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Measuring non-parametric distributions of intravoxel mean diffusivities using a clinical MRI scanner

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Abstract

We measure spectra of water mobilities (i.e., mean diffusivities) from intravoxel pools in brain tissues of healthy subjects with a non-parametric approach. Using a single-shot isotropic diffusion encoding (IDE) preparation, we eliminate signal confounds caused by anisotropic diffusion, including microscopic anisotropy, and acquire *in vivo* diffusion weighted images (DWIs) over a wide range of diffusion sensitizations. We analyze the measured IDE signal decays using a regularized inverse Laplace transform (ILT) to derive a probability distribution of mean diffusivities of tissue water in each voxel. Based on numerical simulations we assess the sensitivity and accuracy of our ILT analysis and optimize an experimental protocol for use with clinical MRI scanners. *In vivo* spectra of intravoxel mean diffusivities measured in healthy subjects generally show single-peak distributions throughout the brain parenchyma, with small differences in peak location and shape among white matter, cortical and subcortical gray matter, and cerebrospinal fluid. Mean diffusivity distributions (MDDs) with multiple peaks are observed primarily in voxels at tissue interfaces and are likely due to tissue partial volume contributions. To quantify tissue-specific MDDs with improved statistical power, we average voxel-wise normalized MDDs in corresponding regions-of-interest (ROIs). This non-parametric, rotation-invariant assessment of isotropic diffusivities of tissue water may reflect important microstructural information, such as cell packing and cell size, and active physiological processes, such as water transport and exchange, which may enhance biological specificity in the clinical diagnosis and characterization of ischemic stroke, cancer, neuroinflammation, and neurodegenerative disorders and diseases.

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Keywords

Mean diffusivity distribution (MDD); isotropic diffusivity; tissue microstructure; MR relaxometry; rotation-invariant; MR diffusometry; mean apparent diffusion coefficient (mADC); mean diffusivity (MD)

1. Introduction

The mean diffusivity (MD) derived from diffusion tensor imaging (DTI) (Basser et al., 1994), is an eloquent and robust clinical biomarker for diagnosing and characterizing ischemic stroke (Lutsep et al., 1997), cancer (Brunberg et al., 1995), brain development (Neil et al., 1998), and numerous neurological disorders and diseases (Werring et al., 1999; Arfanakis et al., 2002; Huisman et al., 2003; McKinstry et al., 2002). MD can be measured as the mean of the diffusion tensor principal diffusivities (or one third of its Trace), and, at low b-values, is equal to the mean apparent diffusion coefficient (mADC) across measurements with large number of orientations uniformly sampling the unit sphere (Basser and Jones, 2002). As an imaging parameter, the MD removes signal modulations arising from bulk diffusion anisotropy and provides an orientationally averaged value of water mobility even in anisotropic white matter (Basser et al., 1994).

Nevertheless, for measurements with large diffusion sensitization (i.e., in the non-Gaussian regime), the value of MD depends on b-value and diffusion time, and reflects contributions from microscopic anisotropy (Mitra, 1995; Callaghan and Komlosh, 2002; Callaghan and Furo, 2004) due to restrictions and tissue architectural heterogeneity. As a result, at high b-values the MD measured with conventional methods may not provide a proper statistical average of the spectrum of mean diffusivities in microscopic tissue water pools within the voxel, resulting in limited biological specificity.

To overcome the effects of microscopic anisotropy on measuring mean diffusivities it is necessary to sensitize the signal to correlations during multiple diffusion periods (Topgaard, 2017; Westin et al., 2016) by modifying conventional spin echo diffusion encoding (Stejskal and Tanner, 1965). Isotropic diffusion encoding (IDE), also referred to as spherical tensor encoding, can be achieved by using specially designed diffusion gradient waveforms (Mori and Van Zijl, 1995; Wong et al., 1995; Eriksson et al., 2013; Sjölund et al., 2015) and provides isotropic weighting (i.e., mean diffusivity weighting) in individual microscopic water pools exhibiting Gaussian diffusion, regardless of their relative shapes, sizes, and orientations (Topgaard, 2017). An elegant application of this method called diffusion variance decomposition (DIVIDE) (Lasi et al., 2014) uses both spherical and linear tensor encoding to quantify, among other parameters, the mean and variance of intravoxel distributions of mean diffusivities, and has been applied *in vivo* (Szczipankiewicz et al., 2015). More recent studies (Topgaard, 2017; de Almeida Martins and Topgaard, 2016) demonstrate the possibility of measuring intravoxel distributions of mean diffusivities (i.e., isotropic diffusivities) across microscopic water pools using model-free, non-parametric approaches, and suggest the possibility of applying such methods to *in vivo* imaging (Dhital et al., 2017; Avram et al., 2018a; Tax et al., 2018).

In this study, we designed and optimized a clinical experiment for measuring *in vivo* diffusion weighted images (DWIs) with IDE over a wide range of diffusion sensitizations (b-values). From these measurements, we estimated the intravoxel mean diffusivity distributions (MDDs) of water in brain tissue. We compared and characterized these rotation-invariant spectra or distributions using region-of-interest analysis (ROIs) in healthy human subjects.

2. Materials and Methods

2.1. Pulse sequence design

All clinical experiments were conducted with a pulse sequence developed in-house to achieve IDE over a large range of diffusion sensitizations or b-values. The sequence in Fig. 1 is based on the original design proposed by Wong et al. (Wong et al., 1995) and was modified to accommodate the presence of a 180° (radio-frequency) RF refocusing pulse and crusher gradients. Specifically, for a desired maximum b-value, b_{max} ; maximum gradient amplitude, G_{max} ; and trapezoidal ramp duration, r , we numerically optimized the values of the trapezoidal pulse widths δ_y , and δ_z on GY, and GZ, along with the ratio R of the gradient amplitude on X to G_{max} (Fig. 1) needed to achieve isotropic diffusion sensitization, i.e., IDE. The width δ_x of the symmetric bipolar gradient pulse on GX was directly determined by the available diffusion time. The resulting pulse sequence uses only trapezoidal gradient pulses, which can be easily programmed and debugged on a conventional clinical MRI scanner and, most importantly, achieves an efficient IDE in a short diffusion preparation time. In addition, the diffusion encoding gradient waveforms are either symmetric or antisymmetric on all physical gradient axes, resulting in less severe encoding artifacts due to concomitant gradient fields (Bernstein et al., 1998; Baron et al., 2012). Fig. 1 shows the gradient waveforms for a $b_{max} = 6 \text{ ms}/\mu\text{m}^2$, or $6000 \text{ s}/\text{mm}^2$, refocusing duration of 5 ms , and ramp time of 3 ms . Once the fidelity of the gradient pulse shapes and timings were verified with an oscilloscope, the number of slices and the repetition time were empirically adjusted to accommodate for the power dissipation requirements and duty cycle limitations of the gradient system. More general and possibly more efficient IDE gradient waveforms may be achieved using the elegant optimization framework proposed by Sjölund et al. (Sjölund et al., 2015).

2.2. Clinical human brain data

Five healthy volunteers were scanned on a 3T Siemens Prisma clinical MRI scanner equipped with a maximum gradient strength of 80 mT/m/axis and a 32-channel RF coil under a clinical protocol approved by the institutional review board (IRB) of the Intramural Research Program of the National Institute of Neurological Disorders and Stroke (NINDS). Whole brain IDE DWIs were acquired with a 22 cm field-of-view (FOV), an in-plane resolution of 2.5 mm , a 5 mm slice thickness, echo time (TE) of 105 ms , and repetition time (TR) of 6 s . Images were acquired at 61 b-values from 0 to $6 \text{ ms}/\mu\text{m}^2$, with up to four averages for images with higher b-values, in a total scan duration of 15 minutes. In addition, we also acquired a DTI scan (using the same scan parameters but a b-value of $1 \text{ ms}/\mu\text{m}^2$ and 35 orientations), and high-resolution anatomical T1-weighted (MPRAGE) and T2-weighted scans. All diffusion data sets were processed with the TORTOISE software package

(Pierpaoli et al., 2010) to correct for echo-planar image (EPI) distortion due to eddy currents and field inhomogeneities, to register all DWI volumes to the high resolution T2-weighted anatomical template, and to resample the corrected images to a 2.5mm isotropic resolution.

2.3. Monte Carlo numerical simulations

We performed numerical simulations to determine the sensitivity and accuracy of the 1-D ILT-based non-parametric reconstruction of MDDs from data acquired with the abovementioned DWI clinical protocol. We started by analytically defining single- or two-peak MDDs using Gaussian functions with different means and variances to serve as the ground truth distributions. We then generated IDE measurements at the same b-values as those used in our clinical experiments. We added Gaussian noise to the real and imaginary channels independently and computed magnitude IDE signal decays with a signal-to-noise ratio (SNR) of 150 : 1 in the non-diffusion attenuated (baseline) image, which was similar to the typical SNR levels obtained with our clinical sequence in brain tissue after 4 averages. Using the ILT method described in the next section, we analyzed 500 independent instances of simulated noisy IDE measurements and estimated MDDs. We assessed the performance of the ILT reconstruction by comparing the mean and standard deviation of the MDDs with the corresponding ground truth distributions.

2.4. Inverse Laplace Transform analysis

The diffusion signal in each voxel can be represented as a summation of many signal components from intravoxel water pools in various microscopic tissue environments. If diffusion of water molecules in each of these pools is Gaussian, we can accurately describe the intravoxel signal components using diffusion tensors with different orientations, shapes, and sizes. Consequently, for an experiment with diffusion encoding gradient waveform $\mathbf{G}(t)$ we can write the total voxel signal as an integral over all microscopic water pools:

$$S(t) = \int_{\mathbf{D} \in V} e^{-\int_0^{TE} (\int_0^t \mathbf{G}(\tau) d\tau)^T \mathbf{D} (\int_0^t \mathbf{G}(\tau) d\tau) dt} p(\mathbf{D}) d\mathbf{D} \quad (1)$$

, where $p(\mathbf{D})$ is the probability density function of intravoxel diffusion tensors. For each intravoxel diffusion tensor the IDE gradient waveform in Fig. 1 provides isotropic weighting by the mean diffusivity, or the tensor Trace (Wong et al., 1995):

$$S(b) = \int_{\mathbf{D} \in V} e^{-b \text{Tr}(\mathbf{D})/3} p(\mathbf{D}) d\mathbf{D} \quad (2)$$

, where the scalar b-value, b , determines the amount of diffusion weighting.

We can acquire IDE DWIs with a wide range of diffusion weightings, b , by varying the diffusion gradient amplitude in Fig. 1 from $G = 0$ to G_{max} . In each voxel the signal decay as a function b can be related to the probability density function of mean diffusivity values in intravoxel water pools, $\mu(D)$, via the one-dimensional Laplace Transform:

$$S(b) = \int_{D \in V} e^{-bD} \mu(D) dD \quad (3)$$

Note that $\mu(D)$ is often referred to as the probability of isotropic diffusivity components $P(D_{iso})$ in the porous media literature, e.g., Eq. 64 in (Topgaard, 2017). From multiple IDE DWIs with different b-values, we can estimate $\mu(D)$ numerically using an inverse laplace transform (ILT) analysis (Provencher, 1982). To obtain a well-behaved solution to this ill-posed problem we used a MATLAB implementation of a non-negative least-squares fit with L2-norm regularization:

$$\hat{\mu} = \underset{\mu > 0}{\operatorname{argmin}} \left\| \begin{bmatrix} \mathbf{M} \\ \lambda \mathbf{I} \end{bmatrix} \mu - \begin{bmatrix} \mathbf{S} \\ \mathbf{0} \end{bmatrix} \right\|_2 \quad (4)$$

$\mathbf{S} = S(b)$ and $\mu = \mu(D)$ are vectors containing the IDE DWI signals at different b-values and the MDD spectral components, respectively; $\mathbf{I}$ is the identity matrix; $\mathbf{0}$ is a vector of zeros; $\mathbf{M}$ is the encoding matrix; and λ is a regularization parameter that controls the smoothness of the solution vector μ and is optimized for each voxel (Fordham et al., 1995). To better approximate a continuous distribution for the solution vector, the elements of $\mathbf{M}$ were defined by integrating over the spectral width, ω , for each spectral component centered at D_j :

$$M_{ij} = \frac{1}{2\omega} \int_{D_j - \omega}^{D_j + \omega} e^{-b_i D} dD \quad (5)$$

2.5. ROI analysis

To spatially resolve features of the sparse MDD maps we integrated the normalized MDDs in each voxel across spectral bands defined for small ($0-0.85 \mu\text{m}^2/\text{ms}$), intermediate ($0.85-2.0 \mu\text{m}^2/\text{ms}$), and large ($>2.0 \mu\text{m}^2/\text{ms}$) mean diffusivity components, and computed images of signal fractions corresponding to these bands. Next, to identify tissue-specific characteristics of MDDs with improved statistical power, we averaged normalized MDDs in ROIs containing: white matter (WM), cortical gray matter (GM), cerebrospinal fluid (CSF), and subcortical gray matter (scGM), including the putamen, caudate nucleus, and globus pallidus. These ROIs were initially defined based on the registered high-resolution T1-weighted (MPRAGE) and T2-weighted scans and subsequently filtered to remove contributions from voxels with significant partial volume effects. Specifically, we removed voxels with more than 5% large diffusivity signal fractions for WM, GM, and scGM ROIs, and voxels with less than 95% large diffusivity signal fractions for the CSF ROI. Tissue-specific MDDs were computed by averaging normalized MDDs from all voxels within each ROI. This approach quantifies tissue-specific MDDs efficiently and accurately by reducing noise through averaging single-voxel MDDs while simultaneously enforcing non-negativity of all spectral components at every location (voxel) within the ROI.

3. Results

3.1. Monte Carlo simulations

The numerical simulations using IDE signal decays with the same experimental design and SNR level as our clinical experiments suggest that the ILT analysis can estimate normalized MDDs from *in vivo* data obtained with a conventional MRI scanner. Fig. 2 shows the numerical simulation results from four didactic examples that illustrate the accuracy, precision, and spectral resolution of the ILT-based MDD estimation:

1. Unimodal MDDs using normal distributions with mean and standard deviations $[\mu, \sigma]$ of $[3.0, 0.15] \mu\text{m}^2/\text{ms}$ and $[0.8, 0.3] \mu\text{m}^2/\text{ms}$, respectively.
2. Bimodal MDDs using mixtures of two normal distributions $[\mu_1, \sigma_1]$, and $[\mu_2, \sigma_2]$, with a signal fraction of 0.4 and parameters $[\mu_1, \mu_2, \sigma_{1,2}] = [0.4, 0.5, 3.0, 0.2] \mu\text{m}^2/\text{ms}$ and $[0.4, 0.5, 1.0, 0.15] \mu\text{m}^2/\text{ms}$, respectively.

For each ground truth distribution, the mean normalized MDD (Fig. 2, black line) follows relatively accurately the ground truth distribution (Fig. 2, red line), with various levels of uncertainty as quantified by the standard deviations at each spectral component (Fig. 2, blue bands around the black line). For unimodal distributions (Fig. 2, top row), both the peak location and width can be estimated with relatively good accuracy and precision (small standard deviation). For bimodal distributions (Fig. 2, bottom row), the uncertainty increases (lower precision), and, while the locations and areas under the curve (AUCs) of individual peaks can still be estimated reasonably well, the widths and shapes of these peaks are resolved with limited accuracy. Because the standard deviation at each spectral component quantifies uncertainty in both amplitude and location across noisy normalized MDDs (Prange and Song, 2009) the precision in estimating bimodal distributions decreases as the separation between peaks increases. The spectral resolution of the estimated MDDs is $\approx 0.45 \mu\text{m}^2/\text{ms}$ (Fig. 2). However, due to the non-linearity of the ILT, and the non-orthonormality of its basis functions (i.e., exponentials), the resolution is not uniform across the spectrum (Whittall and MacKay, 1989; Borgia et al., 1998; Istratov and Vyvenko, 1999; Prange and Song, 2009). In addition, when the ILT is performed numerically, the spectral resolution and accuracy can be further limited by the grouping effect of the L2-norm regularization.

3.2. IDE DWIs in the human brain

In this study, we used a large voxel volume of 31.25 mm^3 and a very efficient IDE gradient waveform that allows scanning with relatively short TE and high b-values to achieve the high SNR levels and wide dynamic range required for non-parametric estimation of MDDs using ILT analysis. IDE DWIs acquired *in vivo* showed excellent SNR and a large signal dynamic range (Fig. 3). Typical values of SNR in the non-diffusion attenuated (i.e., baseline $b = 0 \text{ ms}/\mu\text{m}^2$) images were 150 in WM, 250 in cGM, 105 in scGM, and 300 in CSF. Based on our Monte Carlo simulations, these SNR levels are sufficient for reliable estimation of MDDs in most brain tissues (Fig. 2). While higher SNR levels can be obtained by further reducing spatial resolution (i.e., increasing the FOV), it is worth pointing out some potential sources of errors and imaging artifacts for measuring IDE DWIs *in vivo*.

At high SNR levels sources of errors due to common imaging artifacts (such as Gibbs ringing, Nyquist ghosting, incomplete fat suppression), physiological and subject motion, and tissue susceptibility variations become prominent. For instance, if these confounding signals represent only 2% of the tissue signal, at an SNR of 150, these contributions are well above the noise level (3 : 1) and could bias the MDD estimation. In this study, we deliberately used an anisotropic voxel size with a 2.5 mm in-plane resolution and a 5 mm slice thickness to increase the imaging matrix size and minimize Gibbs ringing artifacts while maintaining good SNR. Gibbs ringing artifact removal using the method proposed by Kellner et al. (Kellner et al., 2016) was also performed on each IDE DWI. To mitigate confounds from chemical shift, we used spatial-spectral RF excitation pulses. Signal instabilities that may arise due to patient and physiological motion were mitigated by keeping the total scan duration relatively short and by instructing subjects to remain still during the duration of the scan. Every IDE DWI was acquired in a single excitation and is inherently orientationally averaged. Overall IDE DWIs were acquired with 61 different diffusion sensitizations (b-values) and a TR=6s, for a total scan duration of 6:30min. In this study, however, to acquisition of IDE DWIs was repeated up to 4 times to improve SNR.

The diffusion gradient waveform in Fig. 1 provides IDE in all intravoxel microscopic water pool whose diffusion properties can be described with an arbitrarily oriented diffusion tensor. Imperfections in the applied gradient waveforms, can alter the effective b-tensor (Westin et al., 2016), leading to deviations from the desired isotropic encoding, especially at high b-values. Such imperfections may be due to:

1. Concomitant field effects that are not refocused by the 180° RF pulse.
2. Eddy currents contributions to the diffusion encoding.
3. Gradient non-linearities due to coil design imperfections (Rogers et al., 2017).
4. Magnetic field inhomogeneities due to tissue components (e.g., iron) that can lead to unwanted cross-terms in the computation of the encoding tensor (Mori and Van Zijl, 1995).

The IDE gradient waveform in Fig. 1 using only trapezoidal pulses and a symmetric diffusion time with respect to the refocusing 180° RF pulse makes it easier to quantify errors from the above-mentioned sources (Baron et al., 2012) and to derive voxel-wise corrections of diffusion encoding values.

3.3. MDDs in the human brain

Our preliminary results reveal a remarkable consistency of intravoxel water mean diffusivity values within healthy human brain tissue. MDDs in single voxels containing healthy brain parenchyma (GM or WM) showed single-peak distributions with only slight differences in peak locations and shapes (Fig.4). In most of the brain parenchyma the IDE signal decays can be modeled reasonably well even with a single exponential decay. Distributions with multiple peaks were measured primarily near tissue interfaces and are likely due to significant partial volume contributions from CSF (Fig. 4). In voxels with negligible CSF partial volume contributions, MDDs revealed slightly smaller intravoxel mean diffusivity values (peak locations) in GM compared to WM, especially in the basal ganglia (scGM).

WM peaks appeared slightly narrower in the corpus callosum. Voxels containing primarily CSF generally showed a distinctive, dominant peak with large diffusivity around $3 \mu\text{m}^2/\text{ms}$ of water at body temperature, as expected.

The ROI analysis showed relatively small variations of tissue-specific MDDs across single-voxel measurements (Fig.5). ROI-averaged MDDs support our findings from Fig. 4, revealing smaller intravoxel mean diffusivity values in GM compared to WM. The large variation across MDDs in the CSF ROI can be explained, in part, due to the larger uncertainty in estimating the locations and amplitudes of high diffusivity spectral components for which only the first few data points in the IDE signal decay (up to $1 \text{ ms}/\mu\text{m}^2$) contain relevant information. This effect was observed in the numerical simulation (Fig. 2). Other factors that may contribute to the variation in the CSF diffusivity components include partial volume effects or signal instabilities arising from physiological (e.g., cardiac) motion.

Signal fractions computed by integrating normalized MDDs across spectral bands of small ($0.85 \mu\text{m}^2/\text{ms}$), intermediate component ($0.85\text{--}2.00 \mu\text{m}^2/\text{ms}$), and large diffusivities ($2.00 \mu\text{m}^2/\text{ms}$) showed good spatial correspondence with tissue structures (Fig. 6). The largest fractions of small diffusivity components, $0.85 \mu\text{m}^2/\text{ms}$, were observed in scGM, in particular, in the putamen, the globus pallidus, and caudate nucleus and, to a lesser extent, the thalamus (Fig. 6). Larger diffusivity components were observed mainly in regions of CSF.

4. Discussion

4.1. Isotropic diffusion encoding

The IDE signal at a given b-value differs from the conventional mADC-, or MD-weighted signal, even though both are rotation-invariant. The mADC-weighted signal computed from multiple DWIs acquired with LTE using the standard diffusion sequence (Stejskal and Tanner, 1965) with gradient orientations uniformly sampling the unit sphere can be modulated by anisotropic restrictions and their orientational dispersion, while the IDE signal provides isotropic encoding in all microscopic water pools, regardless of shape and orientation (Topgaard, 2017; Westin et al., 2016). While mADC-weighted DWIs can be obtained with larger b-values, better SNR, and a well-defined diffusion time (Avram et al., 2018b), IDE DWIs can be acquired faster and provide a more specific and quantitative assessment of intravoxel water diffusivities.

Clinically, these two types of measurements can provide complementary information (Szczepankiewicz et al., 2016). For example, DIVIDE (Lasi et al., 2014) estimates microstructural parameters quantifying features of MDDs, microscopic anisotropy, and orientational order from both LTE and IDE measurements, and shows promise for clinical applications in tumors (Szczepankiewicz et al., 2015). The robustness of DIVIDE relies, in part, on assuming an analytical form for the MDD as a convenient way of estimating its first and second statistical moments from low-b data. Our results generally support the validity of this assumption in healthy brain parenchyma and in voxels with negligible partial volume, but also highlight the need for model-free methods to characterize microstructure in regions

with partial volume and/or pathophysiology by explicitly measuring MDDs with non-parametric approaches, such as the ILT.

4.2. Limitations of the ILT analysis

From the IDE signal decay over a wide range of b-values via the ILT we can estimate rotation-invariant distributions of the mean diffusivities in intravoxel water pools, if we assume that these pools are non-exchanging and undergo Gaussian diffusion. These assumptions may be violated in microenvironments where water is highly restricted at the timescale of the diffusion preparation duration ($\approx 90\text{ms}$), likely leading to a time-dependence of certain MDD components (Peled et al., 1999). For living biological tissues, additional deviations from these assumptions may arise due to microscopic flow, i.e., IVIM (Le Bihan et al., 1986), or exchange between water pools. In addition, the measured MDD spectral components are inherently T2-weighted since the IDE DWIs are acquired with relatively large TEs. This weighting can lead to inconsistencies between MDDs measured with different imaging parameters in future studies. Extending isotropic diffusometry MRI with multidimensional MRI relaxometry analysis (Tax et al., 2018; de Almeida Martins and Topgaard, 2018; Benjamini and Basser, 2017) will help identify and quantify biologically specific microscopic proton pools and characterize their roles in healthy tissue and in disease.

The relatively large uncertainty in estimating the CSF peak *in vivo* can also be attributed to variations in partial volume artifacts across IDE DWIs with different b-values. In DWIs acquired with low diffusion sensitizations ($b = 0.2\text{ms}/\mu\text{m}^2$) the signal intensity in CSF can be significantly larger than that in brain parenchyma due to the higher proton density, longer T2, and closer proximity to the RF receiver array. Even very small changes in partial volume contributions due to subject and physiological motion and can result in signal variations/instabilities that are 4–5 times higher than the noise level in DWIs acquired with low b-values. These signal artifacts can significantly deteriorate the fidelity of the estimated diffusivity spectra. The most contaminated voxels are at the GM/CSF boundary where the high receive sensitivity of the RF coil array further amplifies these signal variations (and SNR), and near the ventricles where physiological motion is most noticeable. We found that for some subjects, excluding measurements with $b = 0.2\text{ms}/\mu\text{m}^2$ from the ILT analysis in voxels with high CSF content stabilizes the position of the CSF peak and prevents artifactual accumulation of diffusion components at the upper bound of the spectral range. In general, the effect of partial volume inconsistencies can be mitigated by:

1. removing outliers from low-b DWIs (e.g., based on the standard deviation of repeated measurements),
2. reducing the total scan duration to minimize the impact of physiological and subject motion, or
3. modeling or filtering out high diffusivity components and fitting spectral diffusivity components over a range limited to parenchymal diffusivities ($0.1\text{--}1.5\mu\text{m}^2/\text{ms}$).

Because the IDE signal decays are inherently analyzed as a sum of multiple exponential functions, isotropic diffusometry can directly perform free water elimination (Pierpaoli and Jones, 2004; Pasternak et al., 2009). However, for *in vivo* imaging, large diffusivity components may also arise from physiological sources other than CSF such as changes in blood flow, glymphatic flow, blood volume, or in microdynamic processes of parenchymal water transport and exchange. For certain pathologies, changes in these high diffusivity spectral components could have great clinical value. Approximating the high diffusivity spectral peak with a single delta function may omit potentially clinically relevant physiological information that could be derived from studying the shape and location of this peak.

4.3. Normalized MDDs in healthy brain parenchyma

The consistency of normalized MDDs in healthy brain tissues (across brain regions and across healthy subjects) may reflect a stability of water microdynamics associated with the homeostatic/energetic balance required for normal cellular metabolism. Normalized MDDs in healthy brain parenchyma show similar single-peak spectra (unimodal distributions) with slightly different locations and shapes. These differences in MDDs could indicate microstructural differences in the size of cellular restrictions, packing, cellularity, properties of extracellular matrix, intracellular hindrances and structures, or differences in active physiological properties such as the microdynamics of axonal transport and water exchange (Pierpaoli et al., 1996). Local susceptibility gradients due to large iron concentrations in the basal ganglia (Pran et al., 2009) may also play an important role in explaining differences between MDDs in scGM, compared to MDDs in WM and GM. The contribution of microscopic restrictions to differences in MDDs will be assessed in future studies investigating the diffusion time dependence of isotropic diffusion encoded MRI.

Accurately quantifying the shape of the unimodal distribution is challenging due to the non-uniqueness of the ILT (Whittall and MacKay, 1989; Borgia et al., 1998; Istratov and Vyvenko, 1999; Prange and Song, 2009), and may depend on the type and amount of regularization. Nevertheless, the shape of MDDs in both GM and WM could still reveal important information about microscopic and molecular scale processes. Water exchange and relaxation occurring over sub-micron length scales would be expected to be in fast-exchange during the typical time course of a diffusion MR experiment, causing many distinct peaks to be blurred into a single broad one (Johnson, 1993). One must therefore consider the relative timescales of diffusion and fast exchange processes in live tissue (Bai et al., 2016) which could result in an apparent merging of multiple water pools, even if they have different mean diffusivities.

Acquiring IDE DWIs with even shorter diffusion times using the next generation clinical MRI gradient systems (Setsompop et al., 2013) may help better resolve the parenchymal water peak and reveal the existence of multiple peaks, if present. Such technological advances will also enable the acquisition of higher b-value IDE DWIs resulting in improved spectral resolution of the estimated MDDs. Nevertheless, as there is always a direction for free diffusion even in axons, the IDE DWI signals decay almost exponentially, resulting in very low SNR levels at higher b-values (Fig. 3). Thus, while increased gradient strength may

allow us to access higher b-values, in order to accurately quantify IDE DWI signals with a precision higher than 150 : 1, it will be necessary to concurrently address imaging artifacts such as Gibbs ringing, ghosting, chemical shift; as well as signal artifacts of biological origin, such as variations in partial volume contributions due to subject motion, physiological pulsation, respiration, or IVIM effects.

4.4. Clinical potential

The biological specificity that arises from measuring distributions of water mobilities in microscopic pools may find clinical applications in stroke, cancer, and many neurodegenerative diseases and neuroinflammation, where conventional mean diffusivity or mADC imaging has already been shown to be clinically valuable. For example, changes in MDDs could reflect disruptions in microstructure (e.g., inflammation, dysmyelination, demyelination) or physiological changes in water exchange and transport in tissues (e.g., as seen in cancer). Future clinical studies will use IDE MRIs to explore how the striking changes in conventional mADC values in brain tissues with disrupted cellular metabolism, e.g., changes in ischemic stroke or cancer therapy, are reflected in diffusivity changes in biologically specific microscopic water pools.

5. Conclusion

Our results show that it is possible to measure *in vivo* MDDs in individual voxels from IDE DWIs acquired with a wide range of b-values. In healthy subjects MDDs are generally unimodal throughout the brain parenchyma, exhibiting only small differences in peak location and shape among WM, GM, scGM, and CSF. Future studies will aim at accurately measuring IDE signal decays *in vivo* with even higher dynamic range, and at establishing the clinical feasibility and utility of measuring MDDs in patient populations. The model-free assessment of rotation-invariant MDDs may provide new information about microstructure and active physiological processes in tissues. The MDD could extend the important role that the mean diffusivity (or the Trace of the diffusion tensor) has in the diagnosis and characterization of ischemic stroke, cancer, neuroinflammation, and neurodegenerative disorders and diseases.

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Figure 1:

Effective diffusion gradient waveform for acquiring clinical IDE DWIs with a wide range of b-values (includes the refocusing effect of the 180° RF pulse). The gradient waveforms GX, GY, and GZ use only trapezoidal pulses with ramp times of 3ms . Pulse widths δ_x , δ_y , and δ_z of the first trapezoid pulses on the X, Y, and Z axes, along with the ratio R of the gradient amplitude on X relative to G_{max} were numerically optimized to achieves a maximum b-value $b_{max} = 6\text{ms}/\mu\text{m}^2$ with a diffusion preparation time of 92ms and $G_{max} = 80\text{mT/m/s}$.

Figure 2:

The accuracy, precision, and spectral resolution of normalized MDDs estimated in a simulated clinical IDE experiment were assessed using the Monte Carlo method. Ground truth (red lines) single-peak (top row) and two-peak (bottom row) MDDs with various peak widths, locations, and separations were defined analytically using Gaussian functions with means μ_1 and μ_2 , standard deviations σ_1 and σ_2 , and signal fraction $f=0.4$, respectively. The mean MDD (black line) computed across MDDs estimated from 500 noisy simulated IDE measurements follows the corresponding ground-truth MDD (red line) with reasonable accuracy. The error bands (blue) illustrate the regions of one standard deviation away from the mean at each spectral component and quantify the precision of the MDD estimation. For an SNR level of 150:1, it is possible to distinguish between single- and double-peak MDDs with a spectral resolution of $\approx 0.45 \mu\text{m}^2/\text{ms}$.

Figure 3:

Maps of *in vivo* IDE signal attenuations over a wide range of diffusion sensitizations ($b = 0 - 6 ms/\mu m^2$) measured in the live human brain.

Figure 4:

Representative IDE signal decays and corresponding normalized MDD spectra from individual voxels containing: CSF (blue), WM (red), GM (green), scGM (yellow) and individual voxels containing partial volume contributions: WM+CSF (magenta) and GM+CSF (cyan). The ILT fits (red lines) are superimposed on the raw voxel signal decays (blue lines) as a function of b-value (from 0 to 6 $ms/\mu m^2$). Locations of representative voxels are shown on a high-resolution T2-weighted anatomical image.

Figure 5:

(Left) Tissue-specific normalized MDDs obtained from multiple voxels in various whole-brain ROIs containing: CSF (blue), WM (red), GM (green) and scGM (yellow). The mean normalized MDDs across all voxels within each ROI (black lines) approximate the tissue-specific MDDs in the healthy brain. The shaded error bands indicate one standard deviation around the mean for each spectral component, computed across all single-voxel normalized MDDs in each ROI. **(Right)** A representative slice showing voxels included in the four tissue-specific ROIs marked with the corresponding colors. Dashed lines indicate thresholds used in Fig. 6 to highlight tissue-specific differences in single-voxel MDD spectra, and their spatial uniformity across tissue types.

Figure 6:

Low, intermediate, and large diffusivity signal components in normalized MDDs measured in live human brain defined using the thresholds from Fig. 5 (dashed lines). Subcortical gray matter in particular, the putamen, the globus pallidus, and caudate nucleus, and to a lesser extent the thalamus contained mostly low diffusivity components $< 0.85 \mu m^2/ms$. Brain regions containing primary WM pathways were dominated by intermediate diffusivity components in the range of $0.85 - 2.0 \mu m^2/ms$. Peaks in GM contained significant signal fractions of small and intermediate mean diffusivities. As expected, large diffusivity values were observed mainly in regions of CSF.