Published in final edited form as:

NMR Biomed. 2016 April ; 29(4): 400–410.

Fast and simplified mapping of mean axon diameter using temporal diffusion spectroscopy

Junzhong Xu^{1,2,3,*}, Hua Li^{1,3}, Ke Li^{1,2}, Kevin D. Harkins¹, Xiaoyu Jiang^{1,2}, Jingping Xie^{1,2}, Hakmook Kang⁴, Richard D. Dortch^{1,2,5}, Adam W. Anderson^{1,2,5}, Mark D. Does^{1,2,5}, and John C. Gore^{1,2,3,5,6}

¹ Institute of Imaging Science, Vanderbilt University, Nashville, TN 37232, USA

² Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN 37232, USA

³ Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37232, USA

⁴ Department of Biostatistics, Vanderbilt University, Nashville, TN 37203, USA

⁵ Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37232, USA

⁶ Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232, USA

Abstract

Mapping axon diameter is of interest for the potential diagnosis and monitoring of various neuronal pathologies. Advanced diffusion-weighted MRI methods have been developed to measure mean axon diameters non-invasively, but suffer major drawbacks that prevent their direct translation into clinical practice, such as complex non-linear data fitting and, more importantly, long scanning times that are usually not tolerable for most human subjects. In the current study, temporal diffusion spectroscopy using oscillating diffusion gradients was used to measure mean axon diameters with high sensitivity to small axons in the central nervous system. Axon diameters have been found to be correlated with a novel metric, $DDR_{\perp}$ (the rate of dispersion of the perpendicular diffusion coefficient with gradient frequency), which is a model-free quantity that does not require complex data analyses and can be obtained from two diffusion coefficient measurements in clinically relevant times with conventional MRI machines. A comprehensive investigation including computer simulations and animal experiments *ex vivo* showed that measurements of $DDR_{\perp}$ agree closely with histological data. In humans *in vivo*, $DDR_{\perp}$ was also found to be correlated well with reported mean axon diameters in human corpus callosum, and the total scan time was only ~8 minutes. In conclusion, $DDR_{\perp}$ may have a potential serve as a fast, simple and model-free approach to map mean axon diameter of white matter in clinics for assessing axon diameter changes.

Graphical Abstract

*Corresponding author: Junzhong Xu, PhD, Vanderbilt University Institute of Imaging Science, 1161 21st Avenue South, AA 1105 MCN, Nashville, TN 37232-2310, United States. junzhong.xu@vanderbilt.edu.

The perpendicular diffusion coefficient dispersion rate $DDR_{\perp}$ was found as a fast, simple and easy-to-use metric to map volume-weighted mean axon diameter AxD noninvasively. The correlation between $DDR_{\perp}$ and AxD was validated with computer simulations based on histology data in rat spinal cord, and also observed in human corpus callosum *in vivo*, which took only ~8 minutes.

Keywords

axon diameter; diffusion MRI; dispersion rate; scanning time; temporal diffusion spectroscopy

Introduction

The extent of axonal damage has been suggested to be a key predictor of outcome in human central nervous system diseases (1,2). It has also been reported that axonal damage is dependent on axon diameter in various neural disorders. For example, for acute and chronic lesions in multiple sclerosis, direct axon counting in post-mortem tissue has suggested that smaller axons might have a greater susceptibility to damage (3). It is also reported that large-diameter axons (type A-alpha) are damaged selectively (4), while small-diameter axons are maldeveloped in autism (5). In addition, traumatic brain injury causes significant axonal swelling (6), and the extent of swelling has been reported to be correlated with survival time (7). These studies all indicate that axon diameter is an important metric to evaluate or to predict the progress of different neurological disorders, and hence measurements of axon diameter have significant potential in many clinical applications. Axon diameters have traditionally been measured using invasive biopsies and destructive histological procedures, which are extremely challenging or impractical *in vivo*, often resulting in significant damage to nervous tissues.

Diffusion-weighted MRI (DWI) provides a powerful approach to measure tissue microstructure non-invasively via detecting the random Brownian motion of water molecules in biological tissues. Water diffusion is restricted or hindered by various microstructures, e.g. cell membranes and organelles, so measurements of the apparent diffusion coefficient (ADC) provide a means to characterize microstructural variations. However, previous attempts to correlate ADC measurements with mean axon diameter have not been successful (8–10), perhaps because of the complication of contributions from multiple compartments and limitations on the pulse sequences used. q-Space imaging is a diffusion imaging

technique that measures the displacement probability profile of water molecules (11–13) and hence mean pore diameters can be obtained (14). However, due to the violation of the short gradient approximation, along with the contribution from extra-axonal diffusion, the mean axon diameter is typically overestimated even with extremely strong gradients, e.g. 48000 mT/m (15,16). Another approach, using the CHARMED model (17), has been developed to include the separation of intra- and extra-axonal diffusion, and from this, the axon diameter distribution within white matter can be fit. Such an approach is named the AxCaliber framework (18,19). However, this approach requires a scanning time usually too long for human subjects: scans of 30 hours for fixed nerves (18) and ~2 hours for the corpus callosum of rat brain *in vivo* (19) have been reported. To reduce scanning times, the ActiveAx model was developed to measure mean axon diameters instead of distributions within ~1 hour for whole brain (20) and around 35 mins for stable parameter estimates in human corpus callosum with known direction, both of which were still long for clinical applications (21). A recent study suggested clinically feasible 11.5 mins for AxCaliber protocol in human corpus callosum(22), but it has been criticized to overestimate axon diameter again (23). Some other approaches have been recently proposed to measure mean axon diameter, such as the d-PFG (24–26) and the OGSE (oscillating gradient spin echo) framework which enhances the sensitivity to small axons (27–29), but these methods also require long scanning times.

All of the above methods have several limitations that prevent directly translating these techniques into practice. Long scanning times ($> \approx 1$ hour) are not feasible for routine MR patient imaging. Moreover, the data analyses on which they rely are all based on *a priori* mathematical models that require complex non-linear data fitting strategies for their interpretation. Therefore, a fast, simple and model-free method is attractive for mapping axon diameters *in vivo* in a clinical environment. We propose a simplified implementation of temporal diffusion spectroscopy via the oscillating gradient spin echo (OGSE) method to map axon diameters. The OGSE method has the ability to achieve very short diffusion times (30), and hence is able to probe microstructure at short length scales (31–34). In neuroimaging, the OGSE method can enhance the detection sensitivity to small axons (28) and provide additional microstructural information (35,36). In a previous temporal diffusion spectroscopy study (28), mathematical modeling and measurements were performed to estimate mean axon size and intra-axonal volume fraction simultaneously. However, that approach requires relatively long scanning time as other axon size mapping approaches (18–20). In the current study, we show a different approach, i.e. without sophisticated non-linear data fitting and long scan time, the mean axon size can be mapped rapidly using a simple metric, i.e. the low frequency dispersion of ADCs over a relatively small range of frequencies can be correlated well with report axon dimensions. Computer simulations, animal *ex vivo*, and human *in vivo* studies were performed to comprehensively evaluate and valid this approach.

Materials and Methods

Temporal diffusion spectroscopy

For a diffusion-sensitizing gradient $\mathbf{g}(t)$, the diffusion-weighted signal can be written as (30)

$$S = S_0 \exp \left[-2 \int_0^\infty \mathbf{D}(f) |\mathbf{F}(f)|^2 df \right], \quad [1]$$

where $\mathbf{D}(f)$ is the temporal diffusion spectrum, the Fourier transform of the autocorrelation function $A(t)$ of the velocities $\mathbf{v}(t)$ of water molecules, i.e. $A(t) = \langle \mathbf{v}(t) \mathbf{v}(t+\tau) \rangle$ (37), while $\mathbf{F}(f)$ is the gradient modulation spectrum, namely

$$\mathbf{F}(f) = \int_{-\infty}^{\infty} dt \exp(i2\pi ft) \int_0^t dt' \gamma \mathbf{g}(t'). \quad [2]$$

If $\mathbf{g}(t)$ is modulated with a cosine waveform with a frequency f_m , $\mathbf{F}(f)$ serves as a sampling function centered at $\pm f_m$ (i.e. the Dirac delta function if the effect of finite gradient duration is ignored (38)) and hence $\mathbf{D}(f_m)$ can be measured directly. Note that the effective diffusion time for such a sequence is not the time between gradient pulses as in PGSE sequences, but is equal to $1/(4f_m)$, which has been validated in neural tissues with low b values (39). Therefore, by selecting different gradient frequencies, the frequency-dependent spectrum $\mathbf{D}(f)$ (corresponding to sampling at different diffusion times) can be obtained, which provides microstructural information at different length scales.

Perpendicular diffusion dispersion rate – DDR_⊥

If velocity autocorrelation functions are exponential decays in time, the whole temporal diffusion spectrum $\mathbf{D}(f)$ of restricted diffusion obtained using the OGSE method over all frequencies can be expressed as a superposition of a series of Lorentzian functions (40). However, the b value of the OGSE method is proportional to G^2/f^2 , and hence the highest achievable frequency in practice is still limited by the gradient strength G . Meanwhile, the lowest achievable frequency is limited by the diffusion gradient duration δ , i.e. $f_{\min} = 1/\delta$. Therefore, $\mathbf{D}(f)$ over only a restricted range of frequencies can be evaluated in practice. Figure 1A shows $\mathbf{D}(f)$ for arrays of impermeable cylinders with different diameters with gradients perpendicular to the main axis. Note that the intra-cylinder diffusion coefficient was assumed to be $1.0 \mu\text{m}^2/\text{ms}$. Due to the limitation of the maximum gradient strength on most current human MRI machines, the practical frequency range is usually quite narrow, e.g. $18 < f < 63$ Hz was used in human *in vivo* OGSE measurements with $G < 60$ mT/m (41,42) and $0 < f < 1000$ Hz was used in rat brain *in vivo* with $G < 1000$ mT/m (43). Considering the small diameter of most axons in the central nervous system, e.g. $1 - 5 \mu\text{m}$, these frequencies are within the intermediate to low frequency range. For example, two frequencies (50 and 100 Hz) were chosen to show the linearity of the diffusion spectra over a narrow frequency range in Figure 1A. More importantly, when considering contributions from both intra- and extra-axonal spaces in real tissues, previous studies have shown that, over a narrow range of intermediate to low frequencies, the diffusion spectrum of simple geometries disperses with the gradient frequency in an approximately linear manner (32), which has also been observed in tumors *in vivo* (44) and in neural tissues *ex vivo* (35,45). In addition, if axons are considered as randomly packed (46), it has been found that the extracellular diffusion coefficient is linearly dependent on f in low frequency range (47). Therefore, a diffusion dispersion rate (DDR), i.e. the slope of such a linear dependence, can be simply defined as

$$\text{DDR} = \frac{dD(f)}{df} \quad [3]$$

over a narrow range of intermediate to low frequencies.

If the diffusion gradient is perpendicular to the main axis of cylindrical axons, the dispersion rate of the perpendicular diffusion coefficient $D_{\perp}$ can be defined as $\text{DDR}_{\perp}$. Figure 1B shows that, despite the discrepancy when the cylinder diameter (AxD) $> 6 \mu\text{m}$, $\text{DDR}_{\perp}$ correlates well with $AxD < 6 \mu\text{m}$, which is the typical axon diameter in the central nervous system. Such feature demonstrates the basis of how measurements of $\text{DDR}_{\perp}$ can be used to estimate mean axon diameter. However, in realistic biological tissues, contributions from extra-axonal space may complicate and sometimes dominate the signal formation with long diffusion times (47). In the current study, we report that $\text{DDR}_{\perp}$ of white matter is a reliable indicator of mean axon diameter in realistic tissues, and hence measurements of $\text{DDR}_{\perp}$ may provide a fast and simple means of mapping mean axon diameter *in vivo*.

Ex vivo study of rat spinal cord

Tissue preparation—The study was approved by the institutional animal care and use committee (IACUC) of Vanderbilt University. Six Sprague Dawley rats (275 - 350 g) were perfused, under deep anesthesia, through the left cardiac ventricle with phosphate buffer followed by 0.5% paraformaldehyde and 4% glutaraldehyde in phosphate buffer. Cervical spinal cord sections $\approx 1 \text{ cm}$ in length were cut out and immediately placed in fixative (0.5% paraformaldehyde and 4% glutaraldehyde in phosphate buffer) for 48 hours. Samples were then washed with phosphate-buffered saline solution for at least 3 days before MRI scanning (48). Each sample was put inside a 5-mm NMR tube filled with fomblin. The details of the tissue preparation can be found in previous reports (49,50).

Magnetic resonance imaging—A 7T Varian MRI scanner was used for all MRI experiments. A 12-mm microgradient coil (Doty Scientific Inc., Columbia, SC) was used to achieve high gradient strength, up to 1126 mT/m in the current study. A 4-shot echo-planar imaging (EPI) sequence with a triple-reference correction (51) was used for all image acquisitions. Other imaging parameters were: FOV = $5 \times 5 \text{ mm}$, matrix diameter = 64×64 (resulting in an in-plane spatial resolution of $78 \mu\text{m}$), and the slice thickness = 2 mm. Conventional diffusion coefficients using pulsed gradients with the stimulated echo sequence were obtained in addition to the OGSE measurements with gradient duration $\delta = 5 \text{ ms}$, separation = 14, 30, 60, 100, 150 ms, echo time = 28 ms, and b value = 1000 s/mm^2 . Apodised cosine waveforms (43) were used for OGSE measurements with TR = 4 sec, TE = 58 ms, gradient duration $\delta = 20 \text{ ms}$, gradient separation = 24 ms, and b value = 1000 s/mm^2 . All diffusion gradients were applied perpendicular to the spinal cord tracts with six frequencies (50, 100, 150, 200, 250, 300 Hz).

Histology—Fixed spinal cord samples were dehydrated in graded ethanol and embedded in epoxy resin after MRI measurements. Several 1- μm -thick axial sections were then cut and stained with 1% Toluidine Blue solution. Note that this tissue preparation protocol may cause a 10% decrease of cross-sectional area (52,53). Oil immersion light microscopy (LM)

was used to acquire digital histological images at a resolution of 0.075 μm . LM images of six separate white matter tracts were collected from each of the six fixed spinal cords, i.e. vestibulospinal tract (VST), fasciculus cuneatus (FC), rubrospinal tract (RST), reticulospinal tract (ReST), funiculus gracilis (FG), and dorsal corticospinal tract (dCST). Thus, altogether 36 white matter tract ROIs (six ROIs per rat) were chosen. The histology section diameter was chosen to include ~200 or more axons in each histological section, i.e. $67.61 \times 67.61 \mu\text{m}^2$ for VST and FC, $45.11 \times 45.11 \mu\text{m}^2$ for RST and ReST, and $33.86 \times 33.86 \mu\text{m}^2$ for FG and dCST. Histological images were then digitally smoothed and semi-automatically segmented into regions of intra-, extra-axonal spaces, and myelin. Note that the mean axon diameters obtained from MRI measurements are actually not the arithmetic mean of axons but rather the volume-weighted mean (20,50), and hence volume-weighted mean axon diameters (AxD's) were obtained for each white matter tract from histology, namely,

$$AxD = \frac{2}{\sqrt{\pi}} \cdot \frac{\sum_{n=1}^N (\sqrt{A_n})^3}{\sum_{n=1}^N A_n}, \quad [4]$$

where N is the total number of axons in each histological image, and A_n is the cross-section area of the n^{th} axon.

Data analysis—Data analysis was performed pixelwise using MATLAB (Mathworks, Natick, MA). All images were co-registered using a rigid-body registration algorithm (54). To investigate the variations of $\text{DDR}_{\perp}$ over the frequency range, the first N ($N = 2, 3$ or 4) frequencies were used in the linear regression, which resulted in three estimates of $\text{DDR}_{\perp}$. For example, $\text{DDR}_{\perp}(N = 2)$ was obtained using a linear fitting with frequencies of 50 and 100Hz, while $\text{DDR}_{\perp}(N = 3)$ used frequencies 50, 150 and 200Hz. Note that with larger frequencies (> 200 Hz), diffusion spectra show curvatures other than linear dependence on frequency (e.g. see Figure 4), so all DDR analysis were restricted with frequencies < 200 Hz. For comparison with histology, the regions of interest (ROI's) of six different white matter tracts (see Figure 2) were manually drawn on the T_2 -weighted image of each spinal cord (yielding altogether 36 ROI's).

Linear mixed-effects models with compound symmetry structure of the correlations were employed to evaluate the relationship between histological mean AxD and the MR parameters, i.e. $D_{\perp}$ and $\text{DDR}_{\perp}$ with animal-specific dependence in each fitted parameters taken into account. For each model, histogram and scatter plots of residuals were visually inspected to assure the normality of each parameter and randomness of the error, respectively.

In silico study based on histology

Computer simulations were performed to show the correlation between simulated $\text{DDR}_{\perp}$ values and mean axon diameters measured from histology of rat spinal cords. The 36 segmented histological images of all white matter tracts of all rat spinal cords were downsampled to a resolution of $0.1125 \times 0.1125 \mu\text{m}$. Each type of white matter tract ($N = 6$) was randomly divided into three groups (two in each), either keeping the original spatial

scale of 0.1125 μm per pixel or doubling or shrinking by half of the original scale, i.e. 0.225 and 0.0563 μm , respectively. By this means, the input structures for computer simulation had a much broader mean axon diameter range (0.55 – 11.96 μm) but with the realistic microstructural features preserved (e.g. axon shape, volume fraction, packing density). A Finite-Difference Time-Domain approach (55) was used to simulate diffusion signals based on representative segmented histological images of six different white matter tracts. The diffusion coefficient of water molecules was assumed to be 1.0 $\mu\text{m}^2/\text{ms}$ everywhere (56), the water exchange between different compartments assumed negligible (17,18), and all other parameters were the same as for the MRI experiments. Note that simulations with water diffusion coefficient of 0.5 and 2 $\mu\text{m}^2/\text{ms}$ were also performed and the main results unchanged qualitatively.

In vivo study of human corpus callosum

Magnetic resonance imaging—The human study was approved by our local Institutional Review Board. Six healthy subjects (five males and one female, age: 31 ± 7) were imaged on a 3.0T Philips Achieva MR scanner (Philips Healthcare, Cleveland, OH) equipped with a dual-channel body coil for RF transmission, a 32-channel head coil for signal reception, and a whole-body gradient coil with strength up to 60 mT/m used in each direction. OGSE imaging data were acquired with a 2D single-shot spin-echo EPI sequence with parameters of TR/TE = 3600/124 ms, field-of-view (FOV) = $192 \times 192 \text{ mm}^2$, in-plane resolution of $2.4 \times 2.4 \text{ mm}^2$ and reconstructed to $2 \times 2 \text{ mm}^2$, partial k-space sampling fraction of 68.6%, number of acquisitions (NSA) of 4, and a total of 8 repetitions to enhance signal-to-noise ratio (SNR). For each diffusion weighting, all images were co-registered before averaged to reduce motion artifacts. A spectral pre-saturation with inversion recovery (SPIR) pulse was used to suppress fat signals. A single slice with thickness of 5 mm was positioned in a sagittal plan through the center of the brain. To reduce any cerebrospinal fluid induced imaging artifacts, a 90_x - 180_y - 90_x composite pre-pulse was applied with inversion recovery time of 1307 ms. The OGSE measurements used cosine-modulated trapezoidal gradients with frequencies of 20 and 40 Hz, and two b values (50 and 400 s/mm^2) to calculate a $D_{\perp}$ for each frequency. The total acquisition time for two $D_{\perp}$ measurements was approximately 8 minutes.

Data analysis—For each set of human data, all diffusion-weighted images were registered to their T2-weighted image using an affine-transformation algorithm written in house. The $D_{\perp}$ values were calculated with a mono-exponential decay equation for each frequency, and $\text{DDR}_{\perp}$ values were then calculated according to Eq.(3). Four regions-of-interest (ROI) were manually drawn within the corpus callosum, guided by the histological mean axon diameter data (see Figure 8A) originally published in (57). Mean $\text{DDR}_{\perp}$ values of each ROI were used for investigating the correlation with expected histological mean axon diameters.

RESULTS

Diffusion simulations based on histological images

Figure 2 shows histology images and the corresponding segmented images used for computer simulations. The histology-derived mean AxD were also provided for each tract.

The simulated apparent diffusion spectra (black pentagrams) are shown in the last row of Figure 2. Linear fits of apparent diffusion spectra are indicated by the solid lines. All R^2 values were larger than 0.92, indicating the apparent diffusion spectra are well represented by linear fits in this frequency range. For a comparison, diffusion spectra arising from intracellular (red circles) and extracellular (blue diamonds) spaces alone are also provided. It is consistent with previous findings that intracellular diffusion spectra decrease more rapidly than extracellular diffusion spectra as mean axon size decreases from VST to dCST. Note that, each column of Figure 2 represents a different white matter tract, and the diffusion gradient was applied in the image plane.

Figure 3 shows the correlations of three types of dispersion rates (i.e. $DDR_{\perp}$ arising from whole space, $DDR_{\perp in}$ from the intra-axonal space only, and $DDR_{\perp ex}$ from the extra-axonal space only) calculated from simulations with the mean AxD measured from histology. Each column represents a different frequency range used to obtain dispersion rate values. A linear fitting was performed in each subfigure in the range of mean axon diameter $< 5 \mu m$ (typical range in the central nervous system.), and the corresponding Spearman correlation coefficients were provided. Note that all correlations had p values < 0.01 , indicating all three types of dispersion rate correlate with AxD. However, the correlation coefficients of all $DDR_{\perp ex}$ were intermediate (< 0.7), while those of $DDR_{\perp}$ and $DDR_{\perp in}$ were high (> 0.92). This suggests that the dependence of $DDR_{\perp}$ on AxD is largely contributed by the intra-axonal space, presumably because of the rapid change of $DDR_{\perp in}$ with AxD while $DDR_{\perp ex}$ varies slowly over the frequency range used here. This may explain why the overall $DDR_{\perp}$ estimates AxD well. We should emphasize that the contribution to the dependence of $DDR_{\perp}$ on AxD is different from the intra-/extra-axonal contribution to $DDR_{\perp}$ itself. Figure 3 shows that when $AxD < 1.5 \mu m$, i.e. when the diffusion time is long compared with the restricting size, $DDR_{\perp ex}$ is much larger (twice or more) than $DDR_{\perp in}$, suggesting $DDR_{\perp}$ is mainly dominated by extra-axonal space when diffusion time is long. This is consistent with previous findings (47). By contrast, when $AxD > 3.5 \mu m$, $DDR_{\perp in}$ becomes larger ($> 50\%$) than $DDR_{\perp ex}$, suggesting $DDR_{\perp}$ is largely contributed by intra-axonal space when diffusion time is relatively shorter. Nonetheless, the overall dependence of $DDR_{\perp}$ on AxD is similar to that of $DDR_{\perp in}$, and this forms the basics of using overall $DDR_{\perp}$ to estimate AxD. Note that, when a different frequency range was used (different columns in Figure 3), the dependence of $DDR_{\perp}$ on AxD remains approximately the same. This may provide extra flexibility for experimental design.

Ex vivo MRI of rat spinal cord

Figure 4 shows the temporal diffusion spectra (data points) of six white matter tracts from a representative rat spinal cord, and the corresponding histology-derived AxD's were provided in the caption. Similar to the simulated data shown in Figure 3, the diffusion spectra disperse with frequency in a linear manner in those white matter tracts with small mean axon diameters, i.e. FG and dCST, while the diffusion spectra are non-linear at higher frequencies (> 200 Hz) in the white matter tracts with large mean axon diameters, i.e. VST. Nevertheless, all diffusion spectra disperse with frequency approximately linearly in the lower frequency range (50 – 200 Hz). Three frequency ranges were used to fit three $DDR_{\perp}$'s in the current study, i.e. $N = 2, 3$ and 4 corresponding to the frequency ranges 50-100 Hz,

50-150Hz and 50-200Hz, respectively. Although there are some small differences in the VST tract, three estimates of $DDR_{\perp}$ using different frequency ranges match well in all other white matter tracts.

The top two rows of Figure 5 show representative $D_{\perp}(f)$ (perpendicular diffusion coefficient) maps with different effective diffusion times obtained using the OGSE and PGSE methods, and the third row shows $DDR_{\perp}$ maps obtained with three different frequency ranges, i.e. $N = 2, 3$, and 4. Although all $D_{\perp}$ maps can differentiate the butterfly-shaped gray matter (indicated by the pink contour) from the surrounding white matter, they are quite homogeneous in the white matter region except the $D_{\perp}$ map with the shortest effective diffusion time (0.83 ms). This suggests $D_{\perp}$ maps with intermediate or long diffusion times cannot differentiate different white matter tracts with different AxD values, which is consistent with previous reports (8–10). By contrast, all three $DDR_{\perp}$ maps show clear contrasts differentiating different white matter tracts, especially distinguishing FC from FG and dCST tracts. The $DDR_{\perp}(N=2)$ map is more noisy than the other two $DDR_{\perp}$ maps, presumably due to the larger variations introduced by using only two frequencies in the linear fitting.

Figure 6A shows the correlations between the histological AxD with $D_{\perp}$ with different effective diffusion times obtained using the OGSE and PGSE sequences, and the correlations between $DDR_{\perp}$ and AxD are depicted in Figure 6B. Each data point represents the mean value of each white matter tract ROI. The correlation coefficient r obtained using the linear mixed-effects model is given for each correlation. Note that the top plots in Figure 6A were obtained from the OGSE measurements, while the bottom plots were from the PGSE measurements with stimulated-echo diffusion sequences. Therefore, a large range of effective diffusion times (0.83 – 148.33 ms) was used to investigate the influence of diffusion time on the correlation of $D_{\perp}$ with the mean axon diameter. It is clear that the correlation coefficient r increases with shorter effective diffusion times. The $D_{\perp}(f)$ with short diffusion times (< 2.5 ms) correlates strongly with the mean axon diameter with $r > 0.84$. For comparison, all correlation coefficients between $DDR_{\perp}$ and histological mean axon diameter are > 0.86 . Figure 6C summarizes all correlation coefficients (r 's) shown in Figure 6A and 6B. Note that $DDR_{\perp}(N=2$ and 3) give highest correlation coefficients, which shows that $DDR_{\perp}$ reports mean axon diameter better than $D_{\perp}$ with longer diffusion times, within the limits of the current study ($< 5 \mu\text{m}$).

Figure 7 shows the Bland-Altman plots of the differences of estimates of $DDR_{\perp}$ with different frequency ranges ($N = 2, 3$ or 4). There is only one data point in Figure 7A and two in Figure 7B that are potential outliers. All other data points are within 1.96 standard deviations, i.e. 95% confidence interval if a Gaussian distribution is assumed. These results suggest that there are no significant differences between different $DDR_{\perp}$'s obtained using different frequency ranges.

In vivo MRI of human corpus callosum

Figure 8 shows the results from the in vivo MRI of human corpus callosum of six healthy subjects. Figure 8A illustrates a typical human corpus callosum cross section, with four separable ROI regions, each was assumed to have a different but uniform mean axon

diameter. The axon diameter data of human corpus callosum were originally published by Aboitiz et al. (57), and later converted to volume-weighted means of different regions (20). Figure 8B shows all six measured $DDR_{\perp}$ maps. Note that some maps $DDR_{\perp}$ are noisy presumably due to relatively low SNR. Figure 8C depicts the correlations between $DDR_{\perp}$ and $D_{\perp}$ (20 and 40 Hz) with histological mean axon diameter, respectively. Neither $D_{\perp}$ at 20 nor 40 Hz provides a significant correlation ($p = 0.88$ and 0.06), while $DDR_{\perp}$ shows $r = 0.73$ and $p < 0.01$, suggesting a good agreement with histology. Note that $D_{\perp}$ (20 Hz) is almost independent with mean axon diameter ($r = 0.02$), while $D_{\perp}$ (40 Hz) shows a slight dependence on mean axon diameter ($r = 0.28$). This is consistent with our *ex vivo* rat spinal cord results shown above that indicate that $D_{\perp}$ values at long diffusion times do not correlate with mean axon diameter but $DDR_{\perp}$ is a reliable indicator of mean axon diameter.

Discussion

Mapping axon diameter is of interest for a variety of pre-clinical and clinical applications. Diffusion MRI is well suited to measure axon diameters non-invasively, but current quantitative diffusion MRI methods are usually based on complex mathematical models that require sophisticated non-linear fitting techniques. Moreover, these methods usually require very long scanning times that are usually not tolerable for human patients *in vivo*. This study introduces a novel model-free metric, $DDR_{\perp}$ (perpendicular diffusion dispersion rate), which is simply the slope of the temporal diffusion spectrum over a relatively low frequency range. The slope (which has dimensions of area) correlates well with axon diameter. The spectrum disperses with gradient frequency approximately linearly over a relatively narrow frequency range. $DDR_{\perp}$ correlates with the mean axon diameter significantly in all white matter tracts with mean axon diameters in the range less than $5 \mu\text{m}$, i.e. the typical range of mean axon diameters in the central nervous system. Note that the ActiveAx model (20), is also capable of reporting an index of mean axon diameter (instead of actual values), but it requires much longer acquisition times. By contrast, our results show that $DDR_{\perp}$ can be obtained with a minimum of two diffusion coefficient measurements taken at a minimum of two frequencies, so that the total scanning time could be reduced to several minutes, depending on the SNR requirement. Therefore, $DDR_{\perp}$ can serve as a simplified metric for fast mapping mean axon diameters of white matter tissues without complex non-linear data fitting, which is very suitable for in-line data processing in practical applications of certain regions, e.g. corpus callosum and spinal cord.

Due to the complex contributions from both intra and extra-axonal diffusion compartments, the accurate quantitative model for $DDR_{\perp}$ is still under development. In a recent study, it has been found that if diffusion time is sufficiently long, extra-axonal diffusion coefficient is dependent on f , while intra-axonal diffusion is dependent on f^2 (47). Therefore, diffusion measurements in such circumstances are dominated by extra-axonal diffusion. This may provide a reasonable alternative explanation (other than axon shrinkage during tissue preparation for histology) that current diffusion measurements e.g. ActiveAx overestimate axon diameter compared with histology. This is consistent with our simulated intra- and extra-axonal ADCs especially for tracts with smaller mean axon diameters. For example, $AxD = 1.10 \mu\text{m}$ for the dCST tract shown in Figure 2, and intra-axonal ADCs are much smaller than extra-axonal ADCs. If $D = 1 \mu\text{m}^2/\text{ms}$, the root mean square displacement

corresponding to the highest frequency 250 Hz (shortest diffusion time) is about 1.41 μm , larger than the corresponding AxD. Therefore, diffusion measurements in these tissue regions are dominated by extra-axonal diffusion. This is also consistent with results shown in Fig.3 that $\text{DDR}_{\perp}$ is mainly determined by $\text{DDR}_{\perp\text{ex}}$ when $\text{AxD} < 1.5 \mu\text{m}$. However, when $\text{AxD} > 3.5 \mu\text{m}$ such as 5.98 μm in VST, the intra-axonal ADC is higher than extra-axonal ADC for frequencies $> 50 \text{ Hz}$ (see Figure 2), and $\text{DDR}_{\perp}$ is similar to $\text{DDR}_{\perp\text{in}}$ in such cases. This indicates that the correlation between $\text{DDR}_{\perp}$ and AxD covers a broad range of diffusion times as well as mean axon diameters, and hence an quantitative model to describe such a correlation should not be limited to long or short diffusion times only. Nonetheless, the experimental observation reported in the current work introduced a promising new metric mapping mean axon size, and may have potential in clinical practice.

Note that simulated ADC values (intra-, extra-axonal and both) of all tracts shown in Figure 2 were fit well with a linear function ($R^2 < 0.93$), in contrast to the prediction that extra-axonal ADC is dependent on f^2 (47). This is presumably because the frequency range used was narrow, and therefore the quadratic dependence of intra-axonal ADC on frequency can be approximated as a linear function in such a narrow frequency range. It would be interesting to investigate the accuracy of the theory developed in (47) using the histology data acquired in the current study.

Our results confirm that the perpendicular diffusion coefficients with relatively long diffusion times ($> 12 \text{ ms}$) are not well correlated with the mean axon diameter, which is consistent with previous reports (8–10). However, for the first time, we report that the perpendicular diffusion coefficients with very short diffusion times (e.g. $< 2.5 \text{ ms}$) are strongly correlated with mean axon diameter ($r > 0.84$, see Figure 6A). This can be explained by considering the characteristic diffusion length (root mean square displacement) of water molecules in each type of measurement. When diffusion time is larger than 12 ms, the characteristic diffusion length of 6.9 μm is much larger than the mean axon diameter of all white matter tracts, so the water molecules in all axons experience the restrictions of the axon walls and the apparent perpendicular diffusion coefficients are not much different in small or large axons. When the diffusion time is $\sim 0.83 \text{ ms}$, the corresponding characteristic diffusion length is $\sim 1.82 \mu\text{m}$, comparable to the smallest mean axon diameter in dCST ($\sim 1.4 \mu\text{m}$), so the measured perpendicular diffusion coefficients would be higher in larger spaces because the water molecules are less restricted than in these smaller axons. However, extremely short diffusion times are usually difficult to achieve in practice, so measuring perpendicular diffusion coefficient with very short diffusion times may be difficult to achieve in practice. By contrast, the $\text{DDR}_{\perp}$ can be obtained with moderately low frequencies which can be readily achieved with conventional human MRI scanners *in vivo*. More importantly, $\text{DDR}_{\perp}$ provides a better correlation with the mean axon diameter than even the perpendicular diffusion coefficient with the shortest diffusion time (0.83 ms) used in the current study. This less stringent requirement on diffusion time and higher correlation with mean axon diameter make $\text{DDR}_{\perp}$ an attractive metric to measure mean axon diameters of white matter tissues *in vivo*.

Because $\text{DDR}_{\perp}$ correlates approximately linearly with the mean axon diameter AxD, a more straightforward approach is to obtain the proportionality constant to convert $\text{DDR}_{\perp}$ directly

to an estimate of AxD. However, the proportionality constant is dependent on the frequency range used in experiments as shown in Figure 3. It is interesting that the frequency ranges using in the current study were very different for rat spinal cord (50 – 300 Hz) and human corpus callosum (20 – 40 Hz), but $DDR_{\perp}$ correlates well with AxD in both cases. This indicates that although the proportionality constant may vary with the frequency range, the linear dependence of $DDR_{\perp}$ on AxD preserves with a brand range of frequencies. Once a careful calibration is done with a fixed frequency range, the direct conversion from $DDR_{\perp}$ to AxD is possible with standardized parameters in practice, which is more desirable to diagnosis purposes.

The effective diffusion time corresponding to the frequencies 20 – 40 Hz used in the human corpus callosum study was 6.25 – 12.5 ms, resulting in root mean square displacements 3.5 – 5 μm if the diffusion coefficient is 1 $\mu\text{m}^2/\text{ms}$. These values are larger than the reported typical axon diameters in the human corpus callosum (57), but the $DDR_{\perp}$ obtained in this frequency range correlated well with the mean AxD. It is possible that the low frequency OGSE measurements of human corpus callosum with low diffusion gradient strengths (< 60 mT/m) were actually more sensitive to relatively larger axons (the higher end tail of axon diameter distribution) and/or extra-axonal diffusion, which correlate with the mean axon size. Nevertheless, the perpendicular ADC values did not correlate with mean axon size while $DDR_{\perp}$ did, indicating that $DDR_{\perp}$ is potentially a surrogate indicator of mean axon diameter in human imaging. Note that with the current advanced gradient systems (58), the frequency range of human imaging can be significantly enhanced, and hence the sensitivity to the relatively small axons can be significantly increased.

The correlation between $DDR_{\perp}$ and mean axon diameter is valid in the regions when the diffusion gradients are perpendicular to axons. By contrast, with the expense of relatively longer scanning time, orientationally invariant estimates of axon diameter can be mapped over the whole brain using other model-based methods, e.g. the ActiveAx model. Therefore, in the current study, rat spinal cord and human corpus callosum were used because of their simple geometric organizations and prior knowledge of axonal directions, and $DDR_{\perp}$ can be obtained with diffusion gradients applied in a single direction chosen *a priori*. For more practical *in vivo* measurements, diffusion tensor imaging (DTI) can be performed first to determine the perpendicular direction of axon fiber bundles, followed by $DDR_{\perp}$ measurements. It would be also interesting to investigate whether the dispersion rate of perpendicular tensor eigenvalues vs frequency correlates with the mean axon diameter.

It should be noted that some physiological complications may affect the implementation of $DDR_{\perp}$ for fast mapping axon size. The dispersion of axon orientations has been reported and modeled previously (59), but its exact influence on $DDR_{\perp}$ remains unclear. In addition, when the volume fraction of extra-axonal space increases significantly when some neurodegenerative diseases occur, the diffusion properties of extra-axonal space may vary significantly, which in turn complicates the simple data analysis of DDR. The influence of varying extra-axonal diffusion on $DDR_{\perp}$ needs to be investigated thoroughly.

Note that the frequency ranges used in the current animal and human studies were different, which was mainly due to the clinically available gradient strength. However, it could be also

problematic to perform both animal and human studies using the same diffusion gradient parameters and frequencies. For example, the tissue properties (e.g. intra- and extra-axonal diffusion coefficient and transcytolemmal exchange rate) can be very different for animal vs human, and *in vivo* vs *ex vivo* conditions. It is interesting to investigate influences of these effects on $DDR_{\perp}$ comprehensively. It is encouraging that $DDR_{\perp}$'s with different frequency ranges showed no statistical differences between each other (see Figure 7), presumably due to the well-behaved linear dispersion of the diffusion spectrum vs frequency over the narrow frequency range considered. For human studies on a regular clinical MRI machine, only relatively low gradient frequencies can be obtained, e.g. up to 40 Hz used in the current study, due to the limitations on gradient hardware and possible peripheral nerve stimulation (58). However, $DDR_{\perp}$ can still be obtained from diffusion measurements in this frequency range, and hence can serve as an indicator of mean axon diameter, as shown in our *in vivo* human corpus callosum study.

One limitation of implementing $DDR_{\perp}$ measurements clinically is SNR. In order to increase diffusion weighting (b value), a long gradient duration (40 ms) was chosen, resulting in a long echo time (124 ms), which significantly decreases SNR. To compensate for this, altogether 32 single-shot acquisitions were averaged to achieve reasonable values. Note that even with this limitation, only ~8 minutes were required to measure $DDR_{\perp}$, which is remarkably less than the previously reported 1 hour whole-brain scan (20) and 35 mins of human corpus callosum only (21). More advanced human gradient coils, such as the Connectome coil with strength up to 300 mT/m for human heads (58), can significantly reduce the echo time and hence enhance SNR. This will further accelerate the measurements of $DDR_{\perp}$ by reducing the number of repetitions, and therefore make it more suitable for clinical applications.

Conclusions

In the current study, we report for the first time that the perpendicular diffusion coefficients with very short diffusion times (< 2.5 ms) obtained using temporal diffusion spectroscopy are significantly correlated with the mean axon diameter. However, due to hardware limitations, this method may not be feasible on human scanners in practice. We thereby propose a novel simple metric, $DDR_{\perp}$ (the dispersion rate of perpendicular diffusion coefficients vs gradient frequency) over a small range of low frequencies, and have shown that this new metric can serve as a fast, simple and easy-to-use metric to map mean axon diameter within white matter with clinical feasibility and potential.

ACKNOWLEDGEMENTS

This study was supported by NIH K25CA168936, R01CA109106, R01CA173593, R01EB001744, P50CA128323, and K25EB013659. The authors thank Dr. William M. Valentine for providing the light microscopy, Drs. E. Brian Welch and Yansong Zhao for stimulating discussions about pulse sequence optimization on Philips MRI systems, and technologists Donna M. Butler, Kristen George-Durrett, Clair Kurtenbach, Leslie McIntosh, and David Pennell for assistance with data acquisition and subject interaction. Tissue preparation for histology was performed in part through the use of the VUMC Cell Imaging Shared Resource.

Abbreviations

AxD	Volume-weighted axon diameter D
D_⊥	Apparent diffusion coefficient perpendicular to axon bundles
DDR_⊥	Diffusion coefficient dispersion rate perpendicular to axon bundles
OGSE	Oscillating gradient spin echo
PGSE	Pulsed gradient spin echo

References

1. Debanne D, Campanac E, Bialowas A, Carlier E, Alcaraz G. Axon Physiology. *Physiol Rev.* 2011; 91:555–602. [PubMed: 21527732]
2. Medana IM, Esiri MM. Axonal damage: a key predictor of outcome in human CNS diseases. *Brain.* 2003; 126:515–530. [PubMed: 12566274]
3. Ganter P, Prince C, Esiri MM. Spinal cord axonal loss in multiple sclerosis: a post-mortem study. *Neuropathol Appl Neurobiol.* 1999; 25:459–67. [PubMed: 10632896]
4. Cluskey S, Ramsden DB. Mechanisms of neurodegeneration in amyotrophic lateral sclerosis. *Mol Pathol.* 2001; 54:386–92. [PubMed: 11724913]
5. Piven J, Bailey J, Ranson BJ, Arndt S. An MRI study of the corpus callosum in autism. *Am J Psychiatry.* 1997; 154:1051–6. [PubMed: 9247388]
6. Smith DH, Meaney DF. Axonal Damage in Traumatic Brain Injury. *Neurosci.* 2000; 6:483–495.
7. Wilkinson, a E.; Bridges, LR.; Sivaloganathan, S. Correlation of survival time with size of axonal swellings in diffuse axonal injury. *Acta Neuropathol.* 1999; 98:197–202. [PubMed: 10442560]
8. Schwartz ED, Cooper ET, Chin C-L, Wehrli S, Tessler A, Hackney DB. Ex vivo evaluation of ADC values within spinal cord white matter tracts. *AJNR Am J Neuroradiol.* 2005; 26:390–7. [PubMed: 15709142]
9. Schwartz ED, Cooper E, Fan Y, Jawad A. MRI diffusion coefficients in spinal cord correlate with axon morphometry. *Neuroreport.* 2005; 16:73–76. [PubMed: 15618894]
10. Gullapalli J, Krejza J, Schwartz ED. In vivo DTI evaluation of white matter tracts in rat spinal cord. *J Magn Reson imaging.* 2006; 24:231–4. [PubMed: 16767707]
11. Assaf Y, Mayk A, Cohen Y. Displacement Imaging of Spinal Cord Using q-Space Diffusion Weighted MRI. *Magn Reson Imaging.* 2000; 44:713–722.
12. Assaf Y, Cohen Y. Assignment of the water slow-diffusing component in the central nervous system using q-space diffusion MRS: implications for fiber tract imaging. *Magn Reson Med.* 2000; 43:191–9. [PubMed: 10680682]
13. Callaghan P, Coy A, MacGowan D. Diffraction-like effects in NMR diffusion studies of fluids in porous solids. *Nature.* 1991; 351:467–469.
14. Cory DG, Garroway AN. Measurement of translational displacement probabilities by NMR: an indicator of compartmentation. *Magn Reson Med.* 1990; 14:435–444. [PubMed: 2355827]
15. Mitra PP, Halperin BI. Effects of Finite Gradient-Pulse Widths in Pulsed-Field-Gradient Diffusion Measurements. *J Magn Reson Ser A.* 1995; 113:94–101.
16. Ong HH, Wright AC, Wehrli SL, Souza A, Schwartz ED, Hwang SN, Wehrli FW. Indirect measurement of regional axon diameter in excised mouse spinal cord with q-space imaging: simulation and experimental studies. *Neuroimage.* 2008; 40:1619–32. [PubMed: 18342541]
17. Assaf Y, Freidlin RZ, Rohde GK, Basser PJ. New modeling and experimental framework to characterize hindered and restricted water diffusion in brain white matter. *Magn Reson Med.* 2004; 52:965–978. [PubMed: 15508168]
18. Assaf Y, Blumenfeld-Katzir T, Yovel Y, Basser PJ. AxCaliber: a method for measuring axon diameter distribution from diffusion MRI. *Magn Reson Med.* 2008; 59:1347–1354. [PubMed: 18506799]

19. Barazany D, Basser PJ, Assaf Y. In vivo measurement of axon diameter distribution in the corpus callosum of rat brain. *Brain*. 2009; 132:1210–1220. [PubMed: 19403788]
20. Alexander DC, Hubbard PL, Hall MG, Moore EA, Ptito M, Parker GJM, Dyrby TB. Orientationally invariant indices of axon diameter and density from diffusion MRI. *Neuroimage*. 2010; 52:1374–89. [PubMed: 20580932]
21. Schneider T, Wheeler-Kingshott C, Alexander D. Mapping the axon diameter index in the corpus callosum is clinically feasible. *Proceedings of the 20th Annual Meeting of ISMRM*. 2012; 20:350.
22. Horowitz A, Barazany D, Tavor I, Bernstein M, Yovel G, Assaf Y. In vivo correlation between axon diameter and conduction velocity in the human brain. *Brain Struct Funct*. 2015; 220:1777–1788. [PubMed: 25139624]
23. Innocenti GM, Caminiti R, Aboitiz F. Comments on the paper by Horowitz et al. (2014). *Brain Struct Funct*. 2015; 220:1789–90. [PubMed: 25579065]
24. Shemesh N, Ozarslan E, Komlosh ME, Basser PJ, Cohen Y. From single-pulsed field gradient to double-pulsed field gradient MR: glean new microstructural information and developing new forms of contrast in MRI. *NMR Biomed*. 2010; 23:757–80. [PubMed: 20690130]
25. Komlosh ME, Özarslan E, Lizak MJ, Horkayne-Szakaly I, Freidlin RZ, Horkay F, Basser PJ. Mapping average axon diameters in porcine spinal cord white matter and rat corpus callosum using d-PFG MRI. *Neuroimage*. 2013; 78:210–6. [PubMed: 23583426]
26. Koch, M a; Finsterbusch, J. Towards compartment size estimation in vivo based on double wave vector diffusion weighting. *NMR Biomed*. 2011; 24:1422–32. [PubMed: 21755551]
27. Siow B, Drobnjak I, Ianus A, Christie IN, Lythgoe MF, Alexander DC. Axon radius estimation with Oscillating Gradient Spin Echo (OGSE) Diffusion MRI. *Diffus Fundam*. 2013; 18:1–6.
28. Xu J, Li H, Harkins KD, Jiang X, Xie J, Kang H, Does MD, Gore JC. Mapping mean axon diameter and axonal volume fraction by MRI using temporal diffusion spectroscopy. *Neuroimage*. 2014; 103:10–19. [PubMed: 25225002]
29. Li H, Gore JC, Xu J. Fast and robust measurement of microstructural dimensions using temporal diffusion spectroscopy. *J Magn Reson*. 2014; 242:4–9. [PubMed: 24583517]
30. Gore JC, Xu J, Colvin DC, Yankeelov TE, Parsons EC, Does MD. Characterization of tissue structure at varying length scales using temporal diffusion spectroscopy. *NMR Biomed*. 2010; 23:745–756. [PubMed: 20677208]
31. Xu J, Does MD, Gore JC. Sensitivity of MR diffusion measurements to variations in intracellular structure: Effects of nuclear size. *Magn Reson Med*. 2009; 61:828–833. [PubMed: 19205020]
32. Xu J, Does MD, Gore JC. Dependence of temporal diffusion spectra on microstructural properties of biological tissues. *Magn Reson Imaging*. 2011; 29:380–390. [PubMed: 21129880]
33. Xu J, Xie J, Jourquin J, Colvin DC, Does MD, Quaranta V, Gore JC. Influence of cell cycle phase on apparent diffusion coefficient in synchronized cells detected using temporal diffusion spectroscopy. *Magn Reson Med*. 2011; 65:920–926. [PubMed: 21413058]
34. Colvin DC, Jourquin J, Xu J, Does MD, Estrada L, Gore JC. Effects of intracellular organelles on the apparent diffusion coefficient of water molecules in cultured human embryonic kidney cells. *Magn Reson Med*. 2011; 65:796–801. [PubMed: 21337411]
35. Aggarwal M, Jones MV, Calabresi PA, Mori S, Zhang J. Probing mouse brain microstructure using oscillating gradient diffusion MRI. *Magn Reson Med*. 2012; 67:98–109. [PubMed: 21590726]
36. Kershaw J, Leuze C, Aoki I, Obata T, Kanno I, Ito H, Yamaguchi Y, Handa H. Systematic changes to the apparent diffusion tensor of in vivo rat brain measured with an oscillating-gradient spin-echo sequence. *Neuroimage*. 2013; 70:10–20. [PubMed: 23274188]
37. Boon, JP.; Yip, S. *Molecular Hydrodynamics*. Courier Dover Publications; 1980.
38. Callaghan PT, Stepisnik J. Frequency-Domain Analysis of Spin Motion Using Modulated-Gradient NMR. *J Magn Reson Ser A*. 1995; 117:118–122.
39. Portnoy S, Flint JJ, Blackband SJ, Stanisz GJ. Oscillating and pulsed gradient diffusion magnetic resonance microscopy over an extended b-value range: Implications for the characterization of tissue microstructure. *Magn Reson Med*. 2012; 69:1131–1145. [PubMed: 22576352]
40. Stepisnik J. Time-dependent self-diffusion by NMR spin-echo. *Phys B*. 1993; 183:343–350.

41. Van AT, Holdsworth SJ, Bammer R. In vivo investigation of restricted diffusion in the human brain with optimized oscillating diffusion gradient encoding. *Magn Reson Med*. 2014; 71:83–94. [PubMed: 23447055]
42. Baron CA, Beaulieu C. Oscillating Gradient Spin-Echo (OGSE) diffusion tensor imaging of the human brain. *Magn Reson Med*. 2014; 72:726–736. [PubMed: 24142863]
43. Does MD, Parsons EC, Gore JC. Oscillating gradient measurements of water diffusion in normal and globally ischemic rat brain. *Magn Reson Med*. 2003; 49:206–215. [PubMed: 12541239]
44. Xu J, Li K, Smith RA, Waterton JC, Zhao P, Chen H, Does MD, Manning HC, Gore JC. Characterizing tumor response to chemotherapy at various length scales using temporal diffusion spectroscopy. *PLoS One*. 2012; 7:e41714. [PubMed: 22911846]
45. Aggarwal M, Burnsed J, Martin LJ, Northington FJ, Zhang J. Imaging neurodegeneration in the mouse hippocampus after neonatal hypoxia-ischemia using oscillating gradient diffusion MRI. *Magn Reson Med*. 2014; 72:829–840. [PubMed: 24123409]
46. Novikov DS, Jensen JH, Helpert JA, Fieremans E. Revealing mesoscopic structural universality with diffusion. *Proc Natl Acad Sci U S A*. 2014
47. Burcaw LM, Fieremans E, Novikov DS. Mesoscopic structure of neuronal tracts from time-dependent diffusion. *Neuroimage*. 2015; 114:18–37. [PubMed: 25837598]
48. Shepherd TM, Thelwall PE, Stanis GJ, Blackband SJ. Aldehyde fixative solutions alter the water relaxation and diffusion properties of nervous tissue. *Magn Reson Med*. 2009; 62:26–34. [PubMed: 19353660]
49. Dula AN, Gochberg DF, Valentine HL, Valentine WM, Does MD. Multiexponential T2, magnetization transfer, and quantitative histology in white matter tracts of rat spinal cord. *Magn Reson Med*. 2010; 63:902–9. [PubMed: 20373391]
50. Harkins KD, Dula AN, Does MD. Effect of intercompartmental water exchange on the apparent myelin water fraction in multiexponential T2 measurements of rat spinal cord. *Magn Reson Med*. 2012; 67:793–800. [PubMed: 21713984]
51. Hu X, Le TH. Artifact reduction in EPI with phase-encoded reference scan. *Magn Reson Med*. 1996; 36:166–171. [PubMed: 8795036]
52. Deneff JF, Cordier AC, Mesquita M, Haumont S. The influence of fixation procedure, embedding medium and section thickness on morphometric data in thyroid gland. *Histochemistry*. 1979; 63:163–71. [PubMed: 115814]
53. Tang Y, Nyengaard JR. A stereological method for estimating the total length and size of myelin fibers in human brain white matter. *J Neurosci Methods*. 1997; 73:193–200. [PubMed: 9196291]
54. Maes F, Collignon A, Vandermeulen D, Marchal G, Suetens P. Multimodality image registration by maximization of mutual information. *IEEE Trans Med Imaging*. 1997; 16:187–98. [PubMed: 9101328]
55. 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]
56. Chin C, Wehrli FW, Hwang SN, Takahashi M, Hackney DB. Biexponential Diffusion Attenuation in the Rat Spinal Cord : Computer Simulations Based on Anatomic Images of Axonal Architecture. *Magn Reson Med*. 2002; 47:455–460. [PubMed: 11870831]
57. Aboitiz F, Scheibel AB, Fisher RS, Zaidel E. Fiber composition of the human corpus callosum. *Brain Res*. 1992; 598:143–153. [PubMed: 1486477]
58. Setsompop K, Kimmlingen R, Eberlein E, Witzel T, Cohen-Adad J, McNab JA, Keil B, Tisdall MD, Hoeft P, Dietz P, Cauley SF, Tountcheva V, Matschl V, Lenz VH, Heberlein K, Potthast A, Thein H, Van Horn J, Toga A, et al. Pushing the limits of in vivo diffusion MRI for the Human Connectome Project. *Neuroimage*. 2013; 80:220–33. [PubMed: 23707579]
59. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage*. 2012; 61:1000–16. [PubMed: 22484410]

Figure 1.

(A) Perpendicular diffusion spectra of impermeable cylinders with different diameters. Two frequencies (50 and 100 Hz, black thick lines) were chosen to calculate $\text{DDR}_\perp$. (B) correlates with the mean axon diameter (AxD).

Figure 2.

Top image: a T_2 -weighted image showing locations of different white matter tracts. The histological images are shown in the second row, and the corresponding segmented images are in the third row as input structures for computer simulations. The black regions represent myelin, white and gray regions are intra- and extra-axonal spaces, respectively. The scale (red) bars represent 10 μm . The bottom plots show simulated perpendicular temporal diffusion spectra $D_{\perp}(f)$ arising from whole space (black pentagrams), intra-axonal only (red circles) and extra-axonal only (blue diamonds) of each white matter tract in the frequency range of 50 – 250 Hz. The lines represent linear fittings of $D_{\perp}(f)$ arising from whole space vs frequency with R^2 values provided.

Figure 3. The simulated correlations of three types of dispersion rates (i.e. $DDR_{\perp}$ arising from whole space, $DDR_{\perp in}$ from the intra-axonal space only, and $DDR_{\perp ex}$ from the extra-axonal space only) calculated from simulations with the mean axon diameter AxD measured from histology. The circles are data, and each solid line represents a linear fit for those with $AxD < 5 \mu m$. All correlations have $p < 0.01$.

Figure 4.

Experimental $D_{\perp}(f)$ (perpendicular temporal diffusion spectrum) of six different white matter tracts obtained using diffusion MRI from a representative rat spinal cord. Circles represent the mean perpendicular diffusion coefficients of individual white matter tracts, and the error bars represent the corresponding standard deviations. Solid lines represent the linear fittings using the first N ($N = 2, 3$ or 4) frequencies (corresponding to highest frequencies 100, 150, or 200 Hz), respectively. The corresponding histological AxD : $5.54 \mu\text{m}$ (VST), $3.46 \mu\text{m}$ (FC), $3.01 \mu\text{m}$ (RST), $2.44 \mu\text{m}$ (ReST), $1.31 \mu\text{m}$ (FG), and $1.10 \mu\text{m}$ (dCST).

Figure 5.

$D_{\perp}$ (perpendicular diffusion coefficient) maps obtained using the OGSE (the first row) and the PGSE (the second row) methods. The bottom images are three $DDR_{\perp}$ maps estimated using different ranges of frequencies in the fittings. The butterfly-shaped gray matter is indicated by the pink contours. N represents the first N ($N = 2, 3$ or 4) frequencies (corresponding to highest frequencies 100, 150, or 200 Hz) were used to fit $DDR_{\perp}$'s.

Figure 6.

(A) Correlations of $D_{\perp}(f)$ at different diffusion times with histological mean axon diameter (AxD) obtained using different effective diffusion times. (B) Correlations of $DDR_{\perp}$ with AxD. Solid lines represent linear fits, and the correlation coefficients (r 's) obtained using the linear mixed-effects models are shown. (C) Summarized correlation coefficients in (A) and (B), suggesting r values are dependent on the diffusion time. For reference, r values for $DDR_{\perp}$'s are also provided.

Figure 7. Bland-Altman plots of differences of different $\text{DDR}_{\perp}$'s. The solid lines are the mean differences for each comparison pair, and the dashed lines are the limits of agreement (mean difference ± 1.96 standard deviation).

Figure 8.

(A) Diagram of human corpus callosum with histological mean axon diameter. Dashed lines indicate four ROI regions used in the analysis. (Data adapted from (20)). (B) DDR $\perp$ maps of six healthy subjects. (C) Correlations between DDR $\perp$ and D $\perp$ (20 and 40 Hz) with histological mean axon diameter shown in (A). The correlation coefficients and p values obtained using the linear mixed-effects model are provided for each correlation.