98,111,112,124,125,128} For example, ALA-PpIX fluorescence provides the surgeon with immediate visualization of a tumor, noting areas with significant PpIX accumulation as a red-pink fluorescence and areas without significant visible levels of fluorescence with a violet-blue background from the excitation light. Current PpIX microscope-based work in mostly high-grade gliomas report sensitivities as low as 20%–30%, specificities > 90%, and overall accuracies 70%–90%.^{116,151,156} A key limitation of current clinical microscope-based visualization tools in fluorescence image-guided surgery is both the limited accuracies and qualitative nature of such fluorescence assessments (see *Quantitative Versus Qualitative Fluorescence*). Sensitivity limitations of microscope-based tools, in part, reside in the geometric constraints of light collection.¹⁵¹ The intensity of the collected fluorescence decreases as the distance from the light source and detector increases from the interrogated area. As such, microscope-based tools are intrinsically less sensitive than contact probe technologies by reason of suboptimal light excitation and collection geometries. Clinical fluorescence microscope-based measurements of the qualitative and subjective visible fluorescence are reliant on current technologies that collect fluorescence on a color charge-coupled device (CCD) camera without any subsequent correction for tissue optical properties. As such, current assessments are subjective and unable to distinguish between objectively high or low levels of fluorescence.¹⁵⁵ Recent developments in advanced microscope-based tools seek to address these limitations, (e.g., use of stronger light excitation, which is limited by how much light tissue can be exposed to before damage occurs; use of more sensitive detectors¹⁵¹).

New Technologies for Fluorescence-Guided Surgery

Additional developments in microscope-based tools that seek to address current limitations with full surgical field of view imaging technologies have been undertaken but are limited. Recent work by Valdés et al. has translated the concepts of quantitative single-point probe fluorescence spectroscopy to full surgical field of view, spectrally-resolved quantitative fluorescence imaging.^{152,155,157} They developed a proof-of-principle system that adapts to current neurosurgical microscopes and collects spectrally resolved data through the native microscope optics unto a standard CCD¹⁵⁵ or CMOS (complementary metal-oxide semiconductor)¹⁵² detector for higher sensitivity. This system used a light correction ratiometric algorithm to derive corrected fluorescence estimates of the tissue PpIX concentrations across the full surgical field of view, noting improved in vivo visualization of human glioma tissue not visualized with standard visible fluorescence clinical systems.^{152,155,157} In the context of the clinical excitement over PpIX and its value as a practical surgical tool, a technological boom has accompanied the field of fluorescence image guidance in neurosurgery, both on

TABLE 3. Diagnostic performance of ALA-PpIX fluorescence*

Authors & Year	Accuracy	Specificity	Sensitivity	NPV	PPV	No. of Pts
Stummer et al., 2000	90	96	89	50	100	52
Roberts et al., 2011	74	71	75	26	95	11
Panciani et al., 2012	90	89	91	91	89	23
Yamada et al., 2015	89	53	95	69	92	99
Stummer et al., 2014†	—	—	—	40	96	33
Stummer et al., 2014‡	—	95 vs 41	72 vs 94	—	—	13
Valdés et al., 2011§	87	92	84	77	95	14
Valdés et al., 2015¶	67	83	58	50	87	6
	38	81	21	30	73	12
Valdés et al., 2014**	84 vs 90	100 vs 81	76 vs 94	67 vs 87	100 vs 91	10
	71	100	61	55	100	15

NPV = negative predictive value; PPV = positive predictive value; Pts = patients.

* Values are percentages unless otherwise indicated.

† Visible fluorescence.

‡ Used a qualitative spectroscopy probe with 2 different thresholds.

§ Mixed histology including low-grade gliomas, high-grade gliomas, meningiomas, and metastases using a quantitative fluorescence probe.

¶ Used a quantitative probe (n = 6) and visible PpIX fluorescence (n = 12) for low-grade gliomas.

** Used a quantitative probe (n = 10 at 2 different thresholds) and visible PpIX fluorescence (n = 15) for meningiomas.

the instrumentation and biomarker development fronts.¹⁵¹ Numerous groups, including ours, have developed hand-held intraoperative spectroscopy tools for acquiring fluorescence and/or white light spectra, with subsequent data processing algorithms, as a means to more accurately detect tumors.^{3,16,17,26,31,37,38,46,56,57,80–84,114,115,145,147,148,154,156,157} Other groups have developed handheld confocal probes to acquire microscopic images of the tumor microstructure, visualizing microscopic features previously not detected intraoperatively.^{119,121,122} Additional developments innovating neurosurgical microscopes aim to create technologies to improve accuracy by means of detecting additional optical features in tissue¹⁵⁵ (Tables 1 and 2).

Spectroscopy

Fluorescence Intensity

Spectroscopy probes have traditionally measured the fluorescence intensity, i.e., the number of emitted photons (y axis) at a specific wavelength (x axis). The fluorescence intensity of a molecule is a function of the intrinsic fluorophore properties, such as fluorophore concentration, absorbing power, and quantum yield over a range of wavelengths at a specified excitation wavelength.¹¹⁰ These

systems usually consist of small, fiberoptic-based devices connected to a spectrometer for spectrally resolved data (e.g., fluorescence, reflectance, Raman) collection and light source(s) for illumination.^{62,110,114,154,156} Collected spectral data can then be used in spectral deconvolution and light transport processing algorithms¹¹⁴ (Figs. 3 and 4).

Other groups have used a spectral processing technique using ratios of 2 fluorescence peaks of PpIX resulting from 2 proposed PpIX states to derive a means for improved detection of low-grade gliomas and infiltrating glioma regions.⁹⁰ In addition, several spectroscopy studies have used the raw fluorescence intensities to demonstrate improved sensitivity for detection of tumor, exploiting tissue autofluorescence or the main PpIX emission peaks.^{80–84,140} Stummer et al.^{129,135} recently used a contact probe spectroscopy system in ALA-PpIX resection, in which they noted a correspondence between the qualitative visible assessments of “strong” and “weak” fluorescence with greater and lower spectroscopic fluorescence intensity signal, respectively, with improved diagnostic capabilities compared with visible fluorescence (Table 3). As expected (i.e., given the improved light delivery–collection geometry), they reported improved detection of

TABLE 4. Diagnostic performance of fluorescein fluorescence*

Authors & Year	Accuracy	Specificity	Sensitivity	NPV	PPV	No. of Pts
Murray, 1982	89	81	96	95	85	23
Acerbi et al., 2014	92	89	94	94	89	9
Moore et al., 1948†	95	92	97	92	97	46
Rey-Dios et al., 2014‡	85	100	79	64	100	6
Díaz et al., 2015	85	91	82	71	95	12

* Values are percentages unless otherwise indicated.

† Limited to nonequivocal samples and those with clear notation of their fluorescence characteristics.

‡ Study involved patients undergoing stereotactic needle biopsy.

FIG. 3. In vivo quantitative fluorescence imaging in gliomas showing ALA-PpIX fluorescence-guided surgery at the beginning (A–C), middle (E–G), and end of surgery (I–K), with visible fluorescence images (B, F, and J) and corresponding quantitative fluorescence images following correction for tissue optical properties noting improved tumor tissue and fluorophore detection (C, G, and K). **D and H:** Spectra showing presence (D) and absence (H) of PpIX signal during and after surgery. **L:** Histologically confirmed tumor tissue corresponding to location in panel G with white arrow. Reproduced with permission from Valdés et al: *Sci Rep* 2:798, 2012.

fluorescence signal that would otherwise have gone undetected with current fluorescence enabled surgical microscopes. Other examples of spectroscopy systems in neurosurgery have been provided by Lin et al. and Toms et al. who used in vivo autofluorescence of tissue coupled with diffuse reflectance measurements in multivariate algorithms to discriminate tumor margins with sensitivities and specificities from 94% to 100% and 76% to 93%, respectively.^{80–84,140} These later approaches use the raw spectral data coupled to machine learning techniques to derive methods for improved tumor tissue classification.

Quantitative Versus Qualitative Fluorescence. Tissue fluorescence is a function of both the absorption and scattering of light that occurs at both the excitation and emission wavelengths, and which itself varies at each spatial location.^{14,114} As such, every assessment of the raw (i.e., “visible”) emitted fluorescence is itself subjective, given the attenuating effects of tissue optical properties at each

spatial location. Such assessments can lead to inaccurate estimates of accumulated tissue fluorophores given the nonlinear relationships between tissue fluorescence signal and accumulated fluorophores. To make truly quantitative and absolute estimates of the levels of fluorescent biomarker, these effects need to be corrected for by using appropriate light-modeling algorithms.¹⁴ A recent study used a light transport modeling algorithm to correct for the distorting effects of tissue optical properties on the “raw” emitted fluorescence to derive quantitative values of the absolute levels of PpIX in tissues.¹⁵⁶ Quantitative estimates have been used to demonstrate a significant improvement in diagnostic accuracy using PpIX as a tumor biomarker across multiple different tumor types, including gliomas, meningiomas, and metastases.^{153,154,156–158} A recent proof-of-principle study demonstrated that quantitative assessments of PpIX significantly improved detection of low-grade gliomas, such that use of quantitative assessments of PpIX for guidance approximated the diagnostic

FIG. 4. Diagram of the intraoperative spectroscopy dual fluorescence system and reflectance probe showing the handheld contact probe, collection of dual fluorescence and reflectance signals, advanced light transport processing algorithms, and machine learning technique for tissue classification. Reproduced with permission from Valdés et al: *J Biomed Opt* 16:116007, 2011.

accuracy of PpIX for low-grade gliomas to that of visible fluorescence for high-grade gliomas.¹⁵⁰ In addition to the application of model-based algorithms to correct for variations in tissue optical properties, correction techniques using ratiometric approaches have also provided a means for estimating the absolute levels of biomarkers in tissues.¹⁵⁷ These techniques generally use a ratio of the fluorescence intensity (e.g., at the main emission peak) over the diffuse reflectance intensity (e.g., at the main excitation wavelength) to provide a corrected fluorescence.¹⁴ An advantage of ratiometric techniques is their ease of implementation, limited assumptions, and low computational power requirements. Yet, a limitation is their need for empirical verification and lack of mathematical and conceptual rigor associated with model-based approaches.

Most of the work to date in fluorescence guidance in neurosurgery uses an approach in which the surgeon visualizes the surgical field and makes assessments on the visibility (or lack thereof) of fluorescence (e.g., pink-red in PpIX or green-yellow with fluorescein sodium). In spectroscopy-based studies, most work to date has also used a qualitative approach, e.g., as in studies by Stummer or Lin, mentioned previously. It is important to compare and contrast between these qualitative approaches and the quantitative spectroscopy studies. Both use spectroscopic, contact probe technologies, which means that they optimize the light delivery and collection efficiency. Spec-

troscopy will demonstrate greater sensitivity for detecting lower levels of fluorescence that could not be detected by noncontact microscope-based techniques. One key distinction that the literature does not clarify pertains to the biophotonic nature of the measurements. All spectroscopy techniques 1) collect data in a spectrally resolved manner and 2) provide a “number” of the arbitrary units of light intensity at each wavelength. The collected light is dependent on numerous factors, including 1) the amount of excitation light, 2) levels of tissue fluorescent marker, 3) tissue optical properties, and 4) system specifics. Most spectroscopy studies, despite providing a “number,” can be divided into truly quantitative (i.e., those that provide correction for tissue optical properties) or qualitative (i.e., those that only collect spectrally resolved data with arbitrary units of intensity without a means of correcting for tissue optical properties). Thus, qualitative probes provide measurements (e.g., derived diagnostic thresholds) that are dependent on variations in tissue optical properties and are relative to system-specific variations, whereas quantitative tools provide measurements that could be applied independent of system specifics, given that they derive measurements of absolute biomarker levels (Fig. 4). These facts highlight the reality that spectroscopy technologies are undergoing exciting developments, and their implementation has yet to be fully exploited in neurosurgery.

Fluorescence Lifetime. In addition to fluorescence intensity measurements in spectroscopy, fluorescence lifetime tools have been developed for neurosurgical guidance.^{15,16,87,170} Lifetime is a property of fluorescent molecules that describes the average time electrons spend in an excited state prior to fluorescence emissions and can range in the order of a few nanoseconds to a fraction of a nanosecond.^{73,110} Fluorescence lifetime measurements are denoted as the fluorescence intensity as a function of time at a specified excitation-emission wavelength pair.¹¹⁰ Fluorescence lifetime measurements provide an alternative means of evaluating the tissue environment and photoactivity independent of the fluorophore concentrations,¹¹⁴ with examples of interrogated endogenous markers including NAD(P)H and flavins. Yong et al.¹⁷⁰ reported a time-resolved fluorescence spectroscopy approach in a series of low- and high-grade gliomas, with detection of the intensity decay profiles in the 370- to 500-nm spectral range. They used the collected data coupled to a linear discriminant analysis (i.e., machine learning) algorithm for diagnostic evaluation, reporting diagnostic accuracies for both low- and high-grade gliomas >90%. Butte et al. noted a similar study using fluorescence lifetime spectroscopy in low-grade gliomas (100% sensitivity and 98% specificity) and high-grade gliomas (47% sensitivity and 94% specificity) using a machine learning algorithm applied on fluorescence lifetime-derived variables, e.g., average lifetime of fluorescence emissions, Laguerre coefficients, and emission intensities at optimally determined excitation-emission wavelength pairs. The authors attributed this classification discrepancy to the higher variability in the high-grade glioma data compared with those of the low-grade gliomas. This work demonstrated *in vivo* implementation of fluorescence lifetime spectroscopy for tumor identification.^{15,16} Fluorescence lifetime techniques provide an additional optical contrast mechanism that takes advantage of differences in the tumor tissue microenvironment and their effect on relevant endogenous or exogenous biomolecules distinct from traditional fluorescence intensity techniques, thus providing the neurosurgeon an additional contrast mechanism and associated tools to implement in the operative theater.

Microscopy

Intraoperative microscopy (i.e., microscopy imaging with cellular and subcellular spatial resolution and millimeter to micrometer field of views) tools have also been used as a means for intraoperative surgical guidance in the resection of brain tumors (Tables 1 and 2). Unlike spectroscopy technologies, which provide a net averaged measurement at 1 location of the fluorescence intensity (e.g., arbitrary units of fluorescence emissions), a derived biomarker concentration, or lifetime parameters, microscopy techniques provide images of the cellular and subcellular architecture based on various techniques, e.g., fluorescence intensity, lifetime, OCT.

Confocal microscopy or confocal laser endomicroscopy handheld probes provide microstructural, *in vivo* images with optical sectioning that enables intraoperative visualization of structures at depth, in 3 dimensions, with histological detail, and without the need for exten-

sive traditional tissue postprocessing.^{27,34,88,91,120,122,123,126,172} Schlosser et al. provided the first report on confocal microscopy in neurosurgery. They examined intraoperatively *ex vivo* samples from 9 patients with GBM immediately following excision and reported the ability of this technology to identify features such as cell density, mitotic figures, necrosis, and microvascular proliferation. Sanai et al.¹²² used a real-time confocal microscopy fluorescence imaging handheld probe with imaging up to 1000× magnification in low-grade gliomas under ALA-PpIX conditions. They noted in their series of 10 patients with WHO Grade I and II gliomas the ability to image microscopic fluorescence with strong histopathological correlations, despite the absence of visible, macroscopic fluorescence in areas of tumor infiltration. In another study,²⁷ 50 patients with both intraaxial and extraaxial tumors were examined after administration of fluorescein sodium using confocal microscopy to identify microscopic features including vascular proliferation, pseudopalisading necrosis, perivascular pseudorosettes, tumor margins, psammoma bodies, and cellular features such as spindle-shaped cells and cellular atypia. The study concluded that confocal microscopy might provide a means for the reliable identification of tumor cells and tumor-brain interfaces. The authors compared their microscopy results with the histopathological analysis and reported diagnostic accuracies of up to 93%.

Similar to fluorescence lifetime spectroscopy, time-resolved fluorescence tools have been used as a means for tissue identification in microscopic imaging. A recent study by Sun et al.¹³⁷ used a fluorescence lifetime imaging microscopy system for intraoperative use in GBM. In this study, the authors used the intrinsic fluorescence emission at 460 nm, corresponding to NADH/NADPH as the fluorescent contrast mechanism, with the hypothesis that tumor versus normal brain would possess varying physiological changes and processes impacting the microenvironment of NADH/NADPH. The authors noted a longer lifetime and weaker fluorescence intensity in GBM versus normal cortex in 3 patients undergoing craniotomy for tumor resection. Their data suggests that GBM tissues have a higher contribution of the protein bound form of NADH/NADPH compared with normal parenchyma.

Advantages and Disadvantages: Spectroscopy, Microscopy, and Wide-Field Imaging

We have discussed the 3 major modes of optical guidance to date in the neurosurgical operating room: wide-field imaging, spectroscopy, and microscopy (Tables 1 and 2). All 3 techniques have specific advantages and disadvantages. Spectroscopy and microscopy both provide multiple advantages compared with standard microscope visualization by providing 1) improved light excitation and collection geometries, given the direct contact with tissue (or closer distance between tissue and detector) and, thus, associated improved signal-to-noise ratios; and 2) improved spatial resolution at the millimeter (e.g., spectroscopy) to cellular/subcellular micrometer level, capable of resolving cytoarchitectural features (e.g., microscopy). Another advantage of spectroscopy techniques is their ability to provide full fluorescence spectra, which enables implementation of deconvolution algorithms for the extraction

of multiple spectral features, parameters, and application of sophisticated algorithms. Nevertheless, the main disadvantages of both technologies are that they 1) interrogate a small (e.g., 1 cm or less) region of tissue; 2) cause varying levels of interruptions to the surgical workflow (i.e., the surgeon is required to pause, pick up the instrument, and interrogate tissue); and 3) provide information to the surgeon that may not be intuitive or validated in larger patient cohorts to justify clinical decision making. Wide-field imaging microscope-based tools provide several key advantages, which include 1) a view of the full surgical field, 2) immediate intraoperative real-time feedback, and 3) ease of use without interruption to the surgical workflow. The disadvantages include 1) a light excitation and collection geometry, leading to greater light loss at both the excitation and emission wavelengths compared with contact point probes; 2) macroscopic resolution usually limited to the millimeter level, unable to resolve cytoarchitectural details; 3) inability to provide spectrally resolved data by the current state-of-the-art clinical systems; and 4) limited clinical availability, implementation, and validation of technologies for fluorophore and/or chromophore quantitation.

Technological advances abound with the exciting development of new fluorescent biomarkers possessing properties such as multimodality, specified targeting capabilities, and improved depth visualization. For example, preclinical work in nanotechnology applied to neurosurgery seeks to develop novel markers for surgical guidance. Specifically, developments at the interface of nanotechnology and optics are creating multimodal nanoagents that incorporate optical contrast agents such as ICG, ALA, or quantum dots; dual fluorescence guidance plus photodynamic tumoricidal agents with ALA-loaded nanoparticles; optically active agents such as gold nanoshells, which induce tissue welding following absorption of NIR light; enhanced optical agents such as surface enhanced Raman scattering nanoparticles for tumor demarcation; nanotubes for thermoablation; and complex multimodal agents for dual fluorescence imaging and MRI visualization. Developments in the field of nanotechnology are vast and beyond the scope of this review, and the readers are referred to various articles noted here.^{30,69,89,118,142} Developments in nanotechnology, to date, are mostly in the preclinical arena, but advances seek to produce tools for clinical applications in the neurological surgery.

In summary, the use of fluorescence in neurosurgery is young, with a plethora of additional biomarkers and technological innovations under development to improve neurosurgical targeting and guidance.^{30,69,89,97,103,118,142,151,162,163} Fluorescence technologies that improve visualization, enhance speed, increase sensitivity, adapt to new technological advances and the surgical workflow, and integrate with incoming technological developments are all exciting innovations that await application in the neurosurgical operating room.

Intrinsic Optical Signals

Exploitation of intrinsic optical signals by use of reflected light provides another means of contrast to extract diag-

nostic information about tissue structure and physiology. The use of reflected light has been applied in such diverse applications as single-point optical spectroscopy probes, full field of view surgical microscopes, and a conglomerate of contact source-detector pairs used in NIR spectroscopy systems.^{114,151} These tools exploit a well-known phenomenon in biomedical optics. Briefly, when tissue is interrogated with light (e.g., white light in the range of 400–720 nm), the reflected white light can be collected either at 1 single wavelength, a group of wavelengths, or in a spectrally resolved manner. The shape (in the case of spectrally resolved acquisition) and the magnitude of the reflected light is a function of the tissue's optical properties (i.e., absorption and scattering).¹¹⁴ Tissue optical properties are themselves a function of variations in tissue microstructure (e.g., cell density, nuclear morphology, mitochondrial density), composition (e.g., necrosis, collagen), and physiology (e.g., highly vascularized with varying levels of hemoglobin and oxygen saturation). Thus, reflectance-based technologies exploit our knowledge of light–tissue interactions to measure tissue optical properties and estimate endogenous markers (e.g., tissue chromophores such as oxy- and deoxyhemoglobin), thus not requiring administration of any exogenous markers (e.g., ALA, fluorescein, ICG). The use of reflectance in neurosurgical guidance can be divided into various tools, including spectroscopy contact probes, microscopy tools (e.g., OCT), and wide-field imaging microscopes.

Wide-Field Reflectance Imaging

The development of wide-field (i.e., surgical microscope) imaging technologies exploits intrinsic signals in tissue, such as variations in oxy- and deoxyhemoglobin, and tissue scattering to provide enhanced optical imaging for neurosurgical guidance. Reflectance-based techniques are perfusion-dependent forms of brain mapping that take advantage of the relationship between neuronal activity and local hemodynamic changes, i.e., neurovascular coupling. Neuronal activity causes local hemodynamic and metabolic changes in blood volume, blood flow, glucose use, oxygen consumption, and concentrations of oxy- and deoxyhemoglobin (which serve as a surrogate markers for neuronal activity), which consequently impact light reflectance secondary to associated intrinsic changes in tissue absorption and scattering.^{104,105} This tool exploits the fact that reflected light of different wavelengths undergoes varying levels of absorption and scattering based on tissue physiology, creating maps of blood volume, blood oxygenation, and corresponding tissue physiological changes. As such, reflectance techniques yield functional contrast by measuring changes in endogenous tissue chromophores at the cortical surface.^{45,104,105} Haglund et al. in 1992 performed a landmark study using intraoperative endogenous optical signals in patients with seizure disorder who were undergoing cognitive tasks.^{45,105} Haglund et al. developed a wide-field reflectance imaging technique that was applied to eloquent cortex to detect intrinsic optical signals for tissue differentiation in epileptogenic brain. The authors noted an increase in optical changes as the magnitude of intensity and duration of stimulation-evoked epileptiform after discharges increased.^{43,45,86} Since this initial work,

intraoperative reflectance has also been used to interrogate the functional topography and plasticity of sensory and language cortices. Cannestra et al. provided an elegant report on topographical specificity by analyzing intraoperative reflectance signals of the somatosensory cortex following contralateral stimulation (e.g., face, thumb, index). The authors noted a non-overlapping cortical brain mass colocalizing with electrocortical stimulation mapping and evoked potentials.¹⁸ Optical signals in Broca's and Wernicke's areas were demonstrated by both Haglund et al.⁴⁵ and Cannestra et al.¹⁹ For example, in the latter study, patients undergoing neurosurgical resection were noted to display a topographical specificity dependent on language tasks. Additional studies in patients with tumors, arteriovenous malformations, cavernous malformations, and epileptogenic foci evaluated such aspects as Spanish and English maps, correlation between blood oxygen level-dependent and optical imaging venous maps, hand representation, and delineation of functional borders.¹⁰⁶

These tools use raw changes in optical signals (e.g., the changes in reflectance signal at the isosbestic points of oxy- and deoxyhemoglobin, i.e., 550 nm and 570 nm, respectively¹⁰⁵). As such, they are similar to nonquantitative spectroscopy studies noting relative changes in optical signal (i.e., signal intensity changes) without explicit measurement of optical markers (i.e., without subsequent data processing in light transport models to quantitate absolute chromophore biomarkers such as oxy- and deoxyhemoglobin levels). New developments such as spatial frequency domain imaging and single snapshot optical properties imaging address this limitation by utilizing patterned illumination coupled with light transport algorithms to derive the absolute tissue optical properties,^{40,41,66,161,168} but are currently not yet implemented in clinical neurosurgical interventions.

Reflectance tools rely on the biophotonics principle of differential light interaction with tissue as a function of tissue physiology and structure and, with application of modeling algorithms, can be used to extract relevant biomarkers for surgical guidance. Exploitation of intrinsic biomarkers has not gained wide application or implementation in neurosurgery as have exogenous markers in fluorescence-guided surgery (e.g., ALA-PpIX). In part, this is due to the issues of specificity, sensitivity, correlated signal-to-noise considerations, and interruptions to surgical workflow. A major limitation to implementation of reflectance imaging has been the difficulty in separating motion and vascular-related noise due to cortical movement during imaging, which can introduce reflectance changes independent of neuronal activity. Such limitations can be addressed, for example, with advanced image processing and registration techniques.¹⁰⁵ With improved light excitation and collection technologies, computational power, and algorithms (e.g., multithreaded implementation of machine learning algorithms for development of high accuracy classification or image registrations algorithms), such limitations are becoming obsolete in delaying clinical implementation of reflectance tools.

Laser Speckle Contrast Imaging

Another optical tool exploiting a form of reflected light is laser speckle contrast imaging (LSCI), which has been

used intraoperatively for the assessment of physiological states, specifically for cerebral blood flow (CBF) monitoring.^{60,100,113} LSCI uses intrinsic tissue contrast by applying a coherent light source (i.e., laser) to measure spatial speckle contrast and quantify the local spatial variance of the speckle pattern from decorrelation of the coherent light in the presence of blood flow. These images can be converted into a quantitative measure of blood flow. Richards et al.¹¹³ report an application of intraoperative LSCI for assessment of CBF during neurosurgical guidance with optimization of signal, correction for motion distortion and associated pulsatile artifacts, and derivation of images of cerebral vasculature and blood flow. This technique provides another means of vascular imaging and extraction of more quantitative parameters independent of exogenous markers (e.g., ICG, fluorescein, iodinated contrast agent).

Optical Coherence Tomography

OCT is an optical imaging modality that detects singly backscattered or back-reflected light to reconstruct depth-resolved, tomographic images of biological tissues with micrometer spatial resolution at millimeter depths.^{7,11,13,36} OCT is analogous to ultrasound in that reflections of light (rather than sound) are detected.¹¹ Backscattered light in OCT is a function of the endogenous tissue optical properties, and, since tissues are highly scattering, OCT is mostly dependent on optical scattering for imaging contrast.³⁶

OCT detection has been implemented across various disciplines including ophthalmology, cardiology, dermatology, urology, dentistry, and in various modes including microscopes, fiberoptic catheters, handheld probes, and endoscopes.^{10,13,36} Boppart et al.¹² reported on an ex vivo use of OCT for the identification of metastatic melanoma in human brain. Bizheva et al. reported one of the first studies on ex vivo human tissues (formalin fixed or fresh tissue) to use OCT to distinguish between normal brain and brain tumors including meningioma, ganglioglioma, and glioblastoma.^{7,9} The authors reported visualization of fine morphological details such as nuclei, cysts, microvasculature, and microcalcifications. Jafri et al.⁵² used a catheter-based OCT system in 25 postmortem human brains for the purpose of identifying and resolving structures of interest for deep brain stimulation (e.g., within the globus pallidus, subthalamic nucleus, and substantia nigra) such as microvasculature and gray-white matter junction or even pathological morphologies (e.g., vacuoles in transmissible spongiform encephalopathies). Assayag et al.⁴ applied a full field of view OCT imaging system, which enabled high spatial resolution of $1.5 \times 1.5 \times 1.0 \mu\text{m}^3$ to depths of approximately 200–300 μm over a 1-cm² field of view and 5-minute acquisition times. The authors examined freshly resected brain tissue specimens in 18 patients with diffuse gliomas (WHO Grades II and III), meningiomas, and metastases. The authors reported the ability to resolve fine structural features including myelinated axon fibers, neuronal cell bodies, vasculature, hippocampal regions (e.g., CA1, CA4), granule and pyramidal neurons, cerebellar folia, and psammoma bodies. The authors report that in their study they were unable to distinguish regions of low cell density (less than ~20%), diffusely infiltrative low-grade gliomas. A recent, elegant study by Kut et al.⁷² reports the

use of OCT on ex vivo human tissue samples of 32 patients with WHO Grade II to IV gliomas. A substantial contribution by the authors is the performance of a diagnostic analysis to derive an attenuation threshold to distinguish tissues with high sensitivity and specificity, and create OCT images discriminating between normal and tumor tissues. The authors report specificities and sensitivities of 100% and 92% for high-grade patients and 80% and 100% for low-grade patients, respectively.

In vivo applications of OCT in neurosurgery are limited. One study involving 9 patients (6 GBM, 2 recurrent GBM, and 1 anaplastic astrocytoma) used a noncontact OCT system at various points during the resection to image surgical planes in vivo, with subsequent biopsy acquisition and histopathological examination. The authors reported distortions in tissue microstructure and light attenuation profiles detected by OCT in regions of tumor infiltration. In summary, the use of OCT in the neurosurgical operating room is mostly limited to ex vivo applications. Nevertheless, there is exciting potential for growth of this modality as an in vivo surgical adjunct given its ability to exploit intrinsic optical contrast, provide micrometer spatial resolution, and detect features at millimeter depths.

Spectroscopy

Optical spectroscopy probes have used either the diffuse reflectance or a combination of the diffuse reflectance and tissue fluorescence from endogenous (e.g., NADPH), exogenous (e.g., fluorescein), or exogenously induced fluorophores (e.g., PpIX).^{103,114,151} Work by Lin et al. at Vanderbilt provides one of the largest experiences in neurosurgery using raw diffuse reflectance intraoperatively. Their group collected the fluorescence and diffuse reflectance spectral peaks (e.g., 460-nm fluorescence emission and 460- and 625-nm reflectance) across a range of brain tumors and developed discrimination algorithms with reported sensitivities and specificities of up to 96% and 93%, respectively.⁸⁴ In an in vivo report involving 26 patients with brain tumors, the authors described a similar use of an intraoperative spectroscopy probe using both autofluorescence and diffuse reflectance in a 2-step discrimination algorithm with sensitivity and specificity of 100% and 76%, respectively.⁸³ In a more recent study of 400 spectra in 12 pediatric patients using only the diffuse reflectance spectra, investigators reported discrimination between normal cortex and brain tumor tissue.⁸² Additional reports used raw fluorescence and/or diffuse reflectance spectral information for tissue discrimination in radiation necrosis^{80,139–141} or neoplastic and epileptogenic pediatric brain.⁸¹ A limitation of these studies rests on the fact that they use raw spectral information. As such, they do not provide explicit quantitation of relevant biomarkers (e.g., oxy- and deoxyhemoglobin) since they measure raw fluorescence and/or reflectance information and optical signal intensity. Thus, interpretation of the biological correlates with respect to the optical data are not intuitive for the surgeon.

A recent proof of principle study addressed these challenges using a combined fluorescence and reflectance approach with a quantitative spectroscopy system.¹⁵⁴ Both the fluorescence and diffuse reflectance in tissue were collected and coupled to a light transport algorithm of the

diffuse reflectance to estimate the absolute tissue optical properties. Tissue optical properties are a function of the absolute levels of tissue biomarkers such as hemoglobin concentration, oxygen saturation, and architectural markers such as scattering. This information was used to correct the emitted fluorescence and extract absolute levels of PpIX. A classification, machine learning algorithm was developed using the derived biological markers, with reported accuracies of up to 94% across a broad range of glioma histologies including low- and high-grade gliomas.¹⁵⁴ This preliminary study highlights the feasibility and value of developing biologically and physiologically intuitive technologies that provide the surgeon with information (i.e., known biomarkers) of physiological relevance.

In summary, reflectance-based technologies exploit variations in intrinsic tissue contrast that arise from histopathological and pathophysiological changes. Reflectance-based technologies have the main advantage of not requiring exogenous contrast administration. Despite this advantage, as noted above, concerns with tissue sensitivity and specificity, as well as technological limitations impacting signal-to-noise, have limited the ability of diffuse reflectance technologies to assume a stronger role in the neurosurgical operative theater. The tools available to the neurosurgeon have yet to be fully developed, and with improved algorithms and hardware a new era in the use of reflectance techniques approaches.

Raman

Raman is a label-free technique that does not require exogenous agents but rather is able to measure the “vibrational fingerprint” of cells and tissues.^{5,21,28,35,53–55,59,64,67,77,117,143,173} Raman is based on the inelastic scattering of photons upon interaction with tissue. Tissue is interrogated with light, and a small amount of this incident light at a specific wavelength is scattered to different wavelengths. The incident and scattered light are a function of the vibrational energies of molecular bonds in tissues, thus providing a way to differentiate cellular structures based on the chemical specificity of lipids, proteins, and DNA.²⁸ A major limitation of Raman is its intrinsically weak signals (1 in approximately 10^7 scattered photons is Raman shifted). As such, various techniques have been developed to improve Raman detection sensitivity, including resonance Raman; surface-enhanced Raman scattering (SERS), which can be used to enhance signals from Raman molecules by a factor of 10^7 – 10^{10} ; and coherent Raman.⁴⁷

The use of Raman in neurosurgery traces a more recent history, with most studies performed on ex vivo specimens. Ex vivo studies using Raman spectroscopy in pediatric brain tumor samples with a support vector machines discrimination algorithm reported > 90% accuracies for tissue identification.⁷⁷ A recent study by Jermyn et al. developed an in vivo intraoperative handheld Raman spectroscopy probe reporting 0.2-second acquisition time, sampling depth of 1 mm, and sensitivity and specificity of > 90% for gliomas in a cohort of 17 patients.^{21,53} In their study, the authors sampled the range from 381 to 1653 cm^{-1} , noting differences in the lipid bands (700–1142 cm^{-1}), nucleic acid bands (1540–1645 cm^{-1}), and the breathing

FIG. 5. Images showing label-free brain tumor imaging with stimulated Raman scattering (SRS) microscopy. **A:** SRS imaging of lipid (at 2854 cm^{-1}), protein (at 2930 cm^{-1}), and red blood cells (at 2800 cm^{-1}) of a fresh brain tumor tissue from an oligodendroglioma surgical case (WHO Grade II). Myelin fibers were visualized based on the strong lipid signals (*green*), cell nuclei and blood vessels were visualized mainly due to their strong protein signals (*blue*), and red blood cells were imaged based on the non-Raman 2-color 2-photon absorption signals (*magenta*). **B:** SRS and H & E imaging of the same frozen section of an entire tissue specimen from an oligoastrocytoma surgical case. Lipid/protein mapping of the brain tumor tissue with SRS provided very similar information to H & E staining with cell-to-cell correlation (B lower zoom images), demonstrating that SRS imaging could be used for label-free neuropathology. Scale bars = $100\text{ }\mu\text{m}$ (A and B upper), 1 mm (B lower). Brain tissue samples were obtained under an institutional review board–approved protocol from the Brigham and Women’s Hospital and Dana-Farber Cancer Institute Neuro-oncology Program Biorepository collection.

mode of phenylalanine in proteins (1005 cm^{-1}). This later study is key in noting intraoperative in situ implementation of Raman techniques for surgical guidance, with minimal disruption to the surgical workflow and excellent diagnostic capabilities (Fig. 5). Given the large amount of spectral information, Raman-based studies have mostly

used machine learning algorithms to classify tissue into normal versus tumor categories.^{21,53–55}

Coherent anti-Stokes Raman scattering (CARS) microscopy is a nonlinear variant of Raman techniques, with much stronger signals than that of conventional Raman spectroscopy. CARS at 2850 cm^{-1} (CH_2 stretching

vibration) was used to image lipids in brain. For example, Uckermann et al. reported on 6 patients with GBM using CARS microscopy, with decreased CARS signal in tumors versus normal tissue.¹⁴³ Romeike et al.¹¹⁷ elaborated on a combined use of 2-photon fluorescence imaging of tissue autofluorescence (e.g., elastin, NAD[P]H or keratin) and CARS (e.g., lipids) microscopy for imaging of ex vivo brain tumor samples and extraction of parameters such as cell density, nucleus to cytoplasm ratio, and nuclear size and shape.

Ji et al.^{54,55} report the use of stimulated Raman scattering (SRS) microscopy for imaging of tissues based on their Raman contrasts. They use a 2-color SRS imaging method to extract specifically lipid and protein signals, such that high lipid content tissue (e.g., white matter) will predominantly appear as 1 color (e.g., green) and high protein content structures as another color (e.g., blue). This work highlights the ability of SRS to image axons, cells, protein/lipid ratios, and tissue histoarchitectural structures. The authors analyzed 14 biopsies from 3 patients undergoing anterior temporal lobectomy and compared these with 39 biopsies from 19 patients with central nervous system tumors and compared SRS and hematoxylin and eosin (H & E) staining results, noting excellent agreement ($\kappa > 80$). They then developed a classifier algorithm applied to human samples to automatically distinguish tissues with varying levels of tumor infiltration with sensitivity and specificity for tumor and nontumor infiltrated brain of 97.5% and 98.5%, respectively. Both Ji et al. and Jermyn et al. note that despite the intrinsic low sensitivity constraints of Raman techniques, with appropriate technological optimization, they can detect tissues with varying degrees of tumor infiltration. Figure 5 shows our SRS imaging of both fresh and frozen human brain tumor tissue with lipid, protein, and blood contrasts. Myelin fibers, cell nuclei, blood vessels, and red blood cells were visualized with both high signal-to-noise ratio and subcellular spatial resolution (Fig. 5A), with strong correlation between SRS and H & E (Fig. 5B). This highlights an example of how SRS imaging could be used for pathological diagnosis of brain tumors.

Implementation of Raman-based technologies in neurosurgery is a new innovation. Most current studies were performed using ex vivo tissue, with only limited experience using Raman in vivo and in situ for brain tumor detection. A major limitation of Raman is the intrinsic low signal, but recent technical developments have addressed this concern by providing high quality data within acceptable timeframes and adequate signals. Future work will seek to further exploit the Raman effect for tissue discrimination and optical guidance, including transition from ex vivo tools to handheld probes with larger field of view tools.

Conclusions

Here we present a survey of optical tools in clinical use for neurosurgical guidance. This paper seeks to provide the audience a key sampling of the major technologies for optical guidance currently available for clinical use to the neurosurgeon as he/she seeks to enhance their armamentarium. We provide an overview of major biomarkers

(e.g., autofluorescence, PpIX, fluorescein), contrast mechanisms (e.g., steady-state vs time-resolved fluorescence, reflectance, Raman), and technologies (e.g., spectroscopy probes, confocal microscopes, and surgical microscopes) available to the neurosurgeon at this time. This report does not seek to provide an exhaustive overview of all studies and results using fluorescence, reflectance, or Raman-based tools in the neurosurgical operating room. Also, this report is not intended to provide a description of the plethora of preclinical tools and agents used in animal models or preclinical studies. In the *References* section we provide a list of important primary and review literature that delve into the minutiae on points of possible interest and further education for the reader such as ALA-PpIX biology²⁰ or principles of optical spectroscopy.¹¹⁴ The field of biomedical optics, with development of optical instrumentation and biomarkers, is a rich and highly prolific field, and here we present some of the clinical tools and markers available in the present day neurosurgical operating room.

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Disclosures

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Author Contributions

Conception and design: Valdés, Roberts, Golby. Acquisition of data: Valdés, Lu. Analysis and interpretation of data: Valdés, Lu, Golby. Drafting the article: Valdés. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Valdés. Administrative/technical/material support: Golby. Study supervision: Valdés, Golby.

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