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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 (MRI) methods provides information on micro-structural 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 (PGSE) 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 intra-cellular 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 micro-structural properties of biological tissues, including cell size, cell membrane permeability, intracellular volume fraction, intra-nucleus and intra-cytoplasm diffusion coefficients, nuclear size and T_2 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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Keywords

apparent diffusion coefficient; magnetic resonance imaging; oscillating gradient; diffusion time; simulation; temporal diffusion spectrum; microstructure

Introduction

The introduction of molecular diffusion explicitly as 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, e.g. [2,3], and subsequently Stejskal and Tanner developed the pulsed gradient spin echo (PGSE) method which made it possible to measure molecular diffusion coefficients directly and quantitatively [4]. These works opened the window to more recent efforts to characterize biological tissues with measurements of water self-diffusion. Diffusion-weighted MRI (DWI) has developed considerably since the 1980's and is able to detect pathophysiological changes in different tissues. For example, Moseley *et al.* reported that diffusion-weighted MRI (DWI) is highly sensitive to the changes occurring after an ischemic stroke [5], Zhong *et al.* showed that diffusion changed as a result of electrical activity in the brain in seizures [6], Prichard *et al.* demonstrated changes in diffusion after electroshock [7], while Zhao *et al.* reported that the apparent diffusion coefficient (ADC) of a tumor could be used as an indicator of tumor response to treatment [8]. Chenevert *et al.* and others have used ADC measurements to monitor the early response of brain tumors [9]. 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 ultra-short diffusion times, making the PGSE method insensitive to ultra-short 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, e.g. 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 between the gradient pulses but instead is related to the period of each gradient oscillation. Values of the ADC can be estimated at each of several discrete oscillation frequencies, producing a temporal diffusion spectrum, from which various structural parameters may be derived. In previous reports we and others have analyzed the theoretical basis of temporal diffusion spectra, have shown how such measurements are expected to behave in various simple geometries [13], and shown the use of this method for providing greater image contrast and detecting increased structural heterogeneity in tissues [14]. The OGSE method has been applied in different applications, including the characterization of porous media [11], ischemic rat brain [15], hyperpolarized gas diffusion [16], tumors [14], intracellular water in cultured cells [17] and detecting

intracellular structure variations in different cell cycle phases [18]. However, there are several independent tissue properties that may affect ADC measurements, and how temporal diffusion spectra depend on specific structural parameters is not always clear.

In previous reports, we have derived analytical expressions of diffusion signals obtained in OGSE measurements from some impermeable simple geometries [13]. Based on those analytical equations, it is possible to predict the apparent temporal diffusion spectra for different tissue micro-structural parameters, such as cell size and intra-cellular diffusion coefficient, and those parameters may be extracted from 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.

Methods

Temporal Diffusion Spectroscopy

For the general OGSE method it is difficult to obtain exact analytical relations between diffusion signals and specific micro-structural parameters. A semi-analytical 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 auto-correlation 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

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

where D is the intracellular diffusion coefficient, σ 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. It should be noted that both Eq.[1] and Eq.[2] are based on the assumption of a Gaussian

distribution to describe the phase variation within the ensemble, but for reasonable gradient strengths achievable in practice, this approximation is still valid for OGSE measurements [13].

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 intra-nuclear, 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 semi-permeable membrane or a freely-permeable nuclear envelope. Although nuclear pores allow small molecules, e.g. 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 , $D_{\text{nuc}}=1.31\mu\text{m}^2/\text{ms}$, $D_{\text{cyto}}=0.48\mu\text{m}^2/\text{ms}$, $D_{\text{ex}}=1.82\mu\text{m}^2/\text{ms}$, $T_{2,\text{nuc}}=63.29\text{ms}$, $T_{2,\text{cyto}}=23.87\text{ms}$, $T_{2,\text{ex}}=150\text{ms}$, cell membrane permeability $P_{\text{m}}=0.024\mu\text{m}/\text{ms}$ 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).

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 $TE=40\text{ms}$ and gradient durations $\sigma=20\text{ms}$ for direct comparison. The gradient waveform in the OGSE method has 1–20 periods, corresponding to 50–1kHz. All ADCs were calculated using two b values, i.e. with $b=0$ and $b=1\text{ms}/\mu\text{m}^2$. 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 $30\times 30\times 30$ grid. The spatial sampling used in the simulation was $\Delta x=0.5\mu\text{m}$ and the temporal increment was $\Delta t=1\mu\text{s}$. 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.

Results

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 $10\mu\text{m}$) when the intracellular diffusion coefficient is constant ($D_{in}=2\mu\text{m}^2/\text{ms}$). 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 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.

Intra-cellular Diffusion Coefficient

The intra-cellular 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= $10\mu\text{m}$) but the intracellular diffusion coefficient varies; the effects of setting $D_{in}=0.5, 1, 2$ and $3\mu\text{m}^2/\text{ms}$ 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 auto-correlation 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.

Echo Time

Fig. 4(a–c) show how apparent temporal diffusion spectra change with different echo times (TE) and T_2 relaxation times. T_2 of extra-cellular space is assumed to be constant at 150ms [22] for all simulations. Three different values of the intra-cellular T_2 relaxation times were considered, and the results at three typical gradient frequencies (100, 500 and 1000Hz) are shown in Fig. 4(d–e). When the T_2 distribution of the whole tissue is homogeneous, the ADC is independent of TE (see Fig. 4f). When the T_2 distribution is heterogeneous, the ADCs obtained by the OGSE method increase slightly ($\sim 3\%$) when TE increases from 40ms to 100ms (Fig. 4(d–e)). 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.

Intra-cellular volume fraction

Fig. 5 shows that apparent temporal diffusion spectra seem to shift up and down but keeps the same shape for different intra-cellular volume fractions (f_i). Different f_i were obtained by adjustment of the cell spacing. Six values of f_i were simulated, i.e. 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. 5(a–c) show that all apparent temporal diffusion spectra are affected in the same way by f_i even when the frequencies are different. Fig. 5(d–f) show 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 intra-cellular 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.

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 $0.1 \mu\text{m/ms}$). 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 the regime 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 regime 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 extra-cellular 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].

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. 7(a–c). Different N/Cs were obtained by swelling the nucleus while keeping the cell size constant. Six values of N/C were simulated: i.e. 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. Figs.7(d–f) show how ADCs at three frequencies (100, 500 and 1000Hz) increase when N/C increases. A homogeneous distribution of T_2 values shows the maximum sensitivity (see Fig. 7(f)) for differentiating tissues with different N/Cs compared with those when the T_2 relaxation distribution is heterogeneous (Figs.7(d–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 intra-cellular T_2 distributions. An exception can be found in Fig. 7e in which ADCs by the PGSE method decreases $\sim 7.9\%$ when $T_{2, \text{nuc}}=63.27$ and $T_{2, \text{cyto}}=23.89$, although this ADC variation is less compared to that shown by the OGSE method, e.g. ADCs increases 13.9% when $f=100\text{Hz}$. 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 phenomena has been reported before [10].

Intra-nuclear diffusion coefficients

The apparent temporal diffusion spectrum was calculated for different values of the intra-nucleus diffusion coefficient (D_{nuc}) and T_2 and the results are shown in Fig. 8(a–c). Four values of D_{nuc} were used in the simulation, i.e. 0.5, 1.0, 1.31 and 1.82 $\mu\text{m}^2/\text{ms}$. 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 $\mu\text{m}^2/\text{ms}$, while ADC increases 18.1% for $T_{2, \text{nuc}}=63.27\text{ms}$ and $T_{2, \text{cyto}}=23.89\text{ms}$, and 14.1% for $T_{2, \text{nuc}}=T_{2, \text{cyto}}=25\text{ms}$. Hence, the mixing between different T_2 components reduces the ability of the OGSE method to detect the variations of intra-nuclear diffusion coefficient. Although different T_2 relaxations may change the ADC values, the ADC obtained by the PGSE method is insensitive to D_{nuc} , which demonstrates again that measurements made using long diffusion times are insensitive to the properties of the intracellular space.

Intra-cytoplasm diffusion coefficients

The apparent temporal diffusion spectrum was also calculated for different values of the intra-cytoplasm diffusion coefficient (D_{cyto}) and T_2 and the results are shown in Fig. 9(a–c). Four values of D_{cyto} were used in the simulation, i.e. 0.5, 1.0, 1.31 and 1.82 $\mu\text{m}^2/\text{ms}$. Similar to the effects of intra-nucleus diffusion coefficient, ADCs obtained by the OGSE method at different frequencies respond differently to the variations of D_{cyto} , and ADCs at higher frequencies are more sensitive to D_{cyto} variations. Compared with the spectra obtained with different values of D_{nuc} (see Fig. 8), the ADC increases more significantly: 44.9% with increasing D_{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 intra-cytoplasm diffusion coefficients, while the measurements with high frequencies (short diffusion times) show a significant sensitivity to such variations.

Discussion

Figures 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 ($\approx < \text{picoseconds}$), so the autocorrelation is essentially infinitesimally narrow, and the spectrum is flat out to a very high frequency, on order of the inter-molecular 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 case in which spins are completely restricted. When diffusion barriers are permeable, the diffusion spectrum tends to a non-zero value of ADC at $f=0$, 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 intra-cellular diffusion coefficients in different ways in different frequency ranges: 1) when the frequency is low, the diffusion spectrum is mainly dependent on cell size, i.e.

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 ($\approx 1/\text{frequency}$), the cell size and the value of diffusion coefficient. Different tissue micro-structural 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 intra-cellular 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 e.g. 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. 6). 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, intra-nuclear and intra-cytoplasm 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 intra-nucleus diffusion coefficients. The intra-cytoplasm 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 intra-nuclear diffusion coefficient despite different T_2 distributions, while the PGSE method shows only a slight dependence on nuclear size and, moreover, is insensitive to intra-nucleus and intra-cytoplasm diffusion coefficients. This is consistent with our experimental findings using synchronized HL-60 cells [18], in which intra-cellular 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 $10,000 \text{ s/cm}^2$.

Transverse relaxation can play an important role and affect both PGSE and OGSE methods. Because published estimates of intra-cellular T_2 values vary widely, a large range of intra-cellular T_2 (25ms – 150ms) 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 intra-cellular 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 intra-cellular 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 intra-cellular 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 intra-cellular structure, while the OGSE method can be “tuned” with proper choices of frequencies to detect changes at sub-cellular scales.

Conclusions

In the current work, the dependence of the apparent temporal diffusion spectrum obtained by the OGSE method on biological tissue micro-structural properties was studied both theoretically and numerically. Compared with the conventional PGSE method, the OGSE method shows some unique features, such as sensitivity to intra-cellular structures while remaining insensitive to the effects of cell membrane permeability. The OGSE method also shows a strong correlation with the intra-cellular 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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References

1. Hahn EL. Spin Echoes. *Phys Rev* 1950;80:580–94.
2. Carr HY, Purcell EM. Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Phys Rev* 1954;94:630–38.
3. Torrey HC. Bloch equations with diffusion terms. *Phys Rev* 1956;104:563–65.
4. Stejskal EO, Tanner JE. Spin diffusion measurements - spin echoes in presence of a time-dependent field gradient. *J Chem Phys* 1965;42:288–92.
5. Moseley ME, Kucharczyk J, Mintorovitch J, Cohen Y, Kurhanewicz J, Derugin N, Asgari H, Norman D. Diffusion-weighted MR imaging of acute stroke: correlation with T2-weighted and magnetic susceptibility-enhanced MR imaging in cats. *AJNR Am J Neuroradiol* 1990;11:423–9. [PubMed: 2161612]
6. Zhong J, Petroff OAC, Prichard JW, Gore JC. Changes in water diffusion and relaxation properties of rat cerebrum during status epilepticus. *Magn Reson Med* 1993;30:241–46. [PubMed: 8366805]
7. Prichard JW, Zhong J, Petroff OA, Gore JC. Diffusion-weighted NMR imaging changes caused by electrical activation of the brain. *NMR Biomed* 1995;8:359–64. [PubMed: 8739272]
8. Zhao M, Pipe JG, Bonnett J, Evelhoch JL. Early detection of treatment response by diffusion-weighted 1H-NMR spectroscopy in a murine tumour in vivo. *Br J Cancer* 1996;73:61–4. [PubMed: 8554985]
9. Chenevert TL, McKeever PE, Ross BD. Monitoring early response of experimental brain tumors to therapy using diffusion magnetic resonance imaging. *Clin Cancer Res* 1997;3:1457–66. [PubMed: 9815831]
10. Xu J, Does MD, Gore JC. Sensitivity of MR diffusion measurements to variations in intracellular structure: Effects of nuclear size. *Magn Reson Med* 2009;61:828–33. [PubMed: 19205020]
11. Schachter M, Does MD, Anderson AW, Gore JC. Measurements of restricted diffusion using an oscillating gradient spin-echo sequence. *J Magn Reson* 2000;147:232–37. [PubMed: 11097814]
12. Parsons EC Jr, Does MD, Gore JC. Temporal diffusion spectroscopy: theory and implementation in restricted systems using oscillating gradients. *Magn Reson Med* 2006;55:75–84. [PubMed: 16342147]
13. Xu J, Does MD, Gore JC. Quantitative characterization of tissue microstructure with temporal diffusion spectroscopy. *J Magn Reson* 2009;200:189–97. [PubMed: 19616979]
14. Colvin DC, Yankeeelov TE, Does MD, Yue Z, Quarles C, Gore JC. New insights into tumor microstructure using temporal diffusion spectroscopy. *Cancer Res* 2008;68:5941–7. [PubMed: 18632649]
15. Does MD, Parsons EC, Gore JC. Oscillating gradient measurements of water diffusion in normal and globally ischemic rat brain. *Magn Reson Med* 2003;49:206–15. [PubMed: 12541239]
16. Carl M, Wilson Miller G, Mugler JP 3rd, Rohrbaugh S, Tobias WA, Cates GD Jr. Measurement of hyperpolarized gas diffusion at very short time scales. *J Magn Reson* 2007;189:228–40. [PubMed: 17936048]
17. Harkins, KD.; Galons, J-P.; Divijak, JL.; Trouard, TP. Experimentally measured intracellular water at very short diffusion times. the 17th Annual Meeting of ISMRM; Honolulu, Hawaii, USA. 2009. p. 449
18. Xu J, Xie J, Jourquin J, Colvin DC, Does MD, Quaranta V, Gore JC. The influence of cell cycle phase on ADC in synchronized cells detected using temporal diffusion spectroscopy. (in revision).
19. Szafer A, Zhong J, Anderson AW, Gore JC. Diffusion-weighted imaging in tissues: theoretical models. *NMR Biomed* 1995;8:289–96. [PubMed: 8739267]
20. Szafer A, Zhong J, Gore JC. Theoretical model for water diffusion in tissues. *Magn Reson Med* 1995;33:697–712. [PubMed: 7596275]
21. Vestergaard-Poulsen P, Hansen B, Ostergaard L, Jakobsen R. Microstructural changes in ischemic cortical gray matter predicted by a model of diffusion-weighted MRI. *J Magn Reson Imaging* 2007;26:529–40. [PubMed: 17685422]
22. Harkins KD, Galons JP, Secomb TW, Trouard TP. Assessment of the effects of cellular tissue properties on ADC measurements by numerical simulation of water diffusion. *Magn Reson Med* 2009;62:1414–22. [PubMed: 19785014]

23. Karger J, Pfeifer H, Heink W. Principles and application of self-diffusion measurement by nuclear magnetic resonance. *Adv Magn Reson* 1988;12:2–89.
24. Anderson AW, Xie J, Pizzonia J, Bronen RA, Spencer DD, Gore JC. Effects of cell volume fraction changes on apparent diffusion in human cells. *Magnetic Resonance Imaging* 2000;18:689–95. [PubMed: 10930778]
25. Callaghan PT. A simple matrix formalism for spin echo analysis of restricted diffusion under generalized gradient waveforms. *J Magn Reson* 1997;129:74–84. [PubMed: 9405218]
26. Sukstanskii AL, Yablonskiy DA. Effects of restricted diffusion on MR signal formation. *Journal of Magnetic Resonance* 2002;157:92–105. [PubMed: 12202137]
27. Stepisnik J. Analysis of Nmr self-diffusion measurements by a density-matrix calculation. *Physica B* 1981;104:350–64.
28. Callaghan PT. Pulsed-gradient spin-echo NMR for planar, cylindrical, and spherical pores under conditions of wall relaxation. *J Magn Reson A* 1995;113:53–59.
29. Maul GG, Deaven L. Quantitative determination of nuclear pore complexes in cycling cells with differing DNA content. *J Cell Biol* 1977;73:748–60. [PubMed: 406262]
30. Talcott B, Moore MS. Getting across the nuclear pore complex. *Trends Cell Biol* 1999;9:312–8. [PubMed: 10407410]
31. Ribbeck K, Gorlich D. Kinetic analysis of translocation through nuclear pore complexes. *EMBO J* 2001;20:1320–30. [PubMed: 11250898]
32. Bowtell RW, Peters A, Sharp JC, Mansfield P, Hsu EW, Aiken N, Horsman A, Blackband SJ. NMR microscopy of single neurons using spin echo and line narrowed 2DFT imaging. *Magn Reson Med* 1995;33:790–4. [PubMed: 7651115]
33. Grant SC, Buckley DL, Gibbs S, Webb AG, Blackband SJ. MR microscopy of multicomponent diffusion in single neurons. *Magn Reson Med* 2001;46:1107–12. [PubMed: 11746576]
34. Hwang SN, Chin CL, Wehrli FW, Hackney DB. An image-based finite difference model for simulating restricted diffusion. *Magn Reson Med* 2003;50:373–82. [PubMed: 12876714]
35. Xu J, Does MD, Gore JC. Numerical study of water diffusion in biological tissues using an improved finite difference method. *Phys Med Biol* 2007;52:N111–26. [PubMed: 17374905]
36. Latour LL, Svoboda K, Mitra PP, Sotak CH. Time-dependent diffusion of water in a biological model system. *Proc Natl Acad Sci U S A* 1994;91:1229–33. [PubMed: 8108392]
37. Staniszc GJ, Szafer A, Wright GA, Henkelman RM. An analytical model of restricted diffusion in bovine optic nerve. *Magn Reson Med* 1997;37:103–11. [PubMed: 8978638]
38. Pfeuffer J, Flogel U, Dreher W, Leibfritz D. Restricted diffusion and exchange of intracellular water: theoretical modelling and diffusion time dependence of ¹H NMR measurements on perfused glial cells. *NMR Biomed* 1998;11:19–31. [PubMed: 9608585]
39. Lyng H, Haraldseth O, Rofstad EK. Measurement of cell density and necrotic fraction in human melanoma xenografts by diffusion weighted magnetic resonance imaging. *Magn Reson Med* 2000;43:828–36. [PubMed: 10861877]
40. Beaulieu C, Allen PS. Determinants of anisotropic water diffusion in nerves. *Magn Reson Med* 1994;31:394–400. [PubMed: 8208115]
41. Meier C, Dreher W, Leibfritz D. Diffusion in compartmental systems. I. A comparison of an analytical model with simulations. *Magn Reson Med* 2003;50:500–9. [PubMed: 12939757]
42. Price WS, Barzykin AV, Hayamizu K, Tachiya M. A model for diffusive transport through a spherical interface probed by pulsed-field gradient NMR. *Biophys J* 1998;74:2259–71. [PubMed: 9591653]
43. Sukstanskii AL, Yablonskiy DA, Ackerman JJ. Effects of permeable boundaries on the diffusion-attenuated MR signal: insights from a one-dimensional model. *J Magn Reson* 2004;170:56–66. [PubMed: 15324758]
44. Hsu CY, Kurman RJ, Vang R, Wang TL, Baak J, Shih Ie M. Nuclear size distinguishes low- from high-grade ovarian serous carcinoma and predicts outcome. *Hum Pathol* 2005;36:1049–54. [PubMed: 16226103]
45. Zink D, Fischer AH, Nickerson JA. Nuclear structure in cancer cells. *Nat Rev Cancer* 2004;4:677–87. [PubMed: 15343274]

Fig. 1.

A packed-sphere tissue model used in the simulation. All spheres are placed on a face-centered-cube (FCC) grid and each sphere represents a cell containing a concentric spherical nucleus.

Fig. 2.
The analytically calculated apparent temporal diffusion spectra inside an impermeable spherical cell change with different cell sizes.

Fig. 3.

The analytically calculated apparent temporal diffusion spectra inside an impermeable spherical cell asymptote but not shape with different intracellular diffusion coefficients. The intracellular space is assumed homogeneous.

Fig. 4.

(a–c) The apparent temporal diffusion spectrum depends on TE and T_2 . Four TEs are simulated, i.e. 40, 60, 80 and 100ms. (d–f) ADCs at three frequencies (100, 500 and 1000Hz) were plotted against TE. For reference, ADCs obtained by the PGSE method were also provided.

Fig. 5.

(a–c) The apparent temporal diffusion spectrum depends on intra-cellular volume fraction (f_i). Altogether six f_i 's were simulated, i.e. 42.4%, 46.4%, 50.9%, 56.0%, 61.8% and 68.5%. (d–f) ADCs at some frequencies (100, 500 and 1000Hz) were plotted against f_i . For reference, ADCs obtained by the PGSE method were also provided.

Fig. 6. (a–c) The apparent temporal diffusion spectrum depends on cell membrane permeability (P_m) and T_2 . Five permeabilities were simulated $P_m=0, 0.01, 0.024, 0.05$ and $0.1 \mu\text{m}/\text{ms}$. (d–f) ADCs at some frequencies (100, 500 and 1000Hz) were plotted against P_m . For comparison, ADCs obtained by the PGSE method were also provided.

Fig. 7. (a–c) Apparent temporal diffusion spectra depend on nucleus-to-cell volume fraction (N/C) and T_2 . Six values of N/C were simulated: i.e. 6.2%, 12.4%, 22.2%, 34.0%, 50.6% and 73.7%. (d–f) ADCs at some frequencies (100, 500 and 1000Hz) were plotted against N/C. For comparison, ADCs obtained by the PGSE method were also provided.

Fig. 8.

(a–c) The apparent temporal diffusion spectrum depends on intra-nucleus diffusion coefficient (D_{nuc}) and T_2 . Four D_{nuc} were used in the simulation, i.e. 0.5, 1.0, 1.31 and 1.82 $\mu\text{m}^2/\text{ms}$. (d–f) ADCs at some frequencies (100, 500 and 1000 Hz) were plotted against D_{nuc} . For comparison, ADCs obtained by the PGSE method were also provided.

Fig. 9.

(a-c) The apparent temporal diffusion spectrum depends on intra-cytoplasm diffusion coefficient (D_{cyto}) and T_2 . Four D_{cyto} were used in the simulation, i.e. 0.5, 1.0, 1.31 and 1.82 $\mu\text{m}^2/\text{ms}$. (d-f) ADCs at some frequencies (100, 500 and 1000Hz) were plotted against D_{nuc} . For comparison, ADCs obtained by the PGSE method were also provided.