INTRODUCTION

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults and has poor prognosis with a median survival of ~14 months despite multimodality therapy with surgery, concurrent chemoradiation therapy, and adjuvant chemotherapy (1, 2). Postcontrast T1-weighted and fluid-attenuated inversion recovery (FLAIR) T2-weighted magnetic resonance imaging (MRI) images are the primary images to guide treatment and assess tumor progression or therapy response (3). However, the postcontrast T1-weighted MRI identifies blood–brain barrier disruption, which is affected by tumor growth but also by radiation, antiangiogenesis drugs, and chemotherapy. Abnormality on T2 FLAIR images is influenced by T2 changes of tumor cells as well as edema that always coexists within GBM. Limitations of conventional MRI in clinical management of GBM have been recognized and motivate investigations of physiological and metabolic MRI.

Diffusion-weighted (DW) imaging is a technique to measure water molecule mobility in the tissue microscopic environment, and is sensitive to cell density and size, cell membrane permeability, and extracellular space tortuosity. Apparent diffusion coefficient (ADC) quantified from conventional DW images with b-values between 0 and 1000 s/mm² using a mono-exponential decay is a commonly reported parameter in literature. The correlation between high cellularity and low ADC in tumor animal models and human cancers motivates investigations of roles of ADC in clinical GBM (4-7). One limitation of DW imaging is that coexistence of edema in clinical GBM results in elevated ADC compared with normal white matter (WM) and gray matter (GM). To overcome this challenge, high b-value DW images and high-order diffusion MR methods have been well as edema that always coexists within GBM. Limitations of FLAIR images is in apparent diffusion coefficient (ADC), apparent restriction size of intracellular water (ARS), cell radius (r), cerebrospinal fluid (CSF), diffusion sensitive signals of intracellular water (S_in), diffusion sensitive signals of extracellular water (S_ex), diffusion-weighted (DW), extracellular diffusion coefficient (D_ex), fractional volume of intracellular water (V_in), fractional volume of water with the small diffusion coefficient (V_s), Gaussian Phase Distribution (GPD), glioblastoma (GBM), intracellular diffusion coefficient (D_in), microstructure model (MSM), mean squared errors (MSE), pulse gradient spin echo (PGSE), signal-to-noise ratio (SNR), tumor volume (TV), volumes of interest (VOIs), white matter (WM).
order diffusion models have been explored in clinical gliomas to differentiate tumor grade and assess therapy response [8–16].

Among the high-order diffusion models, a few models attempt to quantify microstructures in tumors [17, 18]. For example, a model, called VERDICT, has been proposed to quantify microstructural properties of colorectal cancer cell lines [19]. This model considers cell size, vascular volume fraction and associated pseudodiffusivity, and intracellular and extracellular fractional volumes and diffusivities. Owing to complicity of the VERDICT model, prior knowledge of intracellular and extracellular diffusion coefficients is used to fit in vivo DW images in 2 xenograft animal models. Another model, called temporal diffusion spectroscopy, uses oscillating diffusion gradients to probe cellular structures that restrict intracellular water diffusion by assuming that intracellular water is restricted in impermeable cells [20, 21]. Compared with VERDICT, this model simplifies the extracellular water diffusion to a single free diffusion term, and it fits 4 free parameters, including intracellular and extracellular water diffusivities.

Recent studies show that hypercellular tumor volumes (TVs) that can be detected on the DW images with b = 3000 mm²/s in GBM have a prognostic value [22, 23]. This technique used a widely available imaging technique and could be easily translated into a clinical trial. A phase II clinical trial targets this hypercellular TV with intensified radiation doses [24].

In this study, we modified the model described in Jiang et al.’s study [25, 26] and applied it to clinical DW images in the patients with GBM. In our clinic, bipolar pulse diffusion gradient waveforms and a high parallel imaging factor were used to reduce eddy current and geometric distortion in the clinical DW images, respectively. We applied the modified model to the DW images with high b-values to investigate whether there were any significant differences in the quantified microstructure parameters between the hypercellular tumors and normal tissue and edema in the patients with GBM. Similar comparisons were made for the conventional ADC and the parameters quantified from the bi-exponential model. This study was the first step to test the possibility of the application of the model quantifying the microstructure parameters using a widely available diffusion technique on the clinical scanner for GBM.

**MATERIALS AND METHODS**

**Microstructure Model with Bipolar Diffusion Gradients**

We assume a DW signal in tissue that can be considered as a sum of water signals from intracellular and extracellular compartments [21]:

\[
S = S_0 |V_{in} S_{in} + (1 - V_{in}) S_{ex}|
\]

where \(S_0\) is the total magnetization from both water compartments, \(V_{in}\) is the fraction volume of intracellular water, and \(S_{in}\) and \(S_{ex}\) are respective diffusion sensitive signals of intracellular and extracellular water. In the previous works of restricted intracellular diffusion [19, 27, 28], a cell has been modeled as an impermeable sphere, which completely restricted diffusion of intracellular water molecules within the spherical space. The analytical formulae of the restricted diffusion signals have been derived for the conventional monopolar diffusion pulse gradient spin echo (PGSE) and oscillating gradient spin echo sequences using the Gaussian phase distribution (GPD) approximation [20, 29–31]. It has shown that the GPD approximation of restricted diffusion for the conventional PGSE sequence has sufficient accuracy for most experimental conditions and for sphere and parallel-plane geometry assumptions [32]. Therefore, we adopt this formulation to express the restricted diffusion signal of the intracellular water as:

\[
S_{in} = \frac{\gamma^2}{2} \sum_n B_n \int_0^{2\tau} dt_1 g(t_1) \int_0^{2\tau} dt_2 g(t_2) \exp(-D_{in} \lambda_n |t_1 - t_2|) dt_2
\]

where \(\gamma\) is the gyromagnetic ratio of proton spin, \(g(t)\) is the gradient waveform, \(D_{in}\) is the intracellular diffusion coefficient, and \(\lambda_n\) and \(B_n\) are structure–dependent parameters. The analytical formula of \(\lambda_n\) and \(B_n\) for the spherical geometry [provided by a previous work [30]] depend upon the radius \(R\) of the sphere or cylinder and the \(n\)th root of a Bessel function of the first kind. The integral in equation [2] depends upon the specific gradient waveforms \(g(t)\) used in the diffusion pulse sequence. On the clinical scanner, the most commonly used gradient waveform is the conventional monopolar PGSE, and the oscillating diffusion gradient wave forms are not available. However, large eddy currents generated in the monopolar diffusion PGSE can produce artifacts on DW images.

To minimize eddy current–caused artifacts and improve quality of DW images, bipolar diffusion gradient pulse sequences have been introduced on clinical scanners [33]. There are a few variations in bipolar diffusion gradient waveforms that have been implemented on the clinical scanners by different vendors. Three common bipolar gradient waveforms, \(g(t)\), are illustrated in Figure 1. The first one is introduced by Fordham et al. [34], in which bipolar gradient pulses simply replace the monopolar gradients before and after the 180° radiofrequency (RF) pulse. The second one contains 4 diffusion gradient pulses that are placed before, between, and after 2 180° RF pulses. The 4 diffusion gradient pulse durations and time intervals can be tuned to minimize eddy current effects on DW images, resulting in asymmetric waveforms (Figure 1B). We derived \(S_{in}\) in equation [2] for 3 bipolar diffusion gradient waveforms shown in Figure 1 and provided them in the online supplemental Appendix.

Finally, the extracellular diffusion signal is formulated as follows [20]:

\[
S_{ex} = \exp(-b D_{ex})
\]

where \(D_{ex}\) is the extracellular water diffusion, and \(b\) is the b-value and depends upon the gradient waveform. For the 3 bipolar diffusion gradient waveforms, \(b\)-values are given in equations [A3], [A6], and [A9] in the online supplemental Appendix. Note that 4 free parameters \(R, D_{in}, D_{ex}\), and \(V_{in}\) can be estimated by fitting the microstructure model (MSM) to DW images. Here, \(R\) can be considered to be an apparent restriction size (ARS) of intracellular water. Also, whether \(D_{in}\) was sensitive to the cost function in fitting was investigated.

**Bi-Exponential Model**

The bi-exponential diffusion model, considered 2 free diffusion components, has been investigated to differentiate tumor from normal tissue and assess tumor response to therapy [35]. To
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study that has been approved by an institutional review board. All patients had MRI scans post surgery but before chemoradiation therapy.

In Vivo MR Imaging

All scans were performed on a 3.0 T scanner (Skyra, Siemens Healthineers, Erlangen, Germany) using a 20-channel head coil. Conventional MR images, 2D T2-FLAIR images, and 3D pre- and postcontrast T1-weighted images using a MPRAGE sequence were acquired. DW images were acquired by a spin-echo echo-planar pulse sequence with diffusion weighting in 3 orthogonal directions and 11 b-values from 0 to 2500 s/mm$^2$ with an incremental step of 250 s/mm$^2$. A bipolar diffusion gradient waveform (shown in Figure 1B) was used to reduce eddy currents and improve quality of DW images. In this sequence, there were 2 180° RF pulses and 4 diffusion gradient pulses. The four diffusion gradient pulses had durations of $\delta_1 = 9.94$ milliseconds, $\delta_2 = 15.14$ milliseconds, $\delta_3 = 19.8$ milliseconds, and $\delta_4 = 5.28$ milliseconds. The times intervals between the first and second, the first and third, and the first and fourth gradient pulses were $\Delta_1 = 20.84$ milliseconds, $\Delta_2 = 36.64$ milliseconds, and $\Delta_3 = 67.34$ milliseconds, respectively. Other acquisition parameters included the parallel imaging factor of 4 (GRAPPA) (to reduce echo spacing and hence geometric distortion), TE/TR = 93 / 9300 milliseconds, bandwidth of 1040 Hz/pixel, voxel size of $\sim 1.3 \times 1.3 \times 5.2$ mm, 30 slices to cover the whole brain, 1 average, and total scan time of 4.50 minutes. It has been shown that diffusion anisotropy is lost or reduced dramatically in T2 FLAIR abnormalities of GBM owing to tumor infiltration and edema (36). To test the loss or reduction of anisotropic diffusion in GBM, DW images were also acquired in 30 directions with $b = 1000$ s/mm$^2$ and 3 averages. To determine the hypercellular tumor in GBM, a diffusion image volume was acquired at $b = 3000$ s/mm$^2$ and 4 averages.

Definition of Volumes of Interest

First, we investigated whether microstructure and diffusion parameters in solid components of GBM, estimated by this model, were significantly different from ones in edema regions, normal gray matter (GM), and normal WM. Owing to anticipated low SNRs in DW images, we performed this test in several volumes of interest (VOIs). Previous studies of GBM using advanced imaging have shown that solid tumor components can be beyond the contrast-enhanced gross TVs (22, 37). Also, the T2 abnormality volume can consist of tumor, edema, and a mixture of the 2. However, at high b-values, water signals of edema are attenuated much faster than the hypercellular tumor. Based upon this hypothesis, in previous studies, a TV was created by combining automated thresholding on the DW images with $b = 3000$ s/mm$^2$, and then these were viewed and edited by a neuroradiologist with more than 10 years of clinical experience (22). The initial TV was created using a threshold of the mean intensity plus 2 standard deviations calculated from a volume of interest in the normal-appearing tissue most contralateral to the T2-abnormality and performed on the DW images with $b = 3000$ s/mm$^2$. Also, this TV has shown to be significantly correlated with progression-free survival (22). Therefore, we used this TV to characterize microstructure and diffusion parameters by the MSM in this study. Note that this TV is different from the contrast-enhanced TV (Figure 2).

compare the parameters estimated by the MSM, we implemented the bi-exponential model:

\[ S = S_1 \exp(-bD_1) + S_2 \exp(-bD_2) \]  \[ \text{[4]} \]

where $S_1$ and $S_2$ are respective amplitudes of apparent diffusion coefficients of $D_1$ and $D_2$. The fractional volume of water with the small diffusion coefficient is given by:

\[ V_s = S_1/(S_1 + S_2). \]  \[ \text{[5]} \]

Again, 4 free parameters ($D_1$, $D_2$, $S_1$, and $S_2$) are fitted from the bi-exponential model.

Conventional Mono-Exponential Model

Conventional ADC is usually fitted to DW images with $b = 0$ and $b = 1000$ s/mm$^2$ by a mono-exponential diffusion function as

\[ S = S_0 \exp(-bADC) \]  \[ \text{[6]} \]

Patients

Thirty patients (median age, 62 years; males, 19; females, 11) with histologically diagnosed new glioblastoma were included in this
To compare the behavior of this model in edema to solid tumour in GBM, an edema volume was created within the T2-abnormality but had at least 1 cm away from both the TV used in this study and the contrast-enhanced gross TV. In the cases with tumor recurrence, the edema volume was checked and ensured to have no spatial overlap with the recurrent TV. Also, the VOIs of 2 large WM fiber bundles were drawn: one in the frontal lobe and another in the Genu of corpus callosum. To compare to GM, cortex regions in the frontal and parietal lobes were segmented using the fuzzy c-means algorithm on DW images with $b = 0$ (T2-weighted images) and ADC maps. To avoid influence of cerebrospinal fluid (CSF), a deep GM structure, the head of caudate nucleus, was carefully chosen. As a total, 6 VOIs were created (Figure 2).

Before fitting the MSM, we investigated fractional anisotropy (FA) of diffusion in the defined hypercellular TV to determine whether we could fit the MSM using mean diffusivities in the TV. The averaged FA was $0.15 \pm 0.05$ in the TVs, which is consistent with previous reports (36), and $0.41 \pm 0.07$ in the frontal WM. Therefore, it is reasonable to fit the MSM to the mean diffusion signals in the TV using a sphere assumption and omitting anisotropic diffusion. In the WM, the cylinder-shape assumption was used, while the sphere shape was used in other tissue types.

Before the VOI creation, postcontrast T1-weighted images were reformatted into the axial plane with spatial resolution of $1 \times 1 \times 3$ mm. All other images acquired within the same session were reformatted to the postcontrast T1-weighted images using coordinates in the DICOM header.

**Computation of Diffusion Models**

Both the MSM and bi-exponential model were implemented using Matlab. The MSM was fitted to the DW images with 11 b-values using a *Simplex* algorithm in Matlab. We investigated the sensitivity of the objective function to the parameters of $R$, intracellular diffusion coefficient $D_{in}$, intracellular volume fraction $V_{in}$ and extracellular $D_{ex}$. If any parameter was not sensitive to the objective function, we would use a fixed value, which would reduce the number of the free parameters and improve the stability of fitting. *Simplex* was initiated randomly in the ranges of the parameters based on prior knowledge of the physiological parameters given in Table 1. Fitting was run multiple times, and the results were accepted with the minimum mean squared errors (MSE). Similarly, the bi-exponential model was fitted to the same DW images. ADC maps were calculated from DW images with $b = 0$ and 1000 s/mm$^2$ using in-house Functional Image Analysis Tools (*imFIAT*).

**Statistical Analysis**

To evaluate whether the parameters fitted from the MSM can differentiate tumor from other tissue types, Students’ $t$ test was used and a $P$-value of 0.05 was considered significant. Similar analysis was applied to the parameters obtained from the bi-exponential model and ADC.

**RESULTS**

**Parameter Characteristics from the MSM**

When investigating sensitivity of the objective function to the parameter variation, we found that $D_{in}$ had little sensitivity. To test the influence of $D_{in}$ variations on other parameters, we varied $D_{in}$ from 0.1 to 1.0 $\mu$m$^2$/ms. We found that the fitted $R$ in the TVs had no more than 1.5% differences, and $D_{ex}$ and $V_{in}$ did not show significant differences (Figure 3). Therefore, we fixed $D_{in}$ at 0.1 $\mu$m$^2$/ms and varied other 3 parameters in fitting the MSM.

The MSM was fitted to the DW data well in all VOIs, as example curves from TV, WM, GM and edema are shown in

**Table 1. Ranges of Initial Values of Three Parameters for Microstructure Model Fitting**

| Tissue Type     | $R$ ($\mu$m) | $D_{ex}$ ($\mu$m$^2$/ms) | $V_{in}$ |
|-----------------|--------------|--------------------------|---------|
| Tumor           | 5–25         | 1–3                      | 0–1     |
| Normal Tissue   | 0.5–1.5      | 1–3                      | 0–1     |
| Edema           | 0.5–1.5      | 1–3                      | 0–1     |
normal WM, and edema are summarized in Table 2 and Figure 5. For differentiating tumor from edema and normal tissue.

Three parameters, $R$, $D_{ex}$, and $V_{in}$, in the TV were significantly different from normal GM, normal WM, and edema. Specifically, the mean $R$ in the tumor was 28.1 ($\pm$0.48) μm and significantly greater than those in other tissue types (range, 1.1–2.3 μm, $P < .001$). In the latter group, edema had a significantly greater $R$ than normal GM and WM ($P < .001$), which could be because of the existence of a small amount of tumor cells in the edema VOI.

The mean fractional volume of intracellular water, $V_{in}$ in the tumor was 0.42 and significantly increased compared with all other tissue types ($P < .001$). Among normal tissue and edema, as anticipated, $V_{in}$ had the highest value in the large WM fiber bundles (0.21), intermediate value in the GM regions (0.16–0.13), and the lowest value in edema (0.10) that is consistent with the large amount of extracellular water. Most interestingly, the values of $R$ and $V_{in}$ in the tumor had absolutely no overlap with the ones in other tissue types, suggesting potential high sensitivity and specificity of $R$ and $V_{in}$ for differentiating tumor from edema and normal tissue.

The mean $D_{ex}$ in the tumor was 2.03 μm$^2$/ms and significantly greater than that in the normal tissue and in edema ($P < .01$), which is contributed possibly from edema, micronecro- sis, and perfusion mixed in the voxels of GBM. Normal WM and deep GM had $D_{ex}$ values of 1.15–1.31 μm$^2$/ms, which is consistent with other reports (38). Edema had $D_{ex}$ of 1.52 μm$^2$/ms, largely attributing to the great fractional volume of extracellular water. Cortex had $D_{ex}$ of 1.62 μm$^2$/ms, possibly resulting from partial volume average effects with CSF.

**Parameter Characteristics from the Bi-Exponential Model**

Characteristics of the 3 fitted parameters by the bi-exponential model are summarized in Table 3 and Figure 6. None of the 3 parameters in the tumor significantly and completely differed from the values in all other tissue types. $D_1$ (the large diffusion coefficient) in the tumor was $2.02 \pm 0.07 \mu m^2/ ms$, was not significantly different from edema ($1.89 \pm 0.06 \mu m^2/ ms$), but was significantly greater than normal WM and deep GM ($P < .05$). The cortex had the significantly elevated $D_1$ compared to tumor and other normal tissue ($P < .01$). $D_2$ in the tumor was $0.34 \pm 0.01 \mu m^2/ ms$, was not significantly different from edema ($0.33 \pm 0.04 \mu m^2/ ms$), deep GM, and cortex ($0.36 \pm 0.02 \mu m^2/ ms$), but was significantly greater than normal WM ($0.16–0.21 \mu m^2/ ms$, $P < .01$). $V_s$ in the tumor was 0.42, significantly greater than edema (0.29, $P < .05$) and genu (0.33, $P < .05$), and not significantly different from frontal WM and deep GM. Cortex had the highest values in $D_1$, $D_2$, and $V_s$ than tumor and normal WM and GM, possibly owing to the partial volume average with CSF and suggesting that the bi-exponential model is strongly influenced by fluid components.

To test whether combining all 3 parameters ($D_1$, $D_2$, and $V_s$) could differentiate tumor from all other tissue types, binary multivariate logistic regression was applied to the data. Backward rejection with $P > .1$ was used to eliminate the parameters in the logistic regression models. In the first model including the 3 parameters (Table 4), $D_1$ was significant ($P < .05$), but $V_s$ was not significant, and $D_2$ was marginally significant. However, $D_2$ had a large negative coefficient that could offset the $D_1$ contribution in the model. In the second model where $V_s$ was rejected, $D_1$ was
marginally significant, and $D_2$ was not significant. In the final model, $D_1$ was not significant after rejecting $D_2$.

Conventional ADC Model

There were no significant differences of ADC between tumor and any other nontumor tissue types ($P > 0.05$) (Figure 7). In general, ADC in the tumor was greater than WM and deep GM but lower than edema and cortex, consistent with other reports (7).

DISCUSSION

In this study, we modified the model of (20, 25, 26, 29–31) to fit the DW images acquired with a widely available bipolar pulse diffusion gradient imaging and characterize microstructure and diffusion properties of the hypercellular tumor in patients with GBM. We found that 3 parameters ($V_{in}$, $R$, and $D_{ex}$) in the tumor were substantially and significantly different from edema and normal tissue. The bi-exponential diffusion model that does not explicitly model the restricted diffusion of intracellular water could not robustly differentiate GBM from edema and normal brain tissue. ADC that ignores intravoxel heterogeneous diffusion in brain tissue and tumor failed to differentiate GBM from edema and normal tissue. The microstructure model has a great promise to aid in to conventional MRI for GBM diagnosis, image-guide therapy, and response assessment. Further validation with histopathology will warrant the role of the microstructure mode in the clinical management of GBM.

| Table 2. Characteristics of Three Parameters Fitted by the Microstructure Model |
|---------------------------------------------------------------|
| **R (um)** | **$D_{ex}/um^2/ms$** | **$V_{in}$** |
|----------|-----------------|-----------------|
| Tumor    | 28.1 ± 0.48     | 2.03 ± 0.07     | 0.42 ± 0.011 |
| Frontal White Matter  | 1.20 ± 0.01     | 1.15 ± 0.01     | 0.21 ± 0.004 |
| Genu      | 1.13 ± 0.01     | 1.31 ± 0.02     | 0.21 ± 0.005 |
| Deep Gray Matter | 1.19 ± 0.05     | 1.19 ± 0.04     | 0.16 ± 0.004 |
| Cortex    | 1.16 ± 0.02     | 1.62 ± 0.05     | 0.13 ± 0.002 |
| Edema     | 2.32 ± 0.07     | 1.52 ± 0.05     | 0.10 ± 0.007 |

* $R$ was fitted by using a cylinder shape assumption.
In the current study, the fractional volume of intracellular water, $V_{in}$, in GBM estimated by the microstructure model was found substantially different from edema and normal tissue. The $V_{in}$ in the TV had the largest value, which makes possible to differentiate the GBM from surrounding tissue. Also, GBM has enlarged cell size and nucleus, and increased cell density, which can increase $V_{in}$ measured in image voxels, and has micronecrosis and edema, which can reduce $V_{in}$ (39, 40). In the current study, the estimated $V_{in}$ in GBM by the microstructure model was 1.75-fold greater than that reported in a previous study that included primary and metastatic cancers and used the bi-exponential diffusion model (27). Historically, the bi-exponential diffusion model often results in an underestimated $V_{in}$, for which several possible causes have been discussed (38). The underestimation in $V_{in}$ can be caused by the transcytolic/membrane exchange that is omitted in the model as well as low SNR in DW images (41). Also, T2 differences between intracellular and extracellular water could affect the estimated $V_{in}$ values, which will be discussed further in the last paragraph. Diffusion gradient, b-value range, diffusion model, and subject age all have an influence on the estimated $V_{in}$ (4). As expected, the lowest $V_{in}$ value was found in edema, consistent with the notion of a large amount of extracellular water in the region. The $V_{in}$ values in 2 large WM fiber bundles and GM are between the solid GBM and edema, suggesting that the MSM has the potential to differentiate GBM from surrounding tissue. In this study, we evaluated anisotropic diffusion in the hypercellular TV and found low FA (0.15 as a mean value). Therefore, we did not consider anisotropic diffusion in the MSM. In the future study, anisotropic diffusion could be considered in the microstructure model.

Our microstructure model yielded the substantial greater $R$ in GBM than in normal GM, normal WM, and edema. $R$ could be considered as the apparent restriction size of intracellular water and a possible biomarker to differentiate GBM from normal tissue. The $R$ value should be considered as an average value over a distribution. Previous studies have shown the increased radius of tumor cells compared with that of normal tissue using the microstructure model or VERDICT model, and the reasonable correlation between the DW image–estimated cellularity and histopathologically determined cellularity (19, 21). A pathological

**Table 3. Characteristics of Three Parameters Fitted by the Bi-Exponential Model**

| Tissue Type       | $D_1$/um^2/ms (Mean ± SEM) | $D_2$/um^2/ms (Mean ± SEM) | $V_{in}$ (Mean ± SEM) |
|-------------------|-----------------------------|-----------------------------|------------------------|
| Tumor             | 2.02 ± 0.07                 | 0.34 ± 0.01                 | 0.42 ± 0.01            |
| Frontal White Matter | 1.44 ± 0.03               | 0.21 ± 0.02                 | 0.38 ± 0.01            |
| Genu              | 1.55 ± 0.04                 | 0.16 ± 0.02                 | 0.33 ± 0.02            |
| Deep Gray Matter  | 1.73 ± 0.07                 | 0.36 ± 0.02                 | 0.46 ± 0.02            |
| Cortex            | 2.78 ± 0.06                 | 0.55 ± 0.01                 | 0.51 ± 0.02            |
| Edema             | 1.89 ± 0.06                 | 0.33 ± 0.04                 | 0.29 ± 0.04            |
study in GBM shows that the radius of GBM cells can be as large as 20 μm with a mean of 10 μm and a standard deviation of 11 μm (42). The size of the apparent restriction space estimated in our model is larger than the reported cell size. Many factors can cause an overestimation in the restriction size of intracellular water in GBM. It is most likely that the highly permeable cell membrane cannot restrict intracellular water diffusion like a hard boundary. Therefore, the soft boundary increases the estimated radius so that we could call the estimated radius from our MSM as an effective radius. The low SNR in DW images could further cause an overestimate of R (43). Owing to the diffuse nature of GBM, diffused tumor cells can be found in the edema region (44, 45), which could be the cause of the slight but significant increase in the apparent restriction size in the edema region. Further studies could be carried out to investigate whether combining R and V_in in the image voxels with edema can provide information on the GBM cell infiltration and distribution.

In the current study, the value of D_ex in GBM by the microstructure model was found significantly greater than those in normal GM, normal WM and edema, which is similar to one by the bi-exponential diffusion model (Table 3), and also consistent with the values reported by Mulkern et al. (38). D_ex in GBM is affected by the large quantity of extracellular water owing to coexistence of edema, and to a small extent by micronecrosis and perfusion (40). To reduce the number of free parameters in the model, we did not account for the perfusion-caused pseudo diffusion in D_ex. However, we have tested the perfusion effect on D_ex in the TV VOIs in 30 patients using the approach in (46). We estimated that the pseudo diffusion coefficient was ~0.15 μm²/ ms, which was 13.5 times smaller than D_ex (2.03 μm²/ms) in the tumor VOIs. Thus, in the TV VOIs defined in our study, omitting the perfusion effect did not cause a substantial overestimate in D_ex. Note that the TV VOI used in our study is not the contrast-enhanced TV. We believe that the high D_ex in GBM could mainly be due to edema. The relative high values of D_ex in cortex and edema are likely because of the partial volume average of CSF in the cortex VOI and the large amount of extracellular water in the edema region, respectively.

As discussed in Introduction, modeling microstructure and diffusion properties in tumors has been attempted by different models (17, 18, 20, 21, 28). In general, these models fit more free

**Table 4. Multivariate Logistic Regression Using the Parameters from the Bi-Exponential Model**

| Model       | D1     | D2     | V_s   |
|-------------|--------|--------|-------|
| Coefficient | 1215.93| -5009.1| 3.55  |
| P Value     | 0.04   | 0.06   | 0.16  |

| Model       | D1     | D2     |
|-------------|--------|--------|
| Coefficient | 975.2  | -2327.3|
| P Value     | 0.075  | 0.19   |

| Model       | D1     |
|-------------|--------|
| Coefficient | 428.6  |
| P Value     | 0.23   |

**Figure 6.** Bar graphs of estimated parameters of D1 (top left panel), D2 (top right panel), and V_s (bottom left panel) in all tissue types using the bi-exponential diffusion model. * .01 < P < .05; ** .001 < P < .01; *** P < .001. Error bar is SEM.

**Figure 7.** Bar graph of apparent diffusion coefficients in all tissue types using the mono-exponential diffusion model. Error bar is SEM.
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