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Structural Information Revealed by the Dispersion of ADC with Frequency

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Abstract

Diffusion MRI provides a non-invasive means to characterize tissue microstructure at varying length scales. Temporal diffusion spectra reveal how the apparent diffusion coefficient (ADC) varies with frequency. When measured using oscillating gradient spin echo sequences, the manner in which ADC disperses with gradient frequency (which is related to the reciprocal of diffusion time) provides information on the characteristic dimensions of restricting structures within the medium. For example, the dispersion of ADC with oscillating gradient frequency (f ADC) has been shown to correlate with axon sizes in white matter and provide novel tissue contrast in images of mouse hippocampus and cerebellum. However, despite increasing interest in applying frequency-dependent ADC to derive novel information on tissue, the interpretations of ADC spectra are not always clear. In this study, the relation between ADC spectra and restricting dimensions are further elucidated and used to derive novel image contrast related to the sizes of intrinsic microstructures.

Keywords

diffusion; microstructure; diffusion time; oscillating frequency; oscillating gradient; ADC

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Introduction

Diffusion MRI provides a non-invasive means to characterize tissue microstructure, and has been widely used to detect stroke and monitor tumor response to therapy [1–4]. The apparent diffusion coefficients (ADCs) measured at different diffusion times are believed to reflect the structural hindrances and restrictions to free water movement at varying length scales [5]. Conventional pulsed gradient spin echo (PGSE) measurements of ADC in biological tissues usually involve relatively long diffusion times (20–40 ms), so the corresponding one-dimensional root mean square displacements (RMSD) of diffusion molecules are on the order of 10 μm . The ADC measured with PGSE is thus observed to correlate with cellularity in several types of tumors [2, 6, 7].

Oscillating gradient spin echo (OGSE) methods have been used to achieve much shorter effective diffusion times and hence they are able to differentiate smaller structures with higher sensitivity [8, 9]. For example, the measured ADCs at high oscillating frequencies have been shown to convey microstructural variations at sub-cellular scales [10], which may help detect earlier tumor response to treatment before changes in tissue cellularity [11–14]. Moreover, by varying the oscillating frequencies, an apparent diffusion spectrum can be obtained [9]. The manner in which ADC disperses with oscillating frequency provides information on tissue structure over a range of intrinsic length scales and in general may reflect several tissue properties, but some simple features of such spectra have proven empirically useful [5]. For example, the initial rate of change of ADC with frequency at low frequencies has been shown to correlate with axon sizes in white matter [15] and provide novel tissue contrast in images of mouse hippocampus and cerebellum [16–18]. However, despite increasing interest in applying frequency-dependent ADC to derive novel information on tissue [19–22], the interpretations of ADC spectra are not always clear.

Restricted diffusion with generalized time-varying diffusion gradient waveforms has been studied previously [23, 24]. Specifically for water diffusion inside simple geometries using cosine-modulated gradient waveforms, analytical equations describing ADC as a function of frequency have been derived and validated [24–26]. In this study, the theory of water diffusion inside an impermeable cylinder or sphere is re-examined with emphasis on the rate of frequency-dependent changes in ADC at relatively low frequencies. From this, a simple relation between the rate and restricting size can be derived for limited ranges of parameters. Simulations and experiments illustrate this relation. This study may help better understand the information revealed by the behavior of ADC with frequency and suggests a novel type of parametric image that depicts structural dimensions.

Methods

Theory

For an OGSE sequence with a pair of cosine-modulated gradients on either side of a refocusing pulse, the diffusion weighting b-value is:

$$b = \frac{\gamma^2 G^2 \delta}{4\pi^2 f^2} \quad (1)$$

Here γ is the nuclear gyromagnetic ratio, G the maximum gradient amplitude, δ the gradient duration, and f the diffusion gradient oscillation frequency. Based on the equivalence of b -values between OGSE and PGSE sequences [8], the effective diffusion time (Δ_{eff}) for a cosine-modulated OGSE sequence is:

$$\Delta_{\text{eff}} = \frac{1}{4f} \quad (2)$$

Using a Gaussian phase approximation [27, 28], which is accurate enough for most practical applications using OGSE acquisitions [25], the measured ADC for diffusion inside impermeable cylinders can be expressed as

$$\text{ADC}(f, D, R) = 8\pi^2 \sum_k \frac{B_k \lambda_k^2 D^2 f^2}{\delta(\lambda_k^2 D^2 + 4\pi^2 f^2)^2} \times \left\{ \frac{\delta(\lambda_k^2 D^2 + 4\pi^2 f^2)}{2\lambda_k D} - 1 + \exp(-\lambda_k D \delta) + \exp(-\lambda_k D \Delta)(1 - \cosh(\lambda_k D \delta)) \right\} \quad (3)$$

where D is the free diffusion coefficient, R is the cylinder radius, δ is the gradient separation, B_k and λ_k are radius-dependent coefficients [24, 29].

$$B_k = \frac{2(R/\mu_k)^2}{\mu_k^2 - 1} \quad \text{and} \quad \lambda_k = \left(\frac{\mu_k}{R}\right)^2 \quad (4)$$

where μ_k is the k^{th} root of $J_1'(\mu) = 0$ and J_1 is a Bessel function of the first kind. Note that similar expressions describe diffusion inside spheres [24]. The ADC depends strongly on the ratio of the time required to diffuse across the space compared to the period of the gradient. In general we can identify three distinct regimes:

when $f \gg \frac{D}{R^2}$ there is very little dependence on the radius,

when $f \ll \frac{D}{R^2}$ the ADC should vary approximately as R^4 .

The intermediate regime occurs when $f \sim \frac{D}{R^2}$. Note that for tissues if $D \approx 1 \mu\text{m}^2/\text{ms}$ then this regime spans the range of frequencies readily accessible for imaging for structures of a few microns in size. The rate of frequency-dependent changes in ADC ($f_{\text{ADC}} = \text{ADC}/f$) can be readily obtained numerically, and will be shown to first increase and then decrease with frequency. If we define f_0 as the inflection frequency at which f_{ADC} reaches its maximum, the corresponding RMSD can be calculated as

$$\text{RMSD}(f_0) = \sqrt{2D\Delta_{\text{eff}}} = \sqrt{\frac{D}{2f_0}} \quad (5)$$

Using the theoretical expressions, the behavior of $f\text{ADC}$ with frequency, radius and D were evaluated.

Simulation

In biological tissues, $f\text{ADC}$ may be affected by multiple factors, including cell size, cell membrane permeability, intrinsic diffusion coefficient, the presence of organelles and intracellular water fraction. Moreover, because of limitations on gradient hardware and T_2 relaxation, previous experimental studies have usually acquired data over only a narrow range of frequencies (typically < 200 Hz) [12, 13, 18, 20]. Computer simulations were performed to study the effects of cylinder radius (R), intracellular intrinsic diffusion coefficient (D_{in}), and intracellular water fraction (f_{in}) on the measured $f\text{ADC}$. The simulations used an improved finite difference method [30, 31] and tissues were modeled as impermeable cylinders regularly and closely packed on a hexagonal lattice. Initial parameters were: $D_{\text{in}} = 1 \mu\text{m}^2/\text{ms}$, extracellular intrinsic diffusion coefficient $D_{\text{ex}} = 2 \mu\text{m}^2/\text{ms}$, $R = 3 \mu\text{m}$, and $f_{\text{in}} = 76\%$. R , D_{in} , and f_{in} were then varied individually to study their effects on ADC measurements. Following previous experimental parameters [13, 16, 18], the values of ADC were simulated at PGSE($\delta/\tau = 3/50 \text{ ms}$), OGSE(50 Hz), and OGSE(100 Hz). $f\text{ADC}$ at $f = 75 \text{ Hz}$ was approximated as:

$$\Delta_f \text{ADC}(75 \text{ Hz}) \approx \frac{\text{ADC}(100 \text{ Hz}) - \text{ADC}(50 \text{ Hz})}{100 \text{ Hz} - 50 \text{ Hz}} \quad (6)$$

In vitro cell experiments

Murine erythroleukemia (MEL) and human promyelocytic leukemia K562 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 $\mu\text{g}/\text{ml}$ streptomycin (Invitrogen, CA) under standard culture conditions in a humidified incubator maintained at 5% CO_2 and 37 °C. Once enough cells were cultured, they were collected and washed with phosphate-buffered saline (PBS), and then fixed with 4% paraformaldehyde in PBS for over 2 hours. After fixation, cells were centrifuged at 200 g for 2 minutes in a 0.65ml Eppendorf tube to form a pellet, and all the liquid on the top was then carefully removed. There were four samples of each cell type. Using light microscope images, cell radii were estimated to be 5.75 ± 0.51 and $9.49 \pm 0.57 \mu\text{m}$ for MEL and K562 cells, respectively.

NMR diffusion measurements were performed on an Agilent/Varian 7T MRI system with a 12 mm Doty micro-gradient coil. An apodised cosine-modulated OGSE sequence [8] with a gradient duration of 25 ms was used to acquire temporal ADC spectra from 40 Hz to 360 Hz. An additional PGSE-based ADC was also measured with $\delta/\tau = 4/52 \text{ ms}$. For each diffusion frequency/time, the ADC was calculated from four b-values evenly distributed from 0 to $0.75 \text{ ms}/\mu\text{m}^2$. Other parameters were: TR = 5 s, NEX (number of excitations) = 1,

receiver bandwidth = 50 kHz, spectral resolution = 390.625 Hz, TE = 60 ms. The sample temperature was maintained at $\sim 17^\circ\text{C}$ using a cooling water circulation system.

Ex vivo animal experiments

Brain, kidney, and liver samples were freshly excised from six female Athymic nude mice (28–35 g) after intravascular perfusion and euthanasia. Tissue samples were stored in 10% formalin for more than ten days. The samples were then washed in phosphate-buffered saline solution for one week prior to MRI study to wash out fixatives and restore T_2 . For each MRI scan, one sample was put inside a 10-mm NMR tube and immersed in fomblin to reduce susceptibility effects and prevent dehydration.

MR images were acquired on the same scanner as used for the in vitro cell experiments. All images were acquired using a 2-shot echo-planar imaging sequence. For each sample, PGSE data were acquired with $\delta/\gamma = 3/50$ ms, while OGSE data were acquired with six oscillating frequencies evenly distributed from 50 to 300 Hz with a gradient duration of 20 ms. Other parameters were: TR = 5000 ms, TE = 67 ms, NEX = 8, 10 diffusion directions, b-value = $0.8\text{ ms}/\mu\text{m}^2$, FOV = 10×10 mm, slice thickness = 1 mm, matrix size = 64×64 , zero-filled to 128×128 . The maximum gradient strength used in this study was 1.42 T/m. ADC and $f\text{ADC}(75\text{ Hz})$ values were calculated in a pixel-by-pixel manner, and further analyses were performed on regions of interest (ROIs) which were manually selected in brain cortex, kidney cortex, and liver.

Results

Fig 1 shows the numerically calculated values of ADC, $f\text{ADC}$ and $\text{RMSD}(f_0)$ based on Eqs. (3–5) for water diffusion within impermeable cylinders at two typical values of D . It is evident that $f\text{ADC}$ is not a monotonic function of f . $f\text{ADC}$ increases with increasing f at low frequencies but drops after reaching a maximum at $f = f_0$. Fig 1(b) and 1(f) suggest that f_0 shifts to higher frequency as the free diffusion coefficient D increases. This is consistent with Eq.(3) which predicts, under some simplifying assumptions, that f_0 , the inflection point

of the spectrum, occurs at a value proportional to $\frac{D}{R^2}$. The RMSD at f_0 ($\text{RMSD}(f_0)$) is thus found to vary linearly with the cylinder radius. If the ADC spectra were acquired and f_0 were identified, the cylinder radius R could be directly estimated as suggested by Fig 1(c) and 1(g). However, in practice due to gradient hardware and relaxation limitations, only a narrow range of ADC spectra may be obtained. Fig 1(d) and 1(h) show the changes of $f\text{ADC}$ with R at three different oscillating frequencies ($f = 25, 50$ and 100 Hz). The corresponding effective diffusion times are 10, 5 and 2.5 ms respectively, and the vertical dashed lines represent the corresponding RMSDs. Though ADC increases with increasing R at a fixed oscillating frequency as shown in Fig 1(a) and 1(e), $f\text{ADC}$ is not a monotonic function of R , and reaches its maximum when the cylinder radius R is close to the corresponding RMSD. The range of sizes over which there is a monotonic increase in $f\text{ADC}$ with R increases with increasing D and diffusion times.

Fig 2 shows the numerically calculated values of ADC, $f\text{ADC}$ and $\text{RMSD}(f_0)$ for water diffusion within impermeable spheres. Consistent with the 2D case shown in Fig 1,

$\text{RMSD}(f_0)$ is a linear function of the sphere radius R , but the coefficient is a little different. Similarly, $f\text{ADC}$ is not a monotonic function of R , and becomes larger when the sphere radius R is close to the corresponding RMSD.

Fig 3 shows the simulated effects of R , D_{in} and f_{in} on ADC and $f\text{ADC}(75 \text{ Hz})$ in the 2D case. As shown in Fig 3(a), ADC increases with increasing R , but the rate of increase is diffusion time/frequency dependent. Consistent with Figs 1(d), $f\text{ADC}(75 \text{ Hz})$ reaches its maximum when R is close to the RMSD at $D_{\text{in}} = 1 \mu\text{m}^2/\text{ms}$ and $f = 75 \text{ Hz}$. Fig 3(d) shows that $f\text{ADC}(75 \text{ Hz})$ obtains its maximum at $D_{\text{in}} = 2.25 \mu\text{m}^2/\text{ms}$, the corresponding RMSD is 1.3 times larger than the cylinder radius ($R = 3 \mu\text{m}$). The changes of $f\text{ADC}(75 \text{ Hz})$ with D_{in} are in good agreement with the results in Fig 1(b)(c)(f) and (g). Whether D_{in} or f is varied, $f\text{ADC}$ reaches its maximum when the RMSD is about 1.3 times larger than R (Fig 1(c)).

For a two pool model in which the extracellular intrinsic diffusion rate is set to be higher than the intracellular rate, it is not surprising that $f\text{ADC}(75 \text{ Hz})$ depends on the intracellular fractional volume and the average ADC decreases. In our simulation, $f\text{ADC}(75 \text{ Hz})$ increases by about 25% when f_{in} increases 36% to 76%. It should be noted that $f\text{ADC}(75 \text{ Hz})$ may decrease with increasing f_{in} in some cases (data not shown).

The measured ADC spectra for MEL and K562 cells are shown in Fig 4(a). Fig 4(b) shows the comparisons of ADC and $f\text{ADC}$ between those two cell types. $f\text{ADC}$ at 60 Hz was interpolated from two ADC values at 40 and 80 Hz. Though the K562 cell radius ($R \sim 10 \mu\text{m}$) is about twice as large as that of MEL cells ($R \sim 5 \mu\text{m}$), ADC(PGSE) is unable to distinguish them from each other. However, at shorter diffusion times, the ADC values of K562 cells are significantly higher than that of MEL cells ($p < 0.005$), which is in agreement with their size difference. At 17°C , the intracellular intrinsic diffusion coefficient D_{in} is estimated to be about $1 \mu\text{m}^2/\text{ms}$ [32]. Consistent with Fig 1(a), the ADC spectrum of K562 cells increases faster at relatively low frequencies ($< 40 \text{ Hz}$) than that of the MEL cells. However, at a higher frequency ($f = 60 \text{ Hz}$), the RMSD is only $2.9 \mu\text{m}$ and smaller than the cell radii. Then, as predicted, $f\text{ADC}(60 \text{ Hz})$ for K562 cells ($R \sim 10 \mu\text{m}$) is smaller than that of MEL cells ($R \sim 5 \mu\text{m}$). Figs 1(a) and (b) show that $f\text{ADC}$ changes dramatically with frequency in the range from 0 to 50 Hz for larger objects ($R > 5 \mu\text{m}$) and caution needs be displayed in the interpretation of $f\text{ADC}$ values obtained from sparsely sampled spectra.

Fig 5 shows the measured ADC(PGSE) and $f\text{ADC}(75 \text{ Hz})$ maps, and ADC spectra, for brain, kidney, and liver tissues. Consistent with previous reports [16–18], $f\text{ADC}(75 \text{ Hz})$ highlights the granular cell layer of the dentate gyrus (GrDG) in the brain hippocampus. Different from the ADC(PGSE) map, $f\text{ADC}(75 \text{ Hz})$ shows almost no contrast for the kidney cortex and medulla, which suggests that the restricting compartments differ in cell density or other ways but may share similar restricting sizes. Fig 6 shows the ROI-based ADC(PGSE) and $f\text{ADC}(75 \text{ Hz})$ values. Both ADC(PGSE) and $f\text{ADC}(75 \text{ Hz})$ differ between tissues. Typical cell sizes for brain, kidney, and liver are usually 1–3 [33, 34], 10–20 [35, 36], and 15–30 μm [37], respectively, and cell density is expected to vary inversely with size in close packed tissues. In the regime of frequencies and sizes examined the changes of $f\text{ADC}(75 \text{ Hz})$ seen are in agreement with those in Fig 1(d), so a large value of

$f_{\text{ADC}}(75 \text{ Hz})$ does not always mean a large restricting size but depends on other experimental variables.

Discussion

This study re-examined restricted diffusion within an impermeable cylinder or sphere under cosine-modulated OGSE sequences. Our emphasis was to interpret the dispersion rate of ADC with oscillating frequency at relatively low frequencies. Whereas ADC spectra increase continuously with frequency, they also show inflections so that f_{ADC} is not a monotonic function of frequency but reaches a maximum when the second derivative is zero. This occurs at a frequency that in practice is proportional to R^2 . The RSMD value at the point of inflection is itself an indicator of compartment size and varies in direct proportion to R . Measurements of a sufficient range of frequencies around the inflection allow derivation of cell sizes.

At long diffusion times, water in tissues whose cells have non-zero permeability may have encountered restricting barriers or exchanged between the intra- and extracellular spaces many times. The measured ADC then may be relatively insensitive to the restricting size and is mainly determined by cell density, packing and permeability [38, 39]. At extremely short diffusion times, only a small fraction of water may experience the restrictions or hindrances introduced by barriers. The measured ADC then is close to the free diffusion coefficient, which also cannot reflect information on the restricting size. However, when the distance moved in the diffusion time is comparable with the restricting size, the measured ADC varies strongly with the dimensions between the restricting barriers [5]. The frequency at which f_{ADC} reaches its maximum is then an essential feature of ADC spectra that correlates with intrinsic tissue structure. It should be noted that for in vivo imaging it is usually not practical to sample the whole ADC or f_{ADC} spectra because of T_2 limitations on signal.

The relation between f_{ADC} and R derived from the theory can be used to explain previous reports [16–18]. Typical sizes for cells in the cerebellar granule layer (CBGr), the granule layer of the dentate gyrus (GrDG), and hippocampal pyramidal layer (Py) are 5–10 [40], 10–18 [41], and 20 μm [18, 42], respectively. Using the relation between f_{ADC} and R described above, it is not surprising to observe that CBGr had a larger $f_{\text{ADC}}(75 \text{ Hz})$ than GrDG, which in turn had a larger $f_{\text{ADC}}(75 \text{ Hz})$ than Py [18]. Another previous study observed that the values of $f_{\text{ADC}}(75 \text{ Hz})$ decreased in CBGr but increased in brain cortex after mouse death [16]. Though the values of $f_{\text{ADC}}(75 \text{ Hz})$ changed in opposite directions in those regions, both of them were consistent with increased cell size [33, 43] and decreased diffusion coefficient [44–46] after death. CBGr has a larger radius than the RMSD, so the value of $f_{\text{ADC}}(75 \text{ Hz})$ would decrease once cell radius increases. However, the neuronal radii in brain cortex are relatively smaller than the RMSD, so the increased neuronal radii would be more close to the RMSD after death.

This study suggests that measuring ADC spectra at multiple diffusion frequencies/times around f_0 is key to obtaining structural dimensions from diffusion measurements. Axon sizes in the central nervous system may be as small as 2 μm , such as those in the spinal cord white

matter tracts and corpus callosum [47]. OGSE measurements with higher frequencies (> 200 Hz) would be more sensitive to those small axons than conventional PGSE-based measurements [48]. However, for cell sizes in the range of 5 to 20 μm or larger, measurements at much lower frequencies are more suitable. The relative performance of different sequences may be readily interpreted in terms of their temporal spectral representations.

Conclusion

In this study, the theory of water diffusion within an impermeable cylinder was re-examined to derive the relation between f ADC and restricting size. The results indicate that f ADC is not a monotonic function of the oscillating frequency and the inflection frequency f_0 is in theory an indicator of compartment size. Besides, f ADC passes through a maximum when the restricting radius R is close to the corresponding RMSD. The change of f ADC is a reliable indicator of the change of restricting size. However, single values of f ADC should be interpreted with caution, though for low frequencies and larger structures f ADC increases with size. This study may help better understand the information revealed by the behavior of ADC with frequency.

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Fig 1.

The changes of ADC, f_{ADC} and $RMSD(f_0)$ with $D = 1 \mu\text{m}^2/\text{ms}$ (a,b,c,d) and $D = 3 \mu\text{m}^2/\text{ms}$ (e,f,g,h) for 2D water diffusion within impermeable cylinders. (a) and (e) show the ADC spectra with R ranging from 1 to 10 μm . The corresponding f_{ADC} spectra are shown in (b) and (f). f_0 is the frequency at which f_{ADC} reaches its maximum. (c) and (g) show the linear fitting between $RMSD(f_0)$ and the cylinder radius R , fitted parameters are also provided. The changes of f_{ADC} with R at frequencies 25, 50 and 100 Hz are shown in (d) and (h), the vertical dashed lines represent the corresponding values of RMSD.

Fig 2.

The changes of ADC, f_{ADC} and $RMSD(f_0)$ with $D = 1 \mu\text{m}^2/\text{ms}$ (a,b,c,d) and $D = 3 \mu\text{m}^2/\text{ms}$ (e,f,g,h) for 3D water diffusion within impermeable spheres. (a) and (e) show the ADC spectra with R ranging from 1 to 10 μm . The corresponding f_{ADC} spectra are shown in (b) and (f). f_0 is the frequency at which f_{ADC} reaches its maximum. (c) and (g) show the linear fitting between $RMSD(f_0)$ and the cylinder radius R , fitted parameters are also provided. The changes of f_{ADC} with R at frequencies 25, 50 and 100 Hz are shown in (d) and (h), the vertical dashed lines represent the corresponding values of $RMSD$.

Fig 3.

the simulated changes of ADC and $f\text{ADC}(75 \text{ Hz})$ as a function of R (a,b), D_{in} (c,d), and f_{in} (e,f). The effects of R and D_{in} are in good agreement with the theoretical predictions.

Fig 4. the measured ADC spectra for MEL and K562 cells (a). Comparisons of ADC(PGSE), ADC(40 Hz), and $\Delta\text{ADC}(60 \text{ Hz})$ for those two cell types (b). The error-bars indicate the inter-subject standard deviations. Statistical significance was evaluated using the paired-sample t -test (* $p < 0.005$).

Fig 5. ADC(PGSE) maps, f ADC(75 Hz) maps, and ADC spectra for the brain, kidney, and liver. ROIs are shown on the ADC(PGSE) maps. The error-bars represent the standard deviations for six samples.

Fig 6.
Comparisons of ADC(PGSE), and $\Delta_f\text{ADC}(75\text{ Hz})$ for brain, kidney, and liver tissues.