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Abstract. Glioma itself accounts for 80% of all malignant primary brain tumors, and glioblastoma multiforme (GBM) accounts for 55% of such tumors. Diffuse reflectance and fluorescence spectroscopy have the potential to discriminate healthy tissues from abnormal tissues and therefore are promising noninvasive methods for improving the accuracy of brain tissue resection. Optical properties were retrieved using an experimentally evaluated inverse solution. On average, the scattering coefficient is 2.4 times higher in GBM than in low grade glioma (LGG), and the absorption coefficient is 48% higher. In addition, the ratio of fluorescence to diffuse reflectance at the emission peak of 460 nm is 2.6 times higher for LGG while reflectance at 650 nm is 2.7 times higher for GBM. The results reported also show that the combination of diffuse reflectance and fluorescence spectroscopy could achieve sensitivity of 100% and specificity of 90% in discriminating GBM from LGG during *ex vivo* measurements of 22 sites from seven glioma specimens. Therefore, the current technique might be a promising tool for aiding neurosurgeons in determining the extent of surgical resection of glioma and, thus, improving intraoperative tumor identification for guiding surgical intervention. © 2017 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.22.2.027002]

Keywords: optical properties; diffuse reflectance spectroscopy; fluorescence spectroscopy; glioblastoma multiforme; low grade glioma.

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1 Introduction

Glioma is a term used to describe all tumors arising from glial cells in the brain. There are three subgroups—astrocytoma, oligodendroglioma, and ependymoma—representing 30% of all primary brain tumors and 80% of all malignant primary brain tumors.¹ Glioblastoma multiforme (GBM), the highest tumor grade in astrocytoma, accounts for nearly 55% of such tumors and affects over 12,000 people in the United States per year.^{1–4} GBM prognosis is very poor, and its median survival is reported to be from less than 14 months⁵ to 2 years.^{6,7} Currently, neurosurgeons define the extent of a brain tumor resection using techniques such as brain biopsy,^{8,9} intraoperative ultrasound,¹⁰ or visual inspection with the assistance of preoperative magnetic resonance (MR) images.¹¹ Unfortunately, each method has considerable limitations. Brain biopsy is invasive and, hence, limits the number of specimens taken, leading to large sampling errors. Brain tumors frequently resemble normal brain tissues in ultrasound images, causing incomplete removal of margins.¹² In addition, the nonrigidity of brain tissues might cause positional shifts during the operation, making the defined margins on

preoperative MR images unreliable.^{13,14} This problem may lead to either unplanned resection of normal brain tissues or incomplete resection of a tumor.^{15,16} Therefore, a noninvasive method is desired to allow real-time identification of GBM tissue.

Early studies have shown that diffuse reflectance spectroscopy (DRS) has the potential for noninvasive detection of mucosal abnormalities via differences in tissue optical properties^{17–19} and that fluorescence spectroscopy has the ability to detect malignant tissues by analyzing fluorescence characteristics such as emission spectrum^{20,21} and decay lifetime.^{22,23} Optical properties including absorption coefficient (μ_a) and reduced scattering coefficient (μ_s') are wavelength-dependent quantities that govern light propagation in tissues.^{24–27} In human mucosa, the principal absorbers are hemoglobin in blood, collagen crosslinks, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD).^{28–30} Meanwhile, density and the morphology of cells are the main determinants of the scattering signal.³¹ Tissue optical properties are tied to the concentration of absorbers and scatterers present in that tissue. For example, a high number density of cells leads to high μ_s' ³² while high blood vessel density suggests high μ_a .^{33,34} In Lin et al.'s studies, it was shown that

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DRS alone could be used to discriminate brain tumor from normal brain tissues during *in vivo* measurements of 12 patients with a sensitivity of 95% and a specificity of 66%.³⁵

Although the emission peaks in the steady-state fluorescence spectrum could be used to identify the principal fluorophores, the fluorescence signal is usually distorted by absorption and scattering of absorbers and scatterers present in the tissue.^{36,37} To correct the measured fluorescence, and to enable tissue discrimination using steady-state fluorescence, diffuse reflectance (DR) must be used in parallel.^{36,37} By combining steady-state fluorescence and DR, Lin et al. were also able to identify infiltrating tumor margins from normal brain tissues with a sensitivity of 100% and a specificity of 76%.³⁸ In similar studies with the integrated system, Toms et al. could identify glioma margins of 24 glioma patients with a sensitivity of 94% and a specificity of 93%,³⁹ and Valdés et al. were able to achieve a sensitivity and specificity of 94% in differentiating two low grade gliomas (LGG) patients and five GBM patients.⁴⁰ Without using DRS, Butte et al. showed that fluorescence spectroscopy alone had the potential to discriminate LGG with a sensitivity of 100% and a specificity of 98%.^{22,23} Unfortunately, the technique suffered low sensitivity (47%) for detection of high grade gliomas.^{22,23} In these studies, however, the spectral analysis for specific tumor grades, especially for LGG and GBM, was not analyzed separately,^{35,38-40} and the optical properties were not recovered.^{22,23,35,38,39} In general, intraoperative differentiation of GBM and LGG from normal tissues can help neurosurgeons determine the extent of neurosurgical resection and determine a treatment plan.⁴⁰⁻⁴² Knowledge of optical properties can also be used to calculate oxygen saturation, which is expressed as the percentage of oxyhemoglobin in the total hemoglobin.⁴³⁻⁴⁵ Although Asgari et al. were able to show that oxygen saturation was

lower for LGG groups ($36 \pm 21\%$) and higher for GBM ($52 \pm 18\%$) due to the existence of arteriovenous shunts and metabolic shunts in GBM, optical properties were not measured.⁴⁶ Whereas attempts to extract optical properties of healthy brain tissues and gliomas were performed *in vitro* by Gebhart et al.,⁴⁷ and *in vivo* by Valdés et al.,⁴⁰ optical properties were averaged for all gliomas, and separated observations for LGG and GBM were not available.^{40,47}

In this paper, *ex vivo* DR and fluorescence measurements were performed to differentiate GBM from LGG in a total of 22 sites of seven glioma patients. An experimental look-up table (LUT) combined with a fitting routine was used to extract optical properties from 350 to 700 nm. The approach was validated on liquid tissue-simulating phantoms containing hemoglobin, polystyrene microspheres, and India ink.

2 Materials and Methods

2.1 Optical Phantoms and the Inverse Solution

Similar to a previous study,⁴⁸ the inverse solution for extracting optical properties from the measured DR combines an experimental LUT and fitting algorithms. The LUT was generated by measuring the DR from tissue-simulating phantoms with known optical properties. To simulate tissue scattering, polystyrene microspheres with a diameter of $1 \mu\text{m}$ (07310-15, Polysciences Inc., Pennsylvania) were used. To simulate tissue absorption, black India ink (Higgins Ink, Chartpak Inc., Massachusetts) was used. A 6×4 matrix of 24 phantoms consisting of six different concentrations of black India ink (0.025%, 0.05%, 0.1%, 0.2%, 0.35%, and 0.5% of the concentrated ink solution) and four different concentrations of microspheres (0.2%, 0.45%, 0.7%, and 1% w/v) was created [Fig. 1(a)].

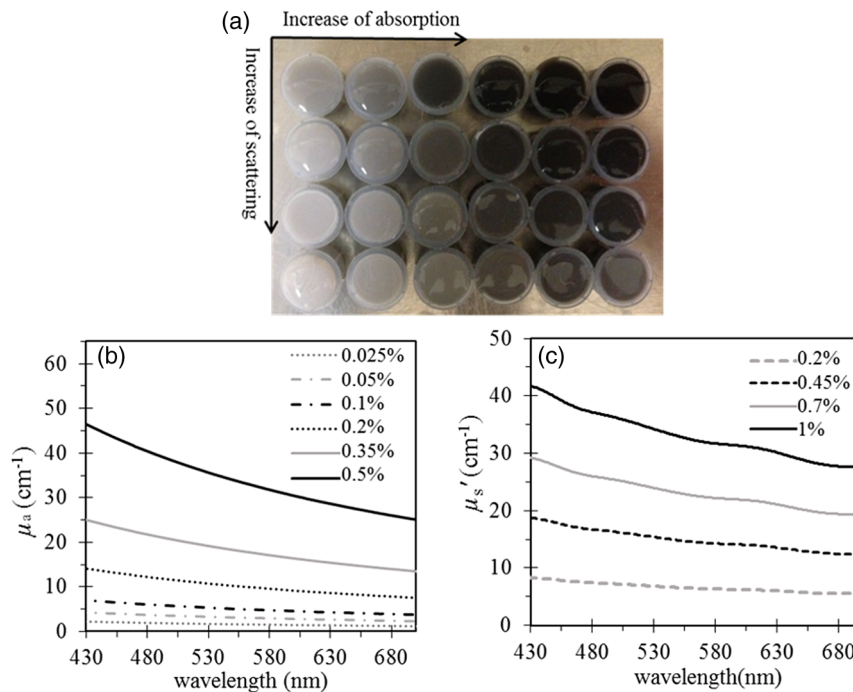


Fig. 1 Matrix of optical phantoms for LUT development: (a) top surface images of 24 phantoms captured with a standard digital camera, (b) absorption coefficients μ_a , and (c) reduced scattering coefficients μ'_s . In (a), six concentrations of black India ink are 0.025%, 0.05%, 0.1%, 0.2%, 0.35%, and 0.5%, and four different concentrations of microspheres are 0.2%, 0.45%, 0.7%, and 1% w/v. These correspond to six spectra of μ_a in (b) and four spectra of μ'_s in (c).

Within the selected concentrations of India ink and microspheres, a μ_a range of 0.05 to 47 cm^{-1} and a μ_s' range of 5 to 42 cm^{-1} were covered for a wavelength range 430 to 700 nm [Figs. 1(b) and 1(c)]. These optical properties were selected based on various reports studying human brain tissues and mucosal tissues.^{20,29,30,47,49} To evaluate the LUT, selected phantoms containing ferrous stabilized hemoglobin (H0267, Sigma-Aldrich, Missouri) and microspheres with different concentrations were created. The DR from these phantoms was measured and the recovered optical properties were compared to target values.

In all phantoms, target optical properties were controlled and calculated by applying Beer–Lambert’s law to the actual absorbance of pure solute absorbers (India ink or hemoglobin) measured with a spectrophotometer (Ultraspec 3000, Pharmacia Biotech Inc., New Jersey) for μ_a , and by applying Mie theory to microsphere concentration for μ_s' .⁵⁰ Hemoglobin H0267 has an absorption spectrum similar to that of human blood with secondary absorption peaks at 540 and 580 nm and stable oxygen saturation.^{49,51,52} Polystyrene microspheres with a diameter of 1 μm were preferred as scatterers because their scattering anisotropy is in a similar range to that of many biological tissues ($g = 0.89$ to 0.93 in UV–vis) and because their well-controlled size and index of refraction permits accurate calculation of scattering properties using Mie theory.^{45,50,53} Black India ink is widely used to simulate secondary absorbers in tissue due to its exponential decrease of absorption with wavelength, low cost, spectral stability, and low-fluorescence.^{54–57}

To fit the optical properties, the least squares fitting routine `fminsearch()` in MATLAB[®] was used, so the absorption coefficients and the reduced scattering coefficients were constrained in the form of Eqs. (1) and (2). This optimization method is based on the Nelder–Mead simplex algorithm and has been used widely for spectral analysis in spectral imaging.^{58–60} The total absorption coefficient, μ_a , accounts for absorption of a primary absorber (human blood) and of secondary absorbers (i.e., NADH, FAD, and collagen).^{20,28,49} In general, the total absorption coefficients of all secondary absorbers can be described as an exponential decay with wavelength^{20,28} while the absorption coefficient of blood is determined predominantly by those of Hb and HbO₂.^{44,61} A similar fitting method for total absorption coefficients can be found elsewhere.^{48,61}

$$\mu_a(\lambda) = A \cdot e^{B \cdot \lambda} + \ln(10) \cdot f_1 \cdot [f_2 \cdot \epsilon_{\text{HbO}_2}(\lambda) + (1 - f_2) \cdot \epsilon_{\text{Hb}}(\lambda)]. \quad (1)$$

In Eq. (1), A and B are fitting coefficients that determine the contribution of secondary absorber so that A (cm^{-1}) is the amplitude constant while B (nm^{-1}) is the rate constant; λ is the wavelength; f_1 (mol/l) is the total concentration of hemoglobin, f_2 (dimensionless) is the oxygen saturation, and ϵ_{HbO_2} ($\text{cm}^{-1} \cdot \text{M}^{-1}$) and ϵ_{Hb} ($\text{cm}^{-1} \cdot \text{M}^{-1}$) are molar extinction coefficients of oxygenated and deoxygenated hemoglobin, respectively. In tissue measurements, A , B , f_1 , and f_2 were calculated by applying the least squares fitting to the LUT-recovered μ_a and the known spectra of ϵ_{HbO_2} and ϵ_{Hb} .⁶² In hemoglobin phantoms without collagen and NADH, A was set to zero and the extracted f_2 should be nearly 100% due to the nature of ferrous-stabilized hemoglobin.⁵² In human tissues, μ_s' is monotonically decreasing with wavelength, and the fitting equation for μ_s' can be expressed in the form of Eq. (2) where a with unit of cm^{-1} is the factor characterizing magnitude of scattering, b (dimensionless) is the factor that characterizes wavelength dependence of scattering, and λ is the wavelength in nanometers (nm).^{30,44,61}

$$\mu_s'(\lambda) = a \cdot \lambda^b. \quad (2)$$

2.2 Brain Tissue Samples

Fresh brain specimens were obtained from brain tissue removed during tumor resection surgery. The study protocol is approved by the McMaster/Hamilton Health Sciences Integrated Research Ethics Board, and patients consented to participate. Prior to the DRS measurement, each specimen was washed with saline, and the spectroscopic measurements were performed within 30 min of the surgery. A total of 22 sites were measured from specimens of seven patients. At each site, reflectance and fluorescence measurements were repeated four times to allow averaging and standard deviation calculation. Following the measurements, each site was marked with tissue marking dyes (Davidson Marking system, Bradley Products Inc., Minneapolis, Minnesota) in different colors. After the optical measurements, the specimens were preserved in formaldehyde and then cut into 5- μm -thick slices with hematoxylin and eosin stain. Tumor grade was assigned by a single pathologist (JP), using World Health Organization guidelines.⁶³ The biopsy results identified four GBM patients (12 sites) and three LGG patients (10 sites). The surface area of tissue samples is at least five times larger than the surface area of the optical probe, which has a diameter of 3 mm. Thickness of tissue samples is at least 0.5 cm and is much larger than the optical penetration depth range of 100 to 300 μm for a human brain tumor at visible wavelengths.⁶⁴

2.3 Instruments

DR signals between 430 and 700 nm were generated using a broadband light source (Dolan-Jenner MI-150, Edmund Optics, New Jersey), while fluorescence signals were generated using a solid-state laser (PNV-001525-140, Teem Photonics, Meylan, France) at 355 nm with 300-ps pulses. Note that optical properties in the 350- to 430-nm range were extrapolated using the calculated parameters (A , B , f_1 , f_2 , a , and b from the fitting results). Measurements of both DR and steady state fluorescence (SSF) signals were performed with the same customized optical probe consisting of one source fiber and three detection fibers at source-detector collection distances (SDD) of 0.23, 0.59, and 1.67 mm. A schematic and detailed description of the system can be found elsewhere.^{65–67} All fibers used in DRS and SSF measurements have a core diameter of 200 μm and numerical aperture of 0.22. After DRS and SSF measurements were performed, the fluorescence decay was recorded using a 400- μm core optical fiber in the bundle.⁶⁸ When observing highly absorbing phantoms, only background noise was collected with the furthest fibers, thus reducing the prediction accuracy of the inverse solution. Therefore, in the current study, only the two detection fibers closest to the source fiber were used to develop the LUT and to extract optical properties from the measured reflectance. To calculate the DR R from the sample, the measured reflectance intensity of the sample was normalized to the reflectance intensity of a reflection standard with 99.9% reflectivity (Labsphere, Inc., New Hampshire) after subtracting background.⁶⁵

3 Results

3.1 Validation of the Inverse Solution

Figure 2(a) shows examples of DR spectra collected from six phantoms with the same microsphere concentration (0.7% w/v)

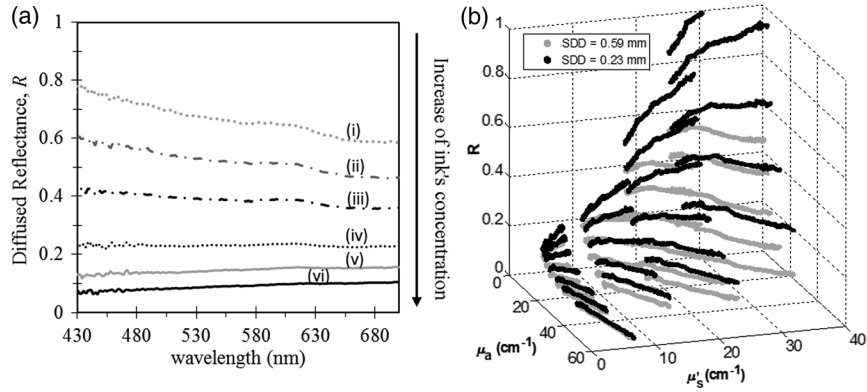


Fig. 2 (a) Examples of diffuse reflectance R for six different ink concentrations while microsphere concentration remains constant and $SDD = 0.23$ mm, and (b) R as a sparse matrix mapped to optical property space $R[\mu_a(\lambda), \mu_s'(\lambda)]$ for $SDD = 0.23$ and 0.59 mm. In (a), concentration of microsphere is 0.7% whereas concentrations of black India ink are 0.025% , 0.05% , 0.1% , 0.2% , 0.35% , and 0.5% , corresponding to six spectra (i) to (vi). In (b), the sparse matrix represents reflectance data per SDD collected from 24 phantoms (six ink concentrations \times four microsphere concentrations).

and different India ink concentrations (from 0.05% to 1%). Figure 2(b) shows the sparse matrix of DR collected from all 24 phantoms at SDD of 0.23 and 0.59 mm. The LUT was evaluated with randomly selected phantoms consisting of hemoglobin and microspheres. Figures 3(a) and 3(b) compare the extracted and the target optical properties spectra for a selected phantom with microsphere concentration of 0.7% w/v and hemoglobin

concentration of 8 mg/ml. The target optical properties are those calculated with Beer-Lambert's law and Mie theory while the extracted values are those calculated from the inverse solution. As shown in Figs. 3(a) and 3(b), the method was able to retrieve μ_a and μ_s' with average errors of 6% and 3% , respectively, from 350 to 700 nm. The intensive evaluation of the inverse solution was performed on a total of 10 hemoglobin

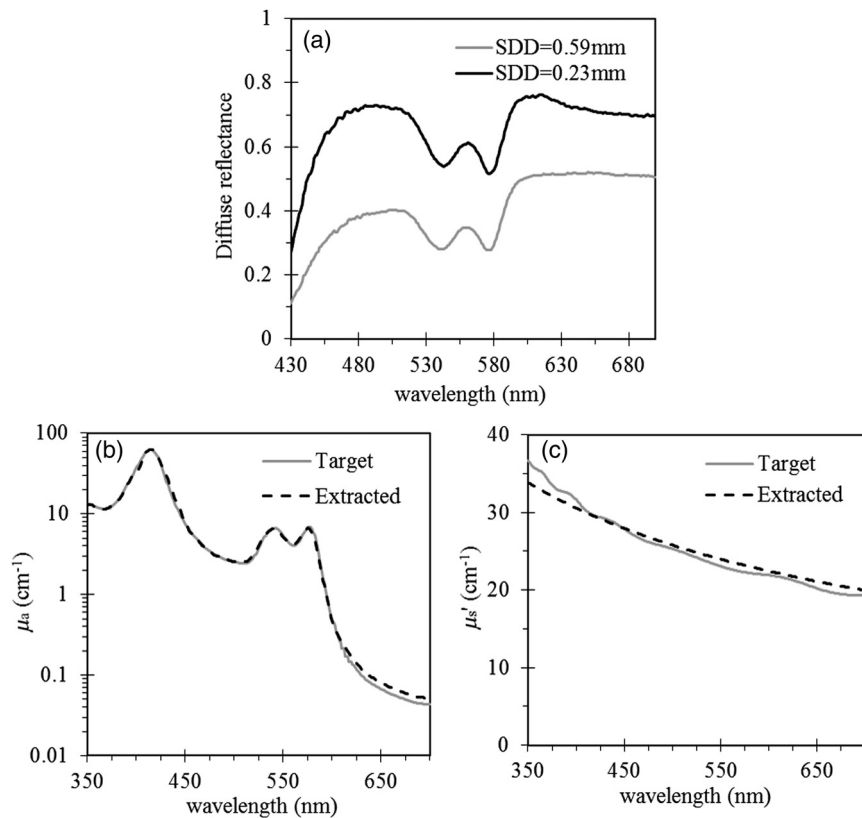


Fig. 3 An example of data analysis for a phantom with Hb concentration of 8 mg/ml, microsphere concentration of 0.7% : (a) diffuse reflectance collected with fiber at SDD of 0.23 and 0.59 mm, (b, c) theoretical (target) versus extracted optical properties. Equations (1) and (2) were used to extrapolate data in 350 to 430 nm. In addition, f_1 and f_2 values of 7.9 ± 0.8 mg/ml and $96\% \pm 3\%$ were obtained by using Eq. (1).

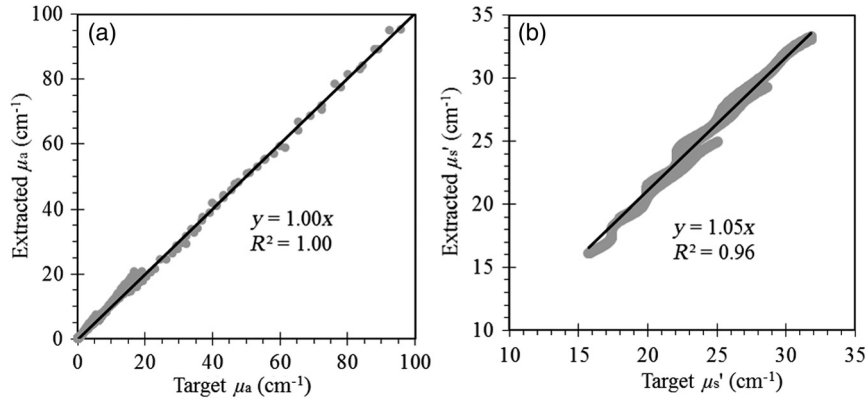


Fig. 4 Evaluation of LUT over 10 different hemoglobin phantoms. In general, average percentage errors of 9% and 6% were obtained for μ_a and μ_s' , respectively.

phantoms with a total of 3500 pairs of target optical properties in 430 to 700 nm (Fig. 4). On average, errors of 9% and 6% were observed for μ_a and μ_s' , respectively. Furthermore, the fitting approach was able to retrieve f_1 and f_2 in all hemoglobin phantoms with average errors of 5.8% and 7%, respectively. For example, the recovered f_1 and f_2 values for the phantom shown in Fig. 3 were 7.9 ± 0.8 mg/ml and $96\% \pm 3\%$, respectively, versus target values of 8 mg/ml and 100%, respectively.

3.2 Brain Tissue Measurements

Figure 5 compares the DR [Fig. 5(a)] and optical properties [Figs. 5(b) and 5(c)] measured for the GBM group and the LGG group. In Fig. 5, data were averaged over all 12 GBM

sites and 10 LGG sites. On average over the entire spectrum (Fig. 5), DR was 3.2 times higher, μ_a was 48% higher, and μ_s' was 140% higher for the GBM group. Data at 650 nm are also shown for comparison (Table 1). Note that 650 nm was selected because this is the region where blood absorption is small, and it is less likely that bleeding during surgery will affect tumor discrimination with optical measurement.³⁵⁻³⁹ The absorption coefficients were determined by the primary absorber (hemoglobin) and secondary absorbers. Below 600 nm, absorption of hemoglobin dominated and determined the shape and intensity of the absorption spectrum. Hemoglobin absorption could be from blood within the tissue that has diagnostic value and/or blood on or close to the tissue surface that is the result of bleeding and has no diagnostic value. Although all

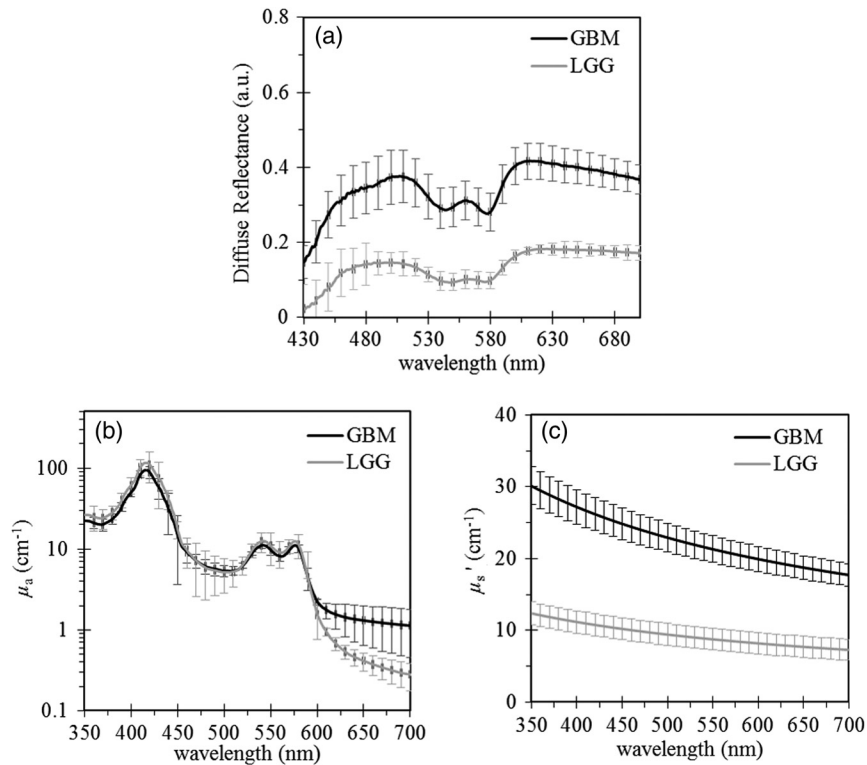


Fig. 5 LGG group versus GBM group average spectral analysis: (a) diffuse reflectance, (b) absorption coefficient μ_a , and (c) reduced scattering coefficient μ_s' . Data were averaged over all sites (12 GBM sites and 10 LGG sites) and error bars are the standard deviation.

Table 1 Average over all GBM and LGG sites: diffuse reflectance at 650 nm (R_{650}), optical properties at 650 nm ($\mu_{a,650}$ and $\mu'_{s,650}$), ratio of fluorescence to reflectance at 460 nm $(F/R)_{460}$, and fluorescence life-time at 460 nm (τ_{460}).

Parameters	GBM	LGG
R_{650} (a.u.)	0.44 ± 0.03	0.16 ± 0.01
$\mu_{a,650}$ (cm^{-1})	1.31 ± 0.11	0.41 ± 0.07
$\mu'_{s,650}$ (cm^{-1})	18.8 ± 1.4	7.7 ± 0.7
$(F/R)_{460}$ (a.u.)	9.9 ± 1.2	26.2 ± 2.7
τ_{460} (ns)	3.2 ± 0.3	3.1 ± 0.05

tissue samples were washed through with saline solution before measurement, a large portion of unwanted blood residues still remained and significantly affected the measured absorption coefficients below 600 nm. As shown in Table 1, at 650 nm DR was 2.8 times higher, μ_a was about 3 times higher, and μ'_s was 2.4 times higher for the GBM group. Figure 6 shows the DR [Fig. 6(a)] and optical properties [Figs. 6(b) and 6(c)] at 650 nm for all GBM and LGG sites. If we define sensitivity as the percentage of GBM sites that were correctly identified as GBM, and specificity as the percentage of LGG sites that were correctly identified as not GBM, the discrimination had a sensitivity of 100% (12/12) and specificity of 80% (8/10) if a cut-off at 20% was applied for DR at 650 nm to optimize the discrimination [Fig. 6(a)]. Sensitivity and specificity of 92% and 80% were achieved if a cut-off of 0.6 cm^{-1} was applied to μ_a at 650 nm [Fig. 6(b)]. These numbers were 100% and 90% if a cut-off of 10 cm^{-1} was applied to μ'_s at 650 nm [Fig. 6(c)]. Although oxygen saturation was calculated at $83.4 \pm 17.3\%$ for GBM and $55.4 \pm 9.9\%$ for LGG using Eq. (1), the results might be affected by a long period of air exposure of the brain tissue specimen and were not used to optimize the discrimination for the *ex vivo* measurements.

Figure 7 shows the average fluorescence intensity with an emission peak at 460 nm [Fig. 7(a)], the average fluorescence lifetime [Fig. 7(b)], and the ratio of fluorescence and diffuse reflectance at 460 nm $(F/R)_{460}$ versus diffuse reflectance at 650 nm (R_{650}) for GBM sites and LGG sites. Although the measured fluorescence signal could identify the characteristic emission peak of brain tissues at 460 nm [Fig. 7(a)], the measured fluorescence signal alone is not able to discriminate tumor types due to high tissue absorption in this wavelength range. To enable tumor discrimination, a graph of the ratio of fluorescence to diffuse reflectance at the emission peak $(F/R)_{460}$ versus R_{650} was used instead.^{38,39} If a cut-off of 20 for F_{460}/R_{460} was applied, sensitivity and specificity of 100% and of 90% were achieved. Although the measured fluorescence signal (F_{460}) is distorted by absorption and F_{460} alone cannot be used to differentiate various brain tumor types, F_{460} can be corrected by using the measured reflection signal and the measured optical properties at the emission wavelength.^{37,65} In general, intrinsic fluorescence (f_{460}) and fluorophore concentration are related to the ratio of (F/R) .^{37,65} As shown in Fig. 7(b) and Table 1, fluorescence life-time alone was not able to discriminate GBM due to the high variation of life-time values, most likely caused by the low signal-to-noise ratio of the autofluorescence and high degree of heterogeneity in GBM.²³ Figure 8 summarizes sensitivity and

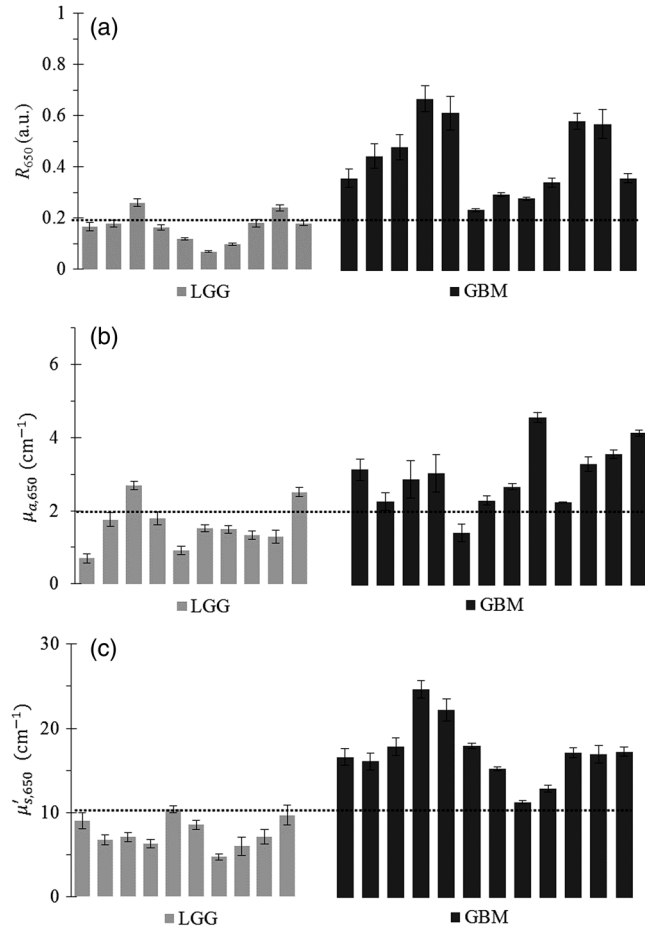


Fig. 6 LGG group versus GBM group: (a) diffuse reflectance at 650 nm (R_{650}), (b) μ_a at 650 nm, and (c) μ'_s at 650nm. Data at 650 nm were selected for due to small blood absorption in this region, and thus it is less likely for blood absorption to affect tumor discrimination.³⁵⁻³⁹

specificity when different parameters were used to discriminate GBM from LGG. In general, R_{650} , $\mu'_{s,650}$ and ratio $(F/R)_{460}$ versus R_{650} could achieve discrimination with a sensitivity of 100%. Combining diffuse reflectance and steady-state fluorescence shows an increase in specificity from 80% to 90%.

4 Discussion and Conclusion

Although the current LUT approach was similar to previous ones that aimed to study skin abnormalities,^{45,69,70} the current LUT covered optical properties of human brain tissues and mucosal tissues. More specifically, μ_a range of 0.05 to 47 cm^{-1} and μ'_s range of 5 to 42 cm^{-1} were covered for a wavelength range 430 to 700 nm. The developed inverse solution was thoroughly evaluated and was able to retrieve optical properties with an average relative error of 9% for μ_a and 6% for μ'_s using phantoms with known optical properties. Furthermore, the developed LUT was used to establish broadband optical properties for GBM and LGG (Figs. 5 and 6). Within the errors of the inverse solution, *ex vivo* measurements showed that μ'_s for GBM was 2.4 times higher than that for LGG [Fig. 5(c)]. Because cell density in GBM is two to three times higher than that in LGG^{71,72} and the scattering probability is proportional to cellular density,³² the GBM group is expected to have higher μ'_s than the LGG group. Furthermore, it is believed that GBM is highly

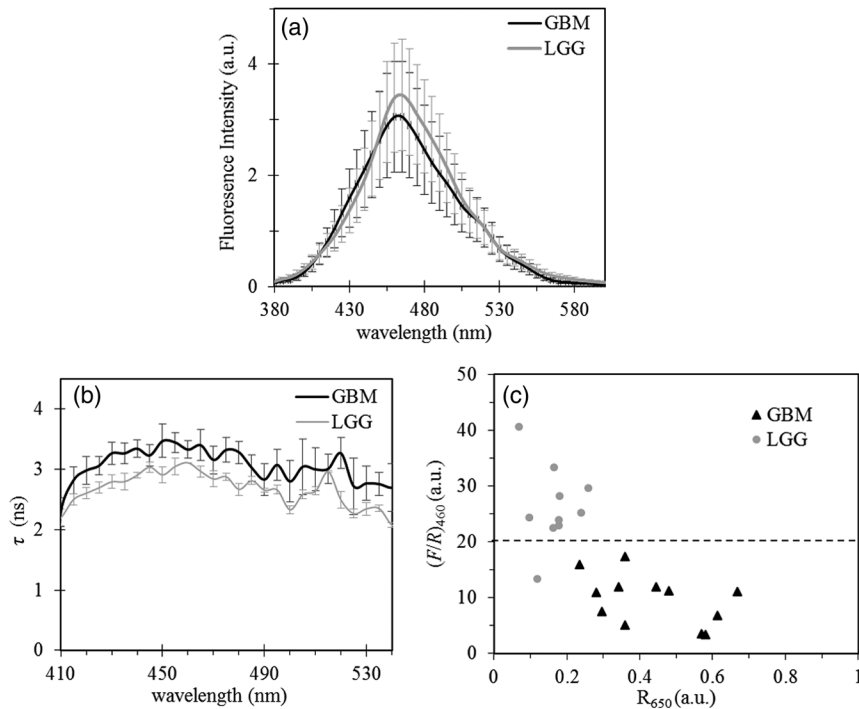


Fig. 7 LGG group versus GBM group: (a) SSF spectrum and (b) fluorescence life-time τ spectrum, (c) ratio of fluorescence to reflectance at 460 nm $(F/R)_{460}$ versus reflectance at 650 nm (R_{650}) . Fluorescence intensity has been normalized to integrating time and laser power corresponding to each measurement. In (a) and (b), data were averaged over all sites (12 GBM sites and 10 LGG sites) and error bars are the standard deviations.

vascular due to high degree of angiogenesis.^{73,74} This may imply that GBM has higher blood content or higher μ_a . In fact, with the observation at 650 nm, the current study showed that μ_a was about three times higher in GBM than that in LGG [Fig. 6(b) and Table 1]. Combining diffuse reflectance and steady-state fluorescence did not change the sensitivity (100%), but increased the specificity from 80% to 90%. Although optical measurements were performed during brain tumor resection, staging glioma (LGG versus GBM) based on their optical characteristics

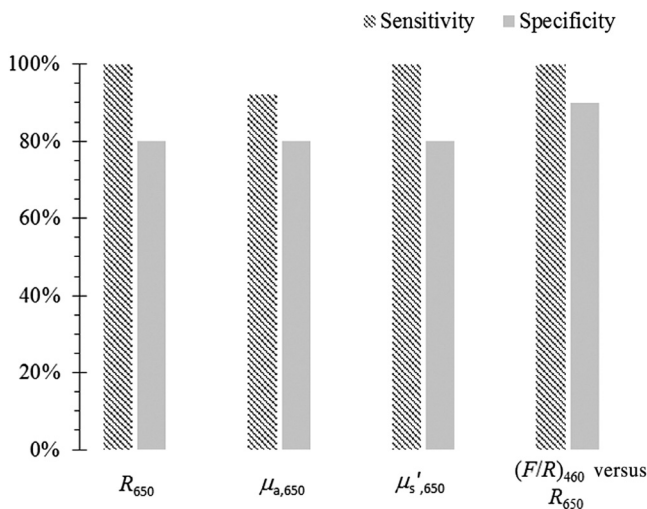


Fig. 8 Summary of sensitivity and specificity when using different parameters for GBM discrimination: diffuse reflectance at 650 nm (R_{650}) , optical properties at 650 nm $(\mu_{a,650}$ and $\mu'_{s,650})$, and ratio $(F/R)_{460}$ versus R_{650} .

has not been attempted. This study has established both optical properties and fluorescence properties for both stages. Similar to previous studies,^{35,38} the current study is limited by the small number of samples due to the limited number of glioma patients enrolled. Nevertheless, the current study has been able to discriminate GBM from LGG by accessing diffuse reflectance, optical properties measurement, and fluorescence (with diffuse reflectance). Although the measured oxygen saturation (f_2) values were higher than that in Asgari et al.'s study, they showed a similar trend so that GBM has higher f_2 than LGG, specifically $83.4 \pm 17.3\%$ for GBM and $55.4 \pm 9.9\%$ for LGG. These numbers were $52 \pm 18\%$ for GBM and $36 \pm 21\%$ in the previous study.⁴⁶ The difference in magnitude of f_2 between the two studies is mainly due to the fact that the current samples have been exposed to air during *ex vivo* measurements while intraoperative measurements were performed in the previous study. Meanwhile, it is believed that arteriovenous shunts and metabolic shunts can cause more oxygen than required in GBM and are responsible for high oxygen saturation levels in GBM.⁷⁵ The preliminary results of the *ex vivo* measurements indicate that the current technique has the potential for *in vivo* discrimination of GBM by accessing the tumor's optical characteristics including diffuse reflectance, SSF, optical properties, and possibly oxygen saturation.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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