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Fast and robust measurement of microstructural dimensions using temporal diffusion spectroscopy

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Abstract

Mapping axon sizes non-invasively is of interest for neuroscientists and may have significant clinical potential because nerve conduction velocity is directly dependent on axon size. Current approaches to measuring axon sizes using diffusion-weighted MRI, e.g. *q*-space imaging with pulsed gradient spin echo (PGSE) sequences usually require long scan times and high *q*-values to detect small axons (diameter <2 μm). The oscillating gradient spin echo (OGSE) method has been shown to be able to achieve very short diffusion times and hence may be able to detect smaller axons with high sensitivity. In the current study, OGSE experiments were performed to measure the inner diameters of hollow microcapillaries with a range of sizes (~ 1.5 – $19.3 \mu\text{m}$) that mimic axons in the human central nervous system. The results suggest that OGSE measurements, even with only moderately high frequencies, are highly sensitive to compartment sizes, and a minimum of two ADC values with different frequencies may be sufficient to extract the microcapillary size accurately. This suggests that the OGSE method may serve as a fast and robust measurement method for mapping axon sizes non-invasively.

Keywords

Temporal diffusion spectroscopy; OGSE; Axon size; Oscillating gradient; Microcapillary

1. Introduction

Mapping the sizes of nerve axons may have significant clinical potential because nerve conduction velocity is directly dependent on axon size [1–3]. Diffusion MRI provides a non-invasive means to characterize the microstructure of biological tissues, and hence may be

suitable for this application. Several approaches have previously been proposed to measure axon sizes using diffusion weighted MRI [3–12]. For example, in q -space imaging, data are acquired with a conventional pulsed gradient spin echo (PGSE) sequence, and the signal attenuation ($E(q)$) is plotted as a function of the wave-vector q (where $q = (2\pi)^{-1} \gamma g \delta$, γ being the nuclear magnetogyric ratio, g and δ are the pulse gradient strength and duration, respectively). Diffusion–diffraction phenomena occur in restricted systems with mono-disperse structure, and by measuring $E(q)$ the compartment size can be extracted [4]. However, such diffusion–diffraction phenomena are usually not resolved in biological systems due to the size poly-dispersity [13]. In such cases, the full-width at half-maximum (FWHM) of the Fourier transform of $E(q)$ may be used to characterize the mean compartment size [5,6]. Another approach using q -space imaging is the AxCaliber framework [3], which acquires multi-dimensional MRI data (q -values and diffusion times). The compartment size distribution is then estimated by modeling the intracellular diffusion using analytical expressions that are based either on the short gradient approximation [14] or on the constant diffusion gradient [15,16]. In addition, a method called the angular double-PGSE has been proposed to measure small compartmental dimensions with relatively low q -values [7,8]. This method usually keeps the q -value fixed but varies the orientation of the second gradient pair. The compartment dimension can be extracted from the angular dependent signal decay. However, the PGSE-based measurements usually encounter long diffusion times (>10 ms) in practice due to gradient hardware limitations, so that the root mean square displacements (RMSD) of diffusing molecules (>6 μm) are much larger than small axon sizes (e.g. diameter $d < 2$ μm). This in turn significantly reduces the sensitivity for detecting effects at small spatial scales. To enhance sensitivity to small scales, much higher q -values and/or more measurements with different degrees of diffusion weighting are usually necessary for PGSE measurements which in turn lead to long scan times.

The oscillating gradient spin echo (OGSE) method has been shown to be able to achieve much shorter diffusion times and hence may be able to detect smaller axons with high sensitivity [17]. Moreover, the OGSE method can probe a range of effective diffusion times by tuning the oscillating gradient frequencies, and thus an apparent diffusion spectrum can be obtained which contains more comprehensive micro-structural information on biological tissues [18]. For example, the OGSE method has previously been used to probe various length scales in biological systems, such as cultured cells [19], healthy rat brain [20], human brain in vivo [21,22], rat and mouse tumors [23–25], and brains after stroke [26]. The sensitivity of the OGSE method to intracellular structure has also been investigated [27,28], and it has been shown that unique microstructure-based contrasts can be obtained which are not available from conventional PGSE experiments [29,30]. Before studying quantitatively the micro-structural properties of biological tissues, it is important to derive and validate the analytical models of OGSE as they may be used for model fitting, parameter estimation, and to optimize experiments [31]. To this end, analytical expressions that predict OGSE signals in some typical structures have been derived and validated with computer simulations [32], which suggests the possibility of extracting small axon sizes using the OGSE method. However, these analytical expressions have not previously been validated or calibrated experimentally using well-characterized physical phantoms with known pore sizes.

In the current study, OGSE experiments were performed to measure the inner diameters of hollow microcapillaries with a range of sizes (~ 1.5 – $19.3 \mu\text{m}$) that mimic axons in the human central nervous system [33]. The fitted sizes were compared with the known values to validate the accuracy of fitting cylinder diameters using the OGSE method. In addition, the limitation of fitting free diffusion coefficients using the OGSE method was investigated. From this, a possible fast method of mapping axon size non-invasively is proposed.

2. Methods

2.1. Theory

The cosine-modulated OGSE waveform was chosen in the current study due to its specific diffusion spectral selectivity [34]. Hence, the effective diffusion gradient is:

$$g(t) = \begin{cases} G \cos[2\pi f t] & 0 < t < \sigma \\ -G \cos[2\pi f(t - \Delta)] & \Delta < t < (\Delta + \sigma) \\ 0 & \text{else} \end{cases} \quad (1)$$

Here G is the gradient amplitude, f the diffusion gradient frequency, σ the gradient duration, the separation of two diffusion gradients. The corresponding b -value is [26]:

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

Under the Gaussian phase approximation [35–38], the restricted diffusion signal attenuation can be described as [39]

$$E = \exp \left(-\frac{\gamma^2}{2} \sum_k B_k \int_0^{2\tau} dt_1 \int_0^{2\tau} dt_2 e^{-D\lambda_k |t_2 - t_1|} g(t_1) g(t_2) \right) \quad (3)$$

where 2τ is the echo time, and B_k and λ_k are structure dependent coefficients. The analytical expressions of B_k and λ_k for some typical geometries such as parallel planes, cylinders, spheres, and spherical shells can be found in the literature [32,39]. In the case of diffusion inside an impermeable cylinder with a diameter d ,

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

where μ_k is the k th root of $J_1'(\mu) = 0$ and J_1 is a Bessel function of the first kind. By substituting Eqs. (1) and (2) into Eq. (3) and assuming the diffusion gradient is perpendicular to the main axis of the cylinder, the measured ADC can be expressed as

$$\text{ADC}(f, D, d) = 8\pi^2 \sum_k \frac{B_k \lambda_k^2 D^2 f^2}{\sigma(\lambda_k^2 D^2 + 4\pi^2 f^2)^2} \times \left\{ \frac{(\lambda_k^2 D^2 + 4\pi^2 f^2) \sigma}{2\lambda_k D} - 1 + \exp(-\lambda_k D \sigma) + \exp(-\lambda_k D \tau) (1 - \cosh(\lambda_k D \sigma)) \right\} \quad (5)$$

where D is the free diffusion coefficient. Eq. (5) shows that ADC has a strong dependence on the gradient oscillating frequency f . If ADC measurements are performed at multiple gradient frequencies, the free diffusion coefficient D and the cylinder diameter d may be extracted simultaneously. This is a much different approach compared with previously reported PGSE-based models.

2.2. Experiment

All NMR diffusion experiments were performed on a Varian 7T scanner with a 12 mm Doty micro-gradient coil. The maximum gradient strength used in this study was 1.88 T/m. Five different types of hollow microcapillaries (Polymicro Technologies, USA) with different inner diameters (1.5–1.6, 4.3–4.5, 9.5–9.7, 14.9–15, 19.0–19.3 μm (lower bound–upper bound provided by the manufacturer)) and the same outer diameter around 150 μm were used as the phantoms. The microcapillaries were cut to ~ 3.5 cm in length and then immersed in distilled water for approximately two weeks. The microcapillaries were then air-dried to remove any residual water outside but retain the water inside the microcapillaries. By this means, a well-characterized one-compartment diffusion system was formed. Each type of microcapillary was packed into 5 mm NMR tubes and aligned parallel to the z -direction of the magnet. The diffusion gradients were applied perpendicular to the microcapillary main axis with a duration of 20 ms on either side of a refocusing pulse. An apodised cosine-modulated waveform which replaced the first and last quarters with half-sine lobes at double frequency was used to avoid very rapid rise times [26]. Temporal diffusion spectra [18] were acquired at 12 oscillating frequencies ranging evenly from 50 Hz to 600 Hz. At each frequency, the ADC was calculated from two acquisitions with $b = 0$ and $b = 700 \text{ s/mm}^2$. The only exception was for the smallest (1.5–1.6 μm) microcapillaries, in which much higher b -values were used ranging from 700 to 50,000 s/mm^2 in order to cause higher diffusion weighting. Other parameters were: TR = 8 s, TE = 60 ms, receiver bandwidth 10 kHz, spectral resolution 78.125 Hz. NEX (number of excitations) varied from 4 to 80 for different microcapillary sizes depending on the signal-to-noise ratio (SNR). The diffusion coefficient of free water was measured with distilled water in a 5 mm NMR tube at the same temperature. The same measurement was also performed with the diffusion gradient along the z -axis in the microcapillary experiments.

2.3. Data analysis

The measured ADC spectra were fit to Eq. (5) with two unknown variables D and d using the *lsqcurvefit* function in Matlab (Mathworks, Natick, MA). To explore the sensitivity of the ADC spectra to the cylinder diameter with a limited range of low frequencies, the ADC spectra were also fit with only a subset of frequencies (ranging from 2 to 12 in number). The fittings were processed with initial values: $D = 1.8 \mu\text{m}^2/\text{ms}$, $d = 15 \mu\text{m}$; lower bounds: $D > 0 \mu\text{m}^2/\text{ms}$, $d > 0 \mu\text{m}$; and upper bounds: $D < 4 \mu\text{m}^2/\text{ms}$, $d < 200 \mu\text{m}$.

3. Results

Fig. 1 shows the measured ADC spectra (markers) and fitted spectra (solid lines) with all the 12 frequencies. Consistent with the theory, the measured ADC increased with the oscillation frequency, approaching the free diffusion coefficient at higher frequencies. Note that the ADC dispersion rate with frequency was strongly dependent on the inner diameter, implying that ADC dispersion rate alone might be a sensitive indicator of diffusion compartment size.

The comparison between the fitted microcapillary inner sizes and the sizes provided by the manufacturer is shown in Fig. 2a. All microcapillary diameters were fit accurately (<6%). Note that the fitted size of the smallest microcapillaries was only -2.6% from the real value, suggesting that the OGSE method can be used to accurately measure small compartment sizes, which are usually overestimated using other methods [10,40]. In addition, all fitted free diffusion coefficients are consistent with the measured diffusion coefficient of free water ($1.82 \mu\text{m}^2/\text{ms}$) except that of the smallest ($1.5\text{--}1.6 \mu\text{m}$) microcapillaries (see Fig. 2b), which deviates ~56% from the true value. All fitted results are summarized in Table 1.

To further investigate the accuracy of the OGSE method to the smallest microcapillaries, the ADC spectrum for the $1.5\text{--}1.6 \mu\text{m}$ microcapillaries was fit using a different strategy that includes a fixed free diffusion coefficient $D = 1.82 \mu\text{m}^2/\text{ms}$ shown in Fig. 3. The new fitted size increased to $1.75 \mu\text{m}$ but was still in reasonably good agreement with the real size. The fitted ADC spectra for the two different types of fitting were almost the same within the frequency range used in this study, although there is significant difference at higher frequencies. This is because the applied gradient frequencies for such a small size are not high enough to probe free diffusion adequately so that the ADC spectra in this low frequency range are still dominated by boundary effects, i.e. are sensitive to restriction size. This is consistent with numerous previous reports [19,41].

Fig. 4 shows the fitted diameters using the first N (ranging from 2 to 12) frequencies. All fitted sizes were consistent with only small fluctuations regardless of the number of frequencies used in fitting. An interesting result is that a minimum of two ADC values at different frequencies is sufficient to estimate the inner diameters of hollow microcapillaries with reasonable accuracy.

Fig. 5 shows the fitted free diffusion coefficients using the first N (ranging from 2 to 12) frequencies. For relatively larger sizes ($d > 9.5 \mu\text{m}$), all the fitted free diffusion coefficients were consistent with the true values regardless of the number of frequencies used in fitting. However, the sensitivity of OGSE spectra to the free diffusion coefficient decreased with decreasing compartment sizes which could be appreciated from the $4.3\text{--}4.5 \mu\text{m}$ microcapillaries. When only the first two frequencies were used, the fitted D was about $1.0 \mu\text{m}^2/\text{ms}$, which was about 45% lower than the true value. However, with more and more high frequencies used in fitting, the fitted D approached quickly to the true value, indicating the free diffusion behavior is then adequately probed. By contrast, the fitted D for the smallest microcapillaries showed a remarkable fluctuation. This is due to two main reasons: (1) the applied gradient frequencies were not high enough so that the sensitivity of the OGSE measurements to free diffusion was low; and (2) the measurement SNRs of the

smallest microcapillaries were about 50 and much lower than those of other microcapillaries due to significantly reduced water content (the intra-microcapillary to total space volume ratio <0.01%). However, even if the fitted D had large errors for the smallest microcapillaries, the fitted size was still in good agreement with the real values as shown in Fig. 4.

4. Discussion

The inner diameters of each sample of the hollow microcapillaries used only vary within a narrow range defined by the lower and upper bounds [42,43]. Therefore, these hollow microcapillaries provide a well-characterized model system to validate the accuracy of different diffusion MRI methods [7,10–12,40]. In this study, the inner diameters of hollow microcapillaries ranging from 1.5 to 19.3 μm (similar to the axon size range in the human central nervous system [33]) have been estimated accurately using the OGSE method. Note that several OGSE-like methodologies [10–12] have already been applied to detect small sizes using similar types of phantoms. To the best of our knowledge, this is the first study that validates the feasibility of using the OGSE diffusion spectra to obtain quantitative structural information in white-matter like systems. The results confirmed that the OGSE method is highly sensitive to measure small sizes non-invasively [19,44,45].

The fitting of the compartment sizes and the free diffusion coefficients is based on Eq. (5), which was derived based on the Gaussian phase approximation [38]. The validity of the Gaussian phase approximation has been analyzed previously [15,46,47], and computer simulations have confirmed that the Gaussian phase approximation is accurate enough for most practical applications [31,32]. Hence, fast ADC measurements can be performed with a few b -values, which avoid long scan times. As shown above, when the frequencies are moderately high, the OGSE measurements can be highly sensitive to the compartment size. Even two ADC values with different frequencies may be sufficient to extract the sizes of the compartments accurately. Thus OGSE may serve as a fast measurement method for mapping axon sizes. The relatively low sensitivity of diffusion measurements in practice to the free diffusion coefficient has also been observed using the angular double- PGSE method [7]. Though the free diffusion coefficient could not be extracted accurately with relatively low frequencies only, it has little influence on the extraction of the compartment sizes. The measured ADC at 50 Hz was about 1.4 $\mu\text{m}^2/\text{ms}$ for the 19 μm microcapillaries, which was 23% lower than the diffusion coefficient of free water at the same temperature. This suggests that, even if the microcapillary boundaries were not fully probed (RMSD $\sim 12 \mu\text{m}$), there was still a significant influence from the boundary restriction on the diffusion signal at such an effective diffusion time. Our results show that with the experimental parameters used in the current study the microcapillary sizes up to 19 μm can be estimated accurately. However, if microcapillaries with even larger sizes are probed with the same experimental parameters, the measured ADC may approach the free diffusion coefficient, i.e. reach the plateau of the ADC spectrum, and, hence, the microcapillary sizes will not be estimated as accurately. Compared with other methods, the OGSE method is capable of detecting small sizes around 1.5 μm accurately. The overestimation using other methods has been tentatively explained by the presence of background magnetic field gradients due to the susceptibility difference between glass and water [10,40]. The OGSE sequence is intrinsically insensitive

to background and imaging gradient cross terms [26,48], so it may be more suitable for extraction of axon sizes in inhomogeneous systems.

Recent analyses have suggested that the ADC measured with oscillating gradient approaches depends not only on the gradient waveforms but also on the number of oscillations [49]. The number of oscillations (n) is implicitly included in Eq. (1) (note that $f = n/\sigma$). The cosine-modulated waveform has been shown to perform better than the sine-modulated waveform to fulfill the validity conditions of the high-frequency expansion of ADC [49]. Our experimental settings have taken both factors explicitly into consideration so that accurate measurement results were obtained.

The implementation of OGSE methods on human scanners is challenging and limited by current gradient systems. Despite the challenge, there have been two published OGSE studies on human scanners with maximum gradient strength around 50 mT/m [21,22]. Instead of the standard cosine waveforms used here, the trapezoidal gradient waveforms have been suggested and implemented to achieve higher diffusion weighting [21,22,31], though with reduced spectral selectivity. Even with frequencies <66 Hz, ADC dispersion with frequency has already been observed in human white matter [21,22]. If more powerful gradient systems are used, e.g. the Connectome gradient coils [50], much higher frequencies and b -values could be achieved in human OGSE measurements. For example, a frequency as high as 100 Hz and a b -value of 700 s/mm² could be possible with a gradient strength of 200 mT/m. Based on current results, the compartment sizes may be extracted in this frequency range ($f < 100$ Hz), which suggests that there is potential to measure axon sizes as small as 1.5 μ m on human scanners using OGSE.

The current study considered only a one-compartment diffusion system which showed the feasibility of OGSE measurements for rapid measurements of small axon sizes. However, in real biological systems, there are several other confounding effects, such as the contribution from the extracellular water, the distribution of axon sizes, and the fiber orientation and dispersion [51,52]. Models similar to the CHARMD model [53] but using the OGSE approach are currently under development, and are predicted to be capable of fast mapping of axon size distributions in vivo.

5. Conclusion

In this study, the inner diameters of hollow microcapillaries over a range of sizes (~ 1.5 – 19.3 μ m) were accurately measured using the OGSE method. The accuracy of fitting cylinder diameters and free diffusion coefficients using the OGSE method was analyzed, and it suggests that OGSE measurements, even with moderately high frequencies, are highly sensitive to compartment sizes. A possible fast method of mapping axon size non-invasively is proposed.

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Fig. 1.

The measured ADC spectra (symbols) and fitted spectra (solid lines) for restricted diffusion inside microcapillaries with different sizes.

Fig. 2.

The fitted size (a) and the fitted free diffusion coefficient (b) versus the real size. The vertical error-bars represent the 95% confidence intervals of the fitted values. The horizontal error-bars represent the lower and upper bounds provided by the manufacturer. The dashed line in (a) is the identity line, and the dashed line in (b) represents the measured diffusion coefficient of free water (1.82 $\mu\text{m}^2/\text{ms}$).

Fig. 3.

Comparison of the fitted ADC spectra for the 1.5–1.6 μm microcapillaries using two different fitting methods, i.e. with and without fixed free diffusion coefficient $D = 1.82 \mu\text{m}^2/\text{ms}$.

Fig. 4.

The fitted diameters against the first N frequencies used in fitting for the microcapillaries of different sizes. Dashed lines represent real values, and markers are fitted results.

Fig. 5.

The fitted free diffusion coefficients against the first N frequencies used in fitting for the microcapillaries of different sizes. Dashed line represents the measured diffusion coefficient of free water ($1.82 \mu\text{m}^2/\text{ms}$), and markers are fitted results.

Table 1

Summary of the sizes and the free diffusion coefficients fitted with Eq. (5). Real sizes are given by lower and upper bounds provided by the manufacturer, and all fitted values are given by mean $\pm$ 95% confidence intervals.

Real size (μm)	Fitted size (μm)	Fitted D ($\mu\text{m}^2/\text{ms}$)
1.5–1.6	1.51 ± 0.17	0.80 ± 0.61
4.3–4.5	4.67 ± 0.08	1.80 ± 0.04
9.5–9.7	9.76 ± 0.87	1.79 ± 0.06
14.9–15.0	15.01 ± 0.88	1.85 ± 0.02
19.0–19.3	18.28 ± 0.87	1.85 ± 0.01