Dependence of temporal diffusion spectra on microstructural properties of biological tissues

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Abstract

The apparent diffusion coefficient (ADC) measured using magnetic resonance imaging methods provides information on microstructural properties of biological tissues, and thus has found applications as a useful biomarker for assessing changes such as those that occur in ischemic stroke and cancer. Conventional pulsed gradient spin echo methods are in widespread use and provide information on, for example, variations in cell density. The oscillating gradient spin echo (OGSE) method has the additional ability to probe diffusion behaviors more readily at short diffusion times, and the temporal diffusion spectrum obtained by the OGSE method provides a unique tool for characterizing tissues over different length scales, including structural features of intracellular spaces. It has previously been reported that several tissue properties can affect ADC measurements significantly, and the precise biophysical mechanisms that account for ADC changes in different situations are still unclear. Those factors may vary in importance depending on the time and length scale over which measurements are made. In the present work, a comprehensive numerical simulation is used to investigate the dependence of the temporal diffusion spectra measured by OGSE methods on different microstructural properties of biological tissues, including cell size, cell membrane permeability, intracellular volume fraction, intranucleus and intracytoplasm diffusion coefficients, nuclear size and T2 relaxation times. Some unique characteristics of the OGSE method at relatively high frequencies are revealed. The results presented in the paper offer a framework for better understanding possible causes of diffusion changes and may be useful to assist the interpretation of diffusion data from OGSE measurements.

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1. Introduction

The introduction of molecular diffusion explicitly has an influence on NMR signals originated in Hahn’s classical paper on spin echoes, in which he noticed that the amplitude of an observed spin echo signal could be reduced by the random thermal motion of spins in the presence of a magnetic field inhomogeneity [1]. Shortly thereafter, a number of reports appeared which described diffusion effects in NMR, for example, Refs. [2,3], and subsequently Stejskal and Tanner [4] developed the pulsed gradient spin echo (PGSE) method which made it possible to measure molecular diffusion coefficients directly and quantitatively. These works opened the window to more recent efforts to characterize biological tissues with measurements of water self-diffusion. Diffusion-weighted magnetic resonance imaging (DWI) has developed considerably since the 1980s and is able to detect pathophysiological changes in different tissues. For example, Moseley et al. [5] reported that diffusion-weighted magnetic resonance imaging (DWI) is highly sensitive to the changes occurring after an ischemic stroke, Zhong et al. [6] showed that diffusion changed as a result of electrical activity in the brain in seizures, Prichard et al. [7] demonstrated changes in diffusion after electroshock, while Zhao et al. [8] reported that the apparent
diffusion coefficient (ADC) of a tumor could be used as an indicator of tumor response to treatment. Chenevert et al. [9] and others have used ADC measurements to monitor the early response of brain tumors. Today, measurements of ADC are widely accepted as a useful biomarker in the diagnosis and monitoring of ischemic stroke and cancer.

Despite their considerable impact, conventional PGSE measurements are limited by some hardware constraints, such as the amplitude and slew rate of practical gradient systems as well as the relatively long durations of slice selective refocusing RF pulses. These limitations prevent the PGSE method from probing ultrashort diffusion times, making the PGSE method insensitive to ultrashort length scales. Therefore, the ADCs obtained by current PGSE methods represent averaged effects over multiple length scales. If restrictions to diffusion occur at long distances and diffusion times, these cannot be distinguished from effects that occur at shorter distances. Thus, when cell membranes are not perfectly permeable, ADC values are limited by and reflect cell density, and measurements of ADC are insensitive to variations of intracellular structure, for example, nuclear size [10]. To probe intracellular changes, much shorter diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the space diffusion times must be used so that water molecules move distances on average much less than a cell diameter. One approach to obtain short diffusion times is to use an oscillating gradient spin echo (OGSE) method [11,12], which replaces two bipolar gradients in the PGSE method with two cosine-modulated gradient waveforms. Short diffusion times can be obtained even at moderate frequencies, and the effective diffusion time is no longer the

experimental data using this model [13]. However, in our earlier studies, in order to highlight the diffusion effect, the \(T_2\) relaxation distribution was assumed uniform and other simplifications were made. We have previously shown the value of elaborate, realistic computer simulations for evaluating the influence of tissue properties on ADC values in PGSE methods including the influence of variations in \(T_2\) between compartments [19,20]. It has also been confirmed more recently that heterogeneous \(T_2\) relaxation values can affect ADC measurements [21,22]. Moreover, there are a large number of other tissue structural parameters that may also affect the ADC, including cell membrane permeability [20,23] and cell volume fraction [24]. We have therefore used computer simulations to investigate how apparent temporal diffusion spectra obtained by the OGSE method are dependent on such parameters. The results presented here offer a framework for better understanding the biophysical factors that affect diffusion measurements and may be useful to assist the interpretation of diffusion data from OGSE measurements.

2. Methods

2.1. Temporal diffusion spectroscopy

For the general OGSE method, it is difficult to obtain exact analytical relations between diffusion signals and specific microstructural parameters. A semianalytical approach can be used to predict the OGSE signals for systems with known diffusion propagators [25,26], but it cannot provide an explicit analytical expression that can be used for extracting parameters. An alternative approach was developed by Stepisnik, which forms the basis of temporal diffusion spectroscopy. He found that the ADC is equivalent to the spectral density of the ensemble-averaged velocity autocorrelation function [27], namely

\[
ADC(f) = \int_0^\infty \langle v(t)v(0) \rangle \exp(-i2\pi ft) dt
\]

where \(v\) is the velocity of diffusing molecules and \(f\) the frequency. By introducing a general expansion of the diffusion propagator [28], we have been able to derive analytical expressions for the ADC obtained using the OGSE method as

\[
ADC(f) = 8\pi^2 \sum_k \frac{B_k a_k^2 D^2 f^2}{\sigma (a_k^2 D^2 + 4\pi^2 f^2)^2} \times \left\{ \frac{\sigma^2 - \exp(-a_k D\sigma)}{2a_k D} + \exp\left(-\frac{1}{2} a_k D \cdot TE \right) \left( 1 - \cosh(a_k D\sigma) \right) \right\}
\]

where \(D\) is the intracellular diffusion coefficient, \(\sigma\) is the gradient duration, \(TE\) is the echo time and \(a_k, B_k\) are structure-dependent constants that can be found in Ref. [13] for some simple geometries, including parallel planes, cylinders, spheres and spherical shells.
2.2. Tissue model

Tissue was modeled as a collection of densely packed spheres (representing cells) on a face-centered cube (FCC). Each cell contains a concentric spherical nucleus (shown as black in Fig. 1). Hence, there are three distinct compartments in the model, the intranuclear, cytoplasmic and extracellular spaces. Each compartment in this model can be ascribed its own intrinsic parameters and is separated from the next compartment by either a semipermeable membrane or a freely permeable nuclear envelope. Although nuclear pores allow small molecules, for example, water, to diffuse across freely [29], the permeability of the nuclear envelope is still limited because of the nuclear pore density [30] and the effective pore diameter [31]. However, the permeability of the nuclear envelope used in the current work was assumed to be infinite in order to emphasize the effects of exchange between water molecules residing in the nucleus and those in cytoplasm.

The lack of available experimental data makes it difficult to more precisely model all parameters inside biological tissues. In the current study, the simulations were initially performed with the following previously published experimental parameters [10,24,32,33]: cell size=10 μm, $D_{\text{nuc}}=1.31 \mu m^2/\text{ms}$, $D_{\text{cyto}}=0.48 \mu m^2/\text{ms}$, $D_{\text{ex}}=1.82 \mu m^2/\text{ms}$, $T_{2,\text{nuc}}=63.29 \text{ms}$, $T_{2,\text{cyto}}=23.87 \text{ms}$, $T_{2,\text{ex}}=150 \text{ms}$, cell membrane permeability $P_{\text{m}}=0.024 \mu m/\text{ms}$ and nucleus to cell volume fraction N/C=34%. Moreover, all parameters are simulated with multiple values over broad ranges to fully understand the diffusion spectra dependence on microstructure (see below).

2.3. Computer simulations

Compared with Monte Carlo algorithms [20], the finite difference method [22,34,35] is more time efficient and suitable for large-scale computing. Here we adapted an improved finite difference method we have described previously to calculate the ADC. The method employs a revised periodic boundary condition that removes the computational edge artifact found using conventional finite difference methods. A parallel computing array was used to enhance the computing efficiency. Further details of the computational aspects of our method have been reported elsewhere [35].

ADCs were simulated for both OGSE and PGSE sequences. The OGSE pulse sequence was identical to the PGSE except for the substitution of two apodized cosine-modulated gradients in place of the two bipolar gradients. Details of the OGSE pulse sequence can be found in Ref. [15]. Both methods employed TE=40 ms and gradient durations $\sigma=20 \text{ ms}$ for direct comparison. The gradient waveform in the OGSE method has $1–20$ periods, corresponding to 50–1 kHz. All ADCs were calculated using two $b$ values, that is, with $b=0$ and $b=1 \mu m^2/\mu s$. The practical consideration of implementation of the OGSE method and its requirement of gradient amplitudes and slew rates can be found in previous publications [10,11,13,15]. The tissue was discretized to be a $30\times30\times30$ grid. The spatial sampling used in the simulation was $\Delta x=0.5 \mu m$ and the temporal increment was $\Delta t=1 \mu s$. It has been reported that computational errors may increase when the gradient amplitude becomes very large [35], and so the spatial and temporal increments were adjusted to finer resolutions in case of large gradient amplitudes to decrease computational errors at the cost of more computing time. All simulation parameters were tested with a pure water model and simulated ADCs had errors less than 1%.

All simulations were performed on the cluster of the Vanderbilt University Advanced Computing Center for Research and Education. The programs were written in C (GCC 4.1.2) with message passing interface (MPICH2) running on a 64-bit Linux operation system and Opteron processor (2.0 GHz) nodes with a Gigabit Ethernet network.

3. Results

3.1. Cell size

Fig. 2 shows how the apparent temporal diffusion spectra of water inside an impermeable ($P_m=0$) spherical cell change with different cell radii ($R=1$, 2, 5 and 10 μm) when the intracellular diffusion coefficient is constant ($D_m=2 \mu m^2/\text{ms}$). The data were calculated analytically by Eq. (2). The spectrum decreases rapidly close to zero frequency, but levels off at high frequencies. This can be understood because as the frequency goes to zero, the effective diffusion time is infinitely long, and the diffusion inside an impermeable system is completely restricted, so the ADC goes to zero. But at high frequencies (short diffusion times), the structure of the system does not affect the autocorrelation of molecular velocities, and the spectrum is flat and
approaches to a constant $D_{in}$. The diffusion spectrum disperses at different rates depending on the cell size: the spectrum disperses faster when the cell size is larger, reflecting the fact that the influence of restriction is strongly dependent on the compartment dimension relative to the frequency.

3.2. Intracellular diffusion coefficient

The intracellular diffusion coefficient has usually been modeled as homogeneous in previous simulations of diffusion in PGSE sequences [20,36,37]. However, the OGSE method with relatively high frequencies is sensitive to intracellular structures, such as nuclear size [10], so it is likely inappropriate to assume a homogeneous intracellular space. However, it is still worthwhile to study how the apparent temporal diffusion spectrum changes assuming an averaged intracellular diffusion coefficient in order to illustrate the sensitivity of the OGSE method to intracellular diffusion properties. Eq. (2) predicts that the apparent temporal diffusion spectrum of an impermeable spherical cell changes when the cell size is fixed (diameter = 10 μm) but the intracellular diffusion coefficient varies; the effects of setting $D_{in} = 0.5, 1, 2$ and 3 $\mu$m²/ms are shown in Fig. 3. The high frequency rates are different and approach the intrinsic intracellular diffusion coefficients, consistent with there being no restrictions. All the temporal diffusion spectra disperse in the same way (i.e., show the same slope of ADC vs. frequency) at low frequencies even with different intracellular diffusion coefficients. This implies that the shape of the autocorrelation function of the velocities of the diffusing spins is mostly determined by the dimensions of the restricting compartments at low frequencies, and the intracellular diffusion coefficient acts as an overall scaling factor.

3.3. Echo time

Fig. 4A–C shows how apparent temporal diffusion spectra change with different echo times (TE) and $T_2$ relaxation times. $T_2$ of extracellular space is assumed to be constant at 150 ms [22] for all simulations. Three different values of the intracellular $T_2$ relaxation times were considered, and the results at three typical gradient frequencies (100, 500 and 1000 Hz) are shown in Fig. 4D–E. When the $T_2$ distribution of the whole tissue is homogeneous, the ADC is independent of TE (see Fig. 4F). However, the ADCs obtained by the PGSE sequence are relatively independent (variation <2%) of TE for all $T_2$ combinations considered. This largely reflects the fact that the measurement of the ADC is dominated by the faster diffusing components, which here already have the higher $T_2$ values.

3.4. Intracellular volume fraction

Fig. 5 shows that apparent temporal diffusion spectra seem to shift up and down but keeps the same shape for different intracellular volume fractions ($f_i$). Different $f_i$ were obtained by adjustment of the cell spacing. Six values of $f_i$ were simulated, that is, 42.4%, 46.4%, 50.9%, 56.0%, 61.8% and 68.5%. The theoretical maximum $f_i$ for spheres on a FCC grid is 74.1%. Fig. 5A–C shows that all apparent temporal diffusion spectra are affected in the same way by $f_i$ even when the frequencies are different. Fig. 5D–F shows a similar dependence of the ADC obtained with the OGSE method despite different frequencies, but with a slightly slower slope compared with those by the PGSE method. Hence, both PGSE and OGSE diffusion methods show a similar dependence on intracellular volume fraction despite
different choices of diffusion times. The ADCs at all frequencies are strongly dependent on the volume fractions of the compartment with lower (restricted) diffusion.

3.5. Cell membrane permeability

Fig. 6 shows the effects of changing cell membrane permeability $P_m$. Five values of the membrane permeability were simulated ($P_m=0, 0.01, 0.024, 0.05$ and $0.1 \, \mu m/\text{ms}$). When $T_2$ relaxation is homogeneous, the apparent temporal diffusion spectrum is independent of the cell membrane permeability over this range of values. Even the highest value still introduces restriction effects at low frequencies ($<100 \, \text{Hz}$). Increasing the permeability is analogous to reducing the restriction boundary of intracellular water, so in

![Diagram](image)

Fig. 4. (A–C) The apparent temporal diffusion spectrum depends on TE and $T_2$. Four TEs are simulated, that is, 40, 60, 80 and 100 ms. (D–F) ADCs at three frequencies (100, 500 and 1000 Hz) were plotted against TE. For reference, ADCs obtained by the PGSE method were also provided.

![Diagram](image)

Fig. 5. (A–C) The apparent temporal diffusion spectrum depends on intracellular volume fraction ($f_i$). Altogether, six $f_i$'s were simulated, that is, 42.4%, 46.4%, 50.9%, 56.0%, 61.8% and 68.5%. (D–F) ADCs at some frequencies (100, 500 and 1000 Hz) were plotted against $f_i$. For reference, ADCs obtained by the PGSE method were also provided.
the regimen in which cell walls hinder free diffusion, an increase in $P_m$ will lead to some averaging of the behaviors of the compartments. In a regimen in which the diffusion time is already short, so that wall effects are less important, $P_m$ has less influence and close-to-intrinsic diffusion properties are probed (see Fig. 6C). However, when $T_2$ relaxation is heterogeneous, the contributions of the shorter $T_2$ components are reduced and the cell membrane permeability changes the ADC spectrum significantly by allowing mixing of different compartments. ADC decreases when $P_m$ increases because more molecules that are slowly diffusing inside cells migrate into the extracellular space (which has long $T_2$) and survive to contribute more signals at the echo time. The PGSE method shows a notable correlation with cell membrane permeability that varies according to the distribution of relaxation times, which is consistent with previous reports [20,22,38].

3.6. Nuclear-to-cell volume fraction

Apparent temporal diffusion spectra were simulated for different nuclear-to-cell volume fractions (N/C) and $T_2$ distributions, and the results are presented in Fig. 7A–C. Different N/Cs were obtained by swelling the nucleus while keeping the cell size constant. Six values of N/C were simulated, that is, 6.2%, 12.4%, 22.2%, 34.0%, 50.6% and 73.7%. ADCs at different frequencies respond differently to the variation of N/C, and ADCs at higher frequencies are more sensitive to N/C variations. Fig. 7D–F shows how ADCs at three frequencies (100, 500 and 1000 Hz) increase when N/C increases. A homogeneous distribution of $T_2$ values shows the maximum sensitivity (see Fig. 7F) for differentiating tissues with different N/Cs compared with those when the $T_2$ relaxation distribution is heterogeneous (Fig. 7D–E). The mixing of components with different $T_2$ relaxation times in the presence of permeable membranes alleviates the ADC differences between tissues with different N/C. In contrast, the PGSE method seems much less sensitive to N/C, and ADC increases <4% when N/C increases from 6.2% to 73.7% for both homogeneous intracellular $T_2$ distributions. An exception can be found in Fig. 7E in which ADCs by the PGSE method decrease ~7.9% when $T_{2,nuc}=63.27$ and $T_{2,cyto}=23.89$, although this ADC variation is less compared to that shown by the OGSE method, for example, ADC increases 13.9% when $f=100$ Hz. It is interesting that the ADCs measured by OGSE increase with N/C while those by the PGSE method behave in an opposite way. This phenomenon has been reported before [10].

3.7. Intranuclear diffusion coefficients

The apparent temporal diffusion spectrum was calculated for different values of the intranuclear diffusion coefficient ($D_{nuc}$) and $T_2$, and the results are shown in Fig. 8A–C. Four values of $D_{nuc}$ were used in the simulation, that is, 0.5, 1.0, 1.31 and 1.82 μm^2/ms. Similar to the response to N/C, ADCs obtained by the OGSE method at different frequencies respond differently to the variation of $D_{nuc}$, and ADCs at higher frequencies are more sensitive to $D_{nuc}$ variations. When $T_2$ is homogeneous, the ADC increases significantly (31.43%) with increasing $D_{nuc}$ from 0.5 to 1.82 μm^2/ms, while ADC increases 18.1% for $T_{2,nuc}=63.27$ ms and
Hence, the mixing between different $T_2$ components reduces the ability of the OGSE method to detect the variations of intranuclear diffusion coefficient. Although different $T_2$ relaxations may change the ADC values, the ADC obtained by the PGSE method is insensitive to $D_{\text{nuc}}$, which demonstrates again that measurements made using long diffusion times are insensitive to the properties of the intracellular space.

### 3.8. Intracytoplasm diffusion coefficients

The apparent temporal diffusion spectrum was also calculated for different values of the intracytoplasm diffusion
coefficient \((D_{\text{cyto}})\) and \(T_2\), and the results are shown in Fig. 9A–C. Four values of \(D_{\text{cyto}}\) were used in the simulation, that is, 0.5, 1.0, 1.31 and 1.82 \(\mu\text{m}^2/\text{ms}\). Similar to the effects of intranucleus diffusion coefficient, ADCs obtained by the OGSE method at different frequencies respond differently to the variations of \(D_{\text{cyto}}\), and ADCs at higher frequencies are more sensitive to \(D_{\text{cyto}}\) variations. Compared with the spectra obtained with different values of \(D_{\text{nuc}}\) (see Fig. 8), the ADC increases more significantly: 44.9% with increasing \(D_{\text{nuc}}\) from 0.5 to 1.82 \(\mu\text{m}^2/\text{ms}\) for homogenous \(T_2\), while ADC increases 27.6% for \(T_2,\text{nuc}=63.27\text{ ms}\) and \(T_2,\text{cyto}=23.89\text{ ms}\), and 23.9% for \(T_2,\text{nuc}=T_2,\text{cyto}=25\text{ ms}\). As expected, the measurements made using long diffusion times (PGSE) are insensitive to the variations of intracytoplasm diffusion coefficients, while the measurements with high frequencies (short diffusion times) show a significant sensitivity to such variations.

4. Discussion

Figs. 2–8 show how the basic features of apparent temporal diffusion spectra vary for different choices of parameters in diffusion-restricted/hindered systems, such as biological tissues. These spectra represent the Fourier components of the autocorrelation of molecular velocities. For free diffusion at body temperatures, water molecules collide and change direction on a very short time scale (\(\approx\)picoseconds), so the autocorrelation is essentially infinitesimally narrow, and the spectrum is flat out to a very high frequency, on order of the intermolecular collision frequency. However, when diffusion is restricted, the autocorrelation function broadens and acquires a negative dip, consistent with a picture in which barriers rectify isotropic diffusion and induce negative correlations between the velocities at different times [12]. Then, the diffusion spectrum is no longer flat but falls off at low frequencies. The manner in which \(D(f)\) disperses with frequency can provide unique information on the structure of the medium. When diffusion barriers are impermeable, the diffusion spectrum goes to zero as frequency goes to zero, corresponding to the tissue ADC that would be measured at long diffusion times. The region of maximal curvature of the temporal diffusion spectrum is expected to reflect the dominant scale over which restrictions occur. The diffusion spectrum is sensitive to cell size and intracellular diffusion coefficients in different ways in different frequency ranges: (1) when the frequency is low, the diffusion spectrum is mainly dependent on cell size, that is, restriction dimension; (2) when the frequency is intermediate, the diffusion spectrum disperses at a rate which is dependent on both cell size and intracellular diffusion coefficient; and (3) at high frequencies, the diffusion spectrum approaches to a constant, the intrinsic diffusion coefficient. In this context, “high” and “low” are determined by the relative sizes of the effective diffusion time (\(\approx\text{1/frequency}\)), the cell size and the value of diffusion coefficient. Different tissue microstructural parameters can be emphasized to different degrees using the diffusion spectrum by “tuning” frequencies corresponding...
to specific length scales, which is one of the potential advantages of the OGSE method.

Theory predicts that the diffusion spectrum will be dependent on both echo time TE and diffusion gradient duration [see Eq. (2)]. However, previous and the current work show that this dependence is subtle when $T_2$ relaxation is not considered. When a heterogeneous distribution of $T_2$ relaxation times is included, different TEs allow different levels of mixing between different $T_2$ compartments, and this may affect the apparent temporal diffusion spectrum slightly as shown in Fig. 4. However, in general, given the choices of diffusion rates and relaxation times considered here, neither PGSE nor OGSE diffusion measurements are very sensitive to echo time.

Both theoretical and experimental studies have suggested that the ADC obtained by the PGSE method has a strong inverse correlation with the intracellular volume fraction $f_i$ [24], as well as cell density [39]. In the current study, the apparent temporal diffusion spectrum shifts up or down with different choices of $f_i$ but keeps the same shape across different frequencies. For all $T_2$ combinations used in the simulation, $f_i$ seems to determine the “baseline” for the diffusion spectrum but not its detailed shape. Thus, the OGSE method shows the same strong dependence on $f_i$ and cell density as PGSE, but this acts as an overall scaling factor and does not interfere with inferences about, for example, cell sizes.

The cell membrane has been suggested as a dominant diffusion restriction factor for the PGSE measurements [40], and numerous theoretical [36,41–43] and computational [20,22,34] models have been developed to describe the effect of cell membrane permeability on diffusion measurements. However, all of these models are based on the PGSE method with relatively long diffusion times. For the OGSE method with relatively short diffusion times, the effect of mixing different diffusion components is small and shows a different behavior to that of the PGSE method. When water $T_2$ values are homogeneous within tissues, the effect of $P_m$ on the diffusion spectrum is negligible at relatively high frequencies, while ADCs obtained by the PGSE method, on the contrary, have a strong positive correlation with $P_m$ (see Fig. 4). This is because the OGSE method with relatively high frequencies probes short diffusion times and the fraction of spins exchanging between different diffusion compartments during such short diffusion recording times is negligible. However, when $T_2$ is heterogeneous, a $T_2$ filtering effect influences the diffusion measurements especially for the relatively long TE considered for the OGSE method, and then the OGSE method shows a strong dependence on $P_m$. This implies that although the cell membrane may not be a dominant diffusion restriction factor at relatively high frequencies, it may still affect OGSE measurements when significant $T_2$ differences are present between different diffusion compartments.

It has been reported before that the OGSE method at moderate to high frequencies has the ability to probe short length scales, and hence is sensitive to intracellular variations such as changes in nuclear size [10]. In the current work, the effects of nuclear size, intranuclear and intracytoplasm diffusion coefficients were studied. Nuclear size has been suggested as an important indicator of tumor grade and state [44], while dynamic variations inside the nucleus [45] during cell proliferation and apoptosis may cause significant variations of intranuclear diffusion coefficients. The intracytoplasm diffusion coefficients are usually reported to be low [33], but those low values might be only apparent rates due to restriction/hindrance effects of massive organelles. Hence, it is important to study the sensitivity of OGSE diffusion measurements to those parameters. In the current simulations, the nuclear envelope is assumed to be completely permeable despite limited nuclear pore density and effective pore diameter. Hence, in our simulation there is massive mixing of spins between the nucleus and cytoplasm during the diffusion measurements, and such an extreme assumption reduces the sensitivity of diffusion measurements to nuclear structures. Even so, the OGSE method still shows a significant sensitivity to both nuclear size and intranuclear diffusion coefficient despite different $T_2$ distributions, while the PGSE method shows only a slight dependence on nuclear size and, moreover, is insensitive to intranuclear and intracytoplasm diffusion coefficients. This is consistent with our experimental findings using synchronized HL-60 cells [18], in which intracellular structural variations during the cell division cycle were detected by the OGSE method, while the PGSE method did not detect any significant differences even with $b$ values up to 10,000 s/cm$^2$.

Transverse relaxation can play an important role and affect both PGSE and OGSE methods. Because published estimates of intracellular $T_2$ values vary widely, a large range of intracellular $T_2$ (25–150 ms) and both homogeneous and heterogeneous $T_2$ distributions were simulated in the current work. The results (see Fig. 6) show that, when $T_2$ is homogeneous, the OGSE method with relatively high frequencies gives the maximum sensitivity to intracellular structures, while it is insensitive to cell membrane permeability over the range considered. However, with an intermediate cell membrane permeability as occurs in biological tissues, mixing between different $T_2$ relaxation components affects the diffusion measurements and reduces the sensitivity of the OGSE method to intracellular scales. On the contrary, the PGSE method with a relatively long diffusion time shows a strong dependence on cell membrane permeability, as reported previously [38], but appears insensitive to intracellular structure variations even with a large range of $T_2$ relaxation values as simulated in the present study. This demonstrates again that the conventional PGSE method with relatively long diffusion times is not useful for probing intracellular structure, while the OGSE method can be “tuned” with proper choices of frequencies to detect changes at subcellular scales.
5. Conclusions

In the current work, the dependence of the apparent temporal diffusion spectrum obtained by the OGSE method on biological tissue microstructural properties was studied both theoretically and numerically. Compared with the conventional PGSE method, the OGSE method shows some unique features, such as sensitivity to intracellular structures while remaining insensitive to the effects of cell membrane permeability. The OGSE method also shows a strong correlation with the intracellular volume fraction, as has been found for the PGSE method. The results reported in the current paper may be helpful to elucidate the biophysical mechanisms underlying diffusion changes and may be useful to assist the interpretation of diffusion data from OGSE measurements.

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