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Quantifying white matter tract diffusion parameters in the presence of increased extra-fiber cellularity and vasogenic edema

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ABSTRACT

The effect of extra-fiber structural and pathological components confounding diffusion tensor imaging (DTI) computation was quantitatively investigated using data generated by both Monte-Carlo simulations and tissue phantoms. Increased extent of vasogenic edema, by addition of various amount of gel to fixed normal mouse trigeminal nerves or by increasing non-restricted isotropic diffusion tensor components in Monte-Carlo simulations, significantly decreased fractional anisotropy (FA) and increased radial diffusivity, while less significantly increased axial diffusivity derived by DTI. Increased cellularity, mimicked by graded increase of the restricted isotropic diffusion tensor component in Monte-Carlo simulations, significantly decreased FA and axial diffusivity with limited impact on radial diffusivity derived by DTI. The MC simulation and tissue phantom data were also analyzed by the recently developed diffusion basis spectrum imaging (DBSI) to simultaneously distinguish and quantify the axon/myelin integrity and extra-fiber diffusion components. Results showed that increased cellularity or vasogenic edema did not affect the DBSI-derived fiber FA, axial or radial diffusivity. Importantly, the extent of extra-fiber cellularity and edema estimated by DBSI correlated with experimentally added gel and Monte-Carlo simulations. We also examined the feasibility of applying 25-direction diffusion encoding scheme for DBSI analysis on coherent white matter tracts. Results from both phantom experiments and simulations suggested that the 25-direction diffusion scheme provided comparable DBSI estimation of both fiber diffusion parameters and extra-fiber cellularity/edema extent as those by 99-direction scheme. An *in vivo* 25-direction DBSI analysis was performed on experimental autoimmune encephalomyelitis (EAE, an animal model of human multiple sclerosis) optic nerve as an example to examine the validity of derived DBSI parameters with post-imaging immunohistochemistry verification. Results support that *in vivo* DBSI using 25-direction diffusion scheme correctly reflect the underlying axonal injury, demyelination, and inflammation of optic nerves in EAE mice.

Introduction

Diffusion tensor imaging (DTI) successfully detects axon and myelin injury through decreased axial diffusivity ($\lambda_{\parallel}$, parallel to white matter tract) and increased radial diffusivity ($\lambda_{\perp}$, perpendicular to white matter tract) in animal models of central nervous system (CNS) diseases and injuries (DeBoy et al., 2007; Kim et al., 2006; Song et al., 2002; Sun et al., 2006). Although decreased DTI-derived fractional anisotropic (FA) has been demonstrated to reflect myelin damage in multiple sclerosis (MS) (Schmierer et al., 2007), it is not a marker specific to myelin damage since other pathological components may also contribute to diffusion anisotropy change (Assaf et al., 2002; Werring et al., 1999). For example, inflammation associated vasogenic edema has been recognized to increase apparent diffusion coefficient (ADC) and underestimate the DTI-derived FA of fiber tracts (Naismith et al., 2010; Pasternak et al., 2009). Increased cellularity has been demonstrated to decrease DTI-derived ADC (Anderson et al., 2000). However, its impact on diffusion anisotropy remains unclear. An *in vivo* experiment of white matter inflammation in rats has suggested the association of changes in DTI-derived ADC with the evolution of pathology

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(Lodygensky et al., 2010). It is clear that both axon/myelin and extra-fiber pathological changes can impact DTI-derived metrics.

DTI assumes that diffusion of water molecules in the CNS white matter follows mono-exponential diffusion weighted signal decay (typically at b-value < 1000 s/mm^2), and was modeled by a single anisotropic tensor. Thus, diffusion anisotropy of white matter tracts in the presence of multiple structural and pathological compartments poses significant challenges in DTI analysis of white matter tracts since non-Gaussian models or multiple diffusion tensors are needed to reflect the tissue and pathological complexity. Various diffusion techniques have been proposed to overcome the limitation of DTI by non-Gaussian modeling of both parametric (model-based) or non-parametric (model-free) approaches. For instance, diffusion spectrum imaging (DSI) resolves crossing or branching fibers by direct evaluation of diffusion displacement probability density function which is the inverse Fourier transform of the diffusion weighted signals, but typically requires a large number of measurements with extensive diffusion weighting (Wedeen et al., 2005); diffusion kurtosis imaging (DKI) quantifies the non-Gaussian diffusion by estimating apparent diffusion kurtosis of diffusion displacement probability distribution (Jensen et al., 2005); generalized diffusion tensor imaging (gDTI) models the white matter tract *via* higher order tensors (Liu et al., 2004); composite hindered and restricted model of diffusion (CHARMED) evaluates an extra-cellular compartment (assigned to hindered diffusion resulting from extra-axonal diffusion weighted signal) and intra-cellular compartments (assigned to restricted diffusion in a cylinder representing individual intra-axonal space) employing a comprehensive diffusion weighting scheme (Assaf and Basser, 2005). Recently, Scherrer et al. proposed multiple fascicle models (MFM) to model an isotropic compartment (assigned to free water diffusion) and multiple anisotropic compartments (assigned to single fascicle) using a cube and sphere (CUSP) acquisition scheme (Scherrer and Warfield, 2012). Zhang et al. proposed neurite orientation dispersion and density imaging (NODDI) to model tissue components. Using high-angular-resolution diffusion imaging (HARDI) acquisition scheme, NODDI assesses intra-cellular (assigned to space within neurites), extra-cellular (assigned to space around the neurites but occupied by glial cells), and CSF compartments for deriving neurite density and orientation dispersion (Zhang et al., 2012). Although these approaches resolve possible fiber orientations and free water diffusion contaminations confounding DTI in the CNS, the restricted water diffusion outside fiber tracts affecting DTI measurements was less commonly dealt with. Glial cells have been modeled as a highly restricted isotropic component in an analytical model (Stanisz et al., 1997). A four-tensor model was proposed to include the restricted isotropic diffusion resulting from cell and the extracellular water components (Alexander et al., 2010), and most recently, restricted isotropic diffusion component has been included as one type of isotropic restricted compartment model in a taxonomy comparison study (Panagiotaki et al., 2012).

The recently-developed diffusion basis spectrum imaging (DBSI) approach models white matter diffusion as the linear combination of multiple discrete anisotropic diffusion tensors describing axonal tracts and a spectrum of isotropic diffusion tensors describing restricted (reflecting cells), and non-restricted (reflecting extra-axonal and extracellular space) diffusion components outside of axonal tracts (Wang et al., 2011). Employing a 99-direction diffusion-encoding scheme, DBSI has shown promise to accurately detect and quantify crossing fibers, axonal injury, demyelination, and inflammation-associated cell infiltration and edema in both *ex vivo* phantom and *in vivo* mouse brain. Although the effect of increased cell infiltration and edema on DTI-derived indices has been demonstrated previously using cuprizone treated mouse model and mouse trigeminal nerve phantoms (Wang et al., 2011), a more comprehensive study was needed to investigate the effect of increased cellularity and vasogenic edema associated with inflammation. In this study, diffusion weighted signals derived from Monte-Carlo simulations and acquired from tissue phantoms of fixed mouse trigeminal nerve and 2% agar gel were employed to demonstrate how cellularity and edema change DTI indices. The accuracy of DBSI to resolve the complication of inflammation was also examined. To image the coherent white matter tracts without fiber crossing, such as optic nerve and spinal cord, a simplified DBSI with one anisotropic diffusion tensor component and a spectrum of isotropic diffusion tensors would be sufficient requiring less diffusion encoding directions. A reduced scanning time can be achieved by significantly reducing the number of diffusion weighted images. Thus, we adopted a 25-direction diffusion encoding scheme (Batchelor et al., 2003) on both fixed tissue phantoms and Monte-Carlo (MC) simulation. Comparisons between 25- and 99-direction DBSI results on both experimental tissue phantom and simulated data were conducted to examine the accuracy of DBSI analysis using the 25-direction diffusion encoding scheme. To further demonstrate the feasibility of the 25-direction scheme for DBSI analysis, *in vivo* DBSI was performed on a group of experimental autoimmune encephalomyelitis (EAE) affected mice at the onset of optic neuritis comparing with the age and gender matched sham control mice. The *in vivo* diffusion MRI data were analyzed using DBSI and verified by post-imaging immunohistochemistry.

Materials and methods

Diffusion-encoding schemes

Both 99- (Wang et al., 2011) and 25-direction (Batchelor et al., 2003) diffusion-encoding schemes were employed for DBSI analysis in this study. The 99 diffusion-encoding directions were selected as prescribed in diffusion spectrum imaging (DSI) where the position vectors are the entire grid points (q_x, q_y, q_z) over the 3-D q-space under the relationship that $(q_x^2 + q_y^2 + q_z^2) \leq r^2$, where $r = 3$ for DBSI (Kuo et al., 2008; Wang et al., 2011; Wedeen et al., 2005). The icosahedral 25-direction sampling scheme was as prescribed by Batchelor et al. with the addition of one extra non-diffusion ($b = 0$) weighted image. See Appendix A and B for the 99- and 25-direction diffusion-encoding schemes.

Fixed trigeminal nerve phantom

Trigeminal nerves (~4 mm long) were dissected from adult female normal C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) after perfusion fixation with 4% paraformaldehyde in 0.01 M phosphate buffered saline (PBS) followed by immersion fixation for 24 h and kept in 0.01 M PBS solution at 4 °C. Twenty trigeminal nerves were employed to generate phantoms of a single trigeminal nerve only ($n = 7$) and a single nerve juxtaposed with different amount of 2% aqueous agar gel to mimic vasogenic edema ($n = 13$). All phantoms were prepared with a nerve gently blotted using Kimwipes® tissue to remove extra solution. Nerves were placed on a microscope slide with an identifier, with or without agar gel, covering with plastic wrap to avoid dehydration.

Diffusion-weighted spectroscopy of fixed trigeminal nerve phantom

Diffusion-weighted data were collected immediately after nerve phantom preparation using a 6-mm inner diameter single-turn surface coil. Diffusion-weighted spin-echo spectroscopy was performed with the following acquisition parameters: repetition time (TR), 2 s; echo time (TE), 32 ms; time between application of gradient pulses (Δ), 16 ms; gradient pulse duration (δ), 8 ms; number of average, 1; the maximum diffusion-weighting factor (b-value), 3200 s/mm^2 for both 99- and 25-direction diffusion weighting schemes at a single setting. Total acquisition time was 4 min 15 s.

Monte-Carlo simulation

Monte-Carlo simulations were performed to evaluate the effect of vasogenic edema and cellularity on DTI and DBSI indices in a computer generated geometric model, mimicking the trigeminal nerve tissue