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T₂*-based fiber orientation mapping

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Abstract

Recent MRI studies at high field have observed that, in certain white matter fiber bundles, the signal in T₂*-weighted MRI (i.e. MRI sensitized to apparent transverse relaxivity) is dependent on fiber orientation θ relative to B₀. In this study, the characteristics of this dependency are quantitatively investigated at 7 T using ex-vivo brain specimens, which allowed a large range of rotation angles to be measured. The data confirm the previously suggested variation of R₂* (= 1/T₂*) with θ and also indicate that this dependency takes the shape of a combination of sin² θ and sin⁴ θ functions, with modulation amplitudes (= ΔR_2^*) reaching 6.44 ± 0.15 Hz (or $\Delta T_2^* = 2.91 \pm 0.33$ ms) in the major fiber bundles of the corpus callosum. This particular dependency can be explained by a model of local, sub-voxel scale magnetic field changes resulting from magnetic susceptibility sources that are anisotropic. As an illustration of a potential use of the orientation dependence of R₂*, the feasibility of generating fiber orientation maps from R₂* data is investigated.

Keywords

T₂* relaxation; R₂*; fiber tracking; magnetic susceptibility anisotropy in white matter; susceptibility tensor imaging (STI); diffusion tensor imaging (DTI)

Introduction

Visualization of white matter structure and assessment of its integrity has important applications in the study of human brain. MRI techniques based on T₂, magnetization transfer, and water diffusion contrasts are sensitive to various aspects of white matter integrity (Miller et al., 1988; Moseley et al., 1990; Dousset et al., 1992), allowing the identification of pathological processes such as edema formation, inflammation and demyelination that may alter the cellular composition and structure of white matter.

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Recently, work performed at high magnetic fields (≥ 7 T) has demonstrated that, even in healthy brain, substantial R_2^* ($= 1/T_2^*$) variations exist that appear to highlight the major fiber bundles (Li et al., 2006). The effect has been partly attributed to the differences in myelin content and microstructure (Li et al., 2009b) and it is also plausible that the preferential axonal orientation in major fiber bundles may have influenced their R_2^* values. Indeed, a few studies have shown that R_2^* weighted images (Wiggins et al., 2008; Schäfer et al., 2009) and calculated R_2^* images (Cherubini et al., 2009; Bender and Klose, 2010; Denk et al., 2011) exhibit contrast that is dependent on the orientation of white matter fibers relative to the main magnetic field (B_0). However, full characterization of this orientation dependence has been hampered by the restricted range of head orientations available for in-vivo studies. To overcome this limitation, and to investigate the full range of fiber orientations, we performed R_2^* -weighted MRI on post-mortem human brain samples. A significant dependence on orientation was found, which could be accurately modeled by the effects of cylindrical susceptibility perturbers with a strength that varies with orientation. Using such a model, we then investigated the feasibility of estimating fiber orientation from the R_2^* data, and verified the results with Diffusion Tensor Imaging (DTI).

Material and Methods

Orientation dependence of white matter R_2^*

In the presence of microscopic susceptibility perturbers, the apparent transverse relaxation in gradient echo (GRE) techniques is accelerated. In the case of randomly distributed point perturbers, the decay of the GRE signal, $S(t)$, is independent of B_0 orientation can be approximated by

$$S(t) = M_0 \cdot \exp(-t/T_2^*) \quad [\text{Eq. 1}]$$

where M_0 (signal intensity at $t = 0$) is proportional to the spin density. The time constant T_2^* (or its reciprocal R_2^*) represents contributions from spin-spin relaxation (represented by T_2) and additional signal dephasing due to local (and mostly static) field inhomogeneities (T_2'):

$$1/T_2^* = 1/T_2 + 1/T_2' \quad \text{or} \quad R_2^* = R_2 + R_2'. \quad [\text{Eq. 2}]$$

For finite perturbers, the quantitative value of R_2' is dependent on perturber strength, size and density.

In white matter fiber bundles, the distribution of perturbers may be non-random and, furthermore, non-isotropic. In fact, compounds such as iron and lipids in ferritin and myelin may contribute significantly to microscopic susceptibility variations (Ogg et al., 1999; Haacke et al., 2005; Li et al., 2006; Li et al., 2009b; Fukunaga et al., 2010), and generally align with axons that may be highly ordered. In white matter, this may lead to an ordered distribution of perturbers, and make the GRE signal decay orientation dependent. Under specific conditions, the signal decay remains close to exponential with an orientation dependence that can be analytically calculated (Yablonskiy and Haacke, 1994). For example, for sufficiently long echo times, the signal decay in sets of parallel cylinders resembling the fiber bundle structures in white matter can be approximated as an exponential with a decay rate

$$R_2^* = c_0 + c_1 \cdot \chi \cdot \sin^2 \theta = c_0 + 0.5 \cdot c_1 \cdot \chi \cdot (1 + \sin(2\theta - \pi/2)) \quad [\text{Eq. 3}]$$

where c_0 and c_1 are constants, χ is magnetic susceptibility difference between the susceptibility perturbers and surrounding medium, and θ is the relative orientation between the structure and B_0 field (Yablonskiy and Haacke, 1994; Yablonskiy et al., 1997; Bender and Klose, 2010).

In addition to this expected $\sin^2 \theta$ dependency of R_2^* , an additional angular dependent term may arise from the recently reported magnetic susceptibility anisotropy of white matter (Lee et al., 2010; Liu, 2010). When susceptibility anisotropy is included in the model, the magnetic susceptibility itself has an angular dependency. If a cylindrical structure is assumed and two dimensional in-plane rotation is used, the susceptibility anisotropy (χ_{aniso}) can be expressed as follows (see Appendix) (Scholz et al., 1984; Hong, 1995):

$$\chi_{\text{aniso}} = \chi \left[(\chi_{\parallel} - \chi_{\perp}) \cdot \sin^2(\theta + \phi) \right] \quad [\text{Eq. 4}]$$

where $\chi_{\parallel}$ and $\chi_{\perp}$ are the volume susceptibility of the cylindrical structure relative to medium when it is parallel and perpendicular to B_0 respectively, and ϕ is the potential phase offset between angular dependence introduced by the distribution of perturbers and the susceptibility anisotropy that results from the perturbers' molecular structure. Hence, the angular dependent R_2^* term mentioned in Eq. 3 becomes:

$$\chi \cdot \sin^2 \theta = (\chi_{\text{iso}} + \chi_{\text{aniso}}) \cdot \sin^2 \theta = [\chi_{\text{iso}} + \chi \left[(\chi_{\parallel} - \chi_{\perp}) \cdot \sin^2(\theta + \phi) \right]] \cdot \sin^2 \theta \quad [\text{Eq. 5}]$$

where the total susceptibility (χ) is divided into an isotropic (χ_{iso}) and an anisotropic (χ_{aniso}) portion. For certain tissue constituents, susceptibility perturber structure and the molecular structure underlying the susceptibility anisotropy may be aligned in parallel or perpendicular fashion. For example, the phospholipid molecule (and possibly other elongated molecules such as cholesterol) that is a major constituent of the axonal myelin sheath has its long axis oriented generally perpendicular to the axonal direction. For any anisotropy resulting from such an arrangement, one would expect to observe a 0 or $\pi/2$ phase offset between the two sinusoids in Eq. 5. In this case, Eq. 5 can be expanded to show a $\sin(4\theta - \pi/2)$ term as well as a $\sin(2\theta - \pi/2)$ term and the resulting R_2^* will show a combination of $\sin 2\theta$ and $\sin 4\theta$ dependencies.

Sample preparation

Two coronal formalin fixed (for approximately 1 year) human brain slabs, derived from an adult patient with no history of neurological disease were used for the experiments. The thickness of the slabs was approximately 10 mm. The slabs were cut into a cylindrical shape (approximately 60 mm diameter). A few days before the MRI scans, they were placed in a cylindrical shaped PVC container (62 mm diameter, 65 mm deep) filled with phosphate buffered saline (Shepherd et al., 2009). For the MRI experiments, the cylindrical axis of the sample container was placed perpendicular to the main magnetic field (anterior-posterior direction in supine position or y-axis) such that rotating the container resulted in a change in the orientation of some of the major fiber bundles (e.g. corpus callosum) relative to the B_0 field (Fig. 1a). To reduce large scale field variations, the sample container was placed in a cavity centered within the shim module. The width of the cavity was marginally larger than the container's diameter to facilitate accurate rotation of the sample container about the

cylindrical axis (Fig. 1b). The shim module ($12 \times 30 \times 4 \text{ cm}^3$) was made out of PVC that has similar susceptibility to water (van Gelderen et al., 2008). Additional shim pieces, each curved on one side, were stacked on the top of the shim module in two layers to further improve field homogeneity.

MRI data acquisition and analysis

R_2^* -weighted MRI data were obtained from a 7 T clinical scanner (General Electric, Milwaukee, WI). Gradient strength and slew rate were 40 mT/m and 150 mT/m/ms respectively. A custom designed single channel 3-inch diameter surface coil with Nova preamplifiers (Nova Medical, Wilmington, MA) was used for signal reception and a Nova Medical quadrature birdcage transmit coil was used for signal excitation.

To demonstrate the orientation dependence of white matter R_2^* values, the samples were scanned at 18 different orientations, each rotated by 10° in the x-z physical plane and covering orientations from 0° to 170° . After acquiring localizer scans and adjusting the B_0 shims, angular R_2^* data were collected sequentially. For each angle, the table was pulled out and the samples container was rotated by 10° , after which the table was put it back to its original position. Note that only in-plane rotation was performed (rather than covering the entire angular space).

Data for the estimation of R_2^* were acquired with a multi-echo 3D GRE sequence. The scan parameters were as follows: TR = 700 ms, first echo time = 4.636 ms, echo spacing = 2.748 ms (for Sample 1) or 2.956 ms (for Sample 2), number of echoes = 12, flip angle = 60° , acquisition bandwidth = $\pm 64 \text{ kHz}$, FOV = $8 \times 8 \times 1.2 \text{ cm}^3$, resolution = $0.625 \times 0.625 \times 0.75 \text{ mm}^3$, and matrix = $128 \times 128 \times 16$. The data acquisition for each angle took 23.9 min and the total acquisition time was 7.2 hours.

From the 3D GRE data, magnitude images were reconstructed, and R_2^* values were estimated by weighted least-square fitting of the multi-echo time series using the magnitude images as a weighting function. The R_2^* images for each angle were aligned to the initial scan (0° data) based on alignment parameters derived from the second echo magnitude images. This was done because the second echo images have high SNR and good contrast. Several steps were performed for the alignment: first, the signal variation due to the sensitivity of the surface coil was removed by dividing by a low-pass filtered image of the original image. After that, the sensitivity corrected image was aligned with the initial scan. The alignment was refined using FLIRT in FSL (Jenkinson and Smith, 2001) applying 2D rigid body transformations. Although this achieved precise alignment of much of the image, small misalignments remained in certain areas presumably due to the changes in field homogeneity and gradient nonlinearity. This issue was subsequently resolved by additional nonlinear image registration (FNIRT, FSL) (Smith et al., 2004).

To estimate the orientation dependence of R_2^* in white matter, two regions of interest (ROI) were selected in the corpus callosum using multiple slices in each sample. The ROIs covered areas where the axonal orientation was expected to be relatively uniform (Fig. 2a and 2d). For each angle, the ROI-averaged R_2^* and its standard deviation were calculated. For each ROI, the angular dependency of the mean R_2^* value was then fitted with the susceptibility anisotropy model as described in Eq. 5 using:

$$R_2^*(\theta) = c_0 + c_1 \cdot \sin(2\theta + \phi_0) + c_2 \cdot \sin(4\theta + \phi_1). \quad [\text{Eq. 6}]$$

This model does not assume alignment between magnetic susceptibility perturber structure and magnetic susceptibility anisotropy structure. As a result, the phases of the two sinusoids are independent.

For comparison, the data was also fitted without including the anisotropy term (isotropic model) using:

$$R_2^*(\theta) = c_0 + c_1 \cdot \sin(2\theta + \phi_0) \quad [\text{Eq. 7}]$$

Least-square fit results were used to compare the validity of the two models. An adjusted R^2 value that takes into account the numbers of regressors used in each model, was calculated to check the goodness of the fit. To estimate the statistical significance of the additional regressors in the susceptibility anisotropy model, an F-test was performed for the two models in the four ROIs (the two ROIs in the two samples). The resulting F-values were averaged and a p-value was calculated from F-distribution.

After fitting the data to the two models, phase coherence was observed between the two sinusoids of the susceptibility anisotropy model (see Fig. 3). Hence a new anisotropic model with phase coherence was designed as following:

$$R_2^*(\theta) = c_0 + c_1 \cdot \sin(2\theta + \phi_0) + c_2 \cdot \sin(4\theta + 2\phi_0 - \pi/2). \quad [\text{Eq. 8}]$$

This model was fitted to the average R_2^* values in each ROI, after which the adjusted R^2 value was calculated and an F-test (with this model and the isotropic susceptibility model) was performed.

In order to investigate the feasibility to calculate fiber orientation maps using the angular dependence of R_2^* , the parameters c_0 , c_1 and c_2 were calculated by averaging the parameters from the two ROIs in each tissue and setting ϕ_0 to $-\pi/2$ to have a peak R_2^* at a 90° angle. The resulting R_2^* values from Eq. 8 were inverted to T_2^* values and this resulted in angular dependent T_2^* curves for each of the two tissue samples. These curves were normalized to have a peak-to-peak value of 1. Subsequently, the curve from Sample 1 was used in generating the orientation map of Sample 2 and vice versa. Note that the inversion from R_2^* to T_2^* was only performed to facilitate the comparison with DTI data (see Results).

To generate a T_2^* -based fiber orientation map, the normalized T_2^* curve was cross-correlated on a voxel-by-voxel basis with a 4D T_2^* data set (3D space + 18 aligned orientations). First, the peak correlation coefficient in each voxel was calculated to generate a correlation coefficient map. The angle at which peak correlation occurred provided a " T_2^* angle" map. Then the size of T_2^* variation (i.e. ΔT_2^*) at this angle was estimated by a linear regression to generate a ΔT_2^* map. After that, the T_2^* angle map was color coded similarly to what is common practice in DTI: in each voxel, an orientation angle was decomposed into x and z contributions and graded red and blue colors were assigned proportionally to the magnitudes of the x and z components, respectively. Finally, the resulting color map was multiplied by the ΔT_2^* map to highlight the strongest angular dependencies. This map was referred to as a T_2^* orientation map. A mask based on T_2^* values ($10 \text{ ms} < T_2^* < 30 \text{ ms}$) was used to select white matter regions.

For comparison, fiber orientation data were also obtained with DTI using a 7 T animal MRI system (30 cm bore size, Bruker BioSpin, Ettlingen, Germany). The scanner was equipped with a 15-cm gradient set (Resonance Research, Billerica, MA) capable of delivering 450

mT/m gradients within 130 μ s rise time. A four-element custom designed phased array coil (28 mm diameter each) was used for signal reception.

For the DTI scan, the sample was positioned using a three-plane localizer and ROI-based B_0 shimming (MAPSHIM, Bruker BioSpin) was performed. After that, DTI data were acquired using a spin-echo sequence (line-by-line) with the following parameters: TR = 1000 ms, TE = 57.43 ms, flip angle = 70°, acquisition bandwidth = ± 10 kHz, FOV = $8 \times 8 \times 1.2$ cm³, resolution = $0.625 \times 0.625 \times 0.75$ mm³, and matrix = $128 \times 128 \times 16$. The number of diffusion gradient directions was 20, and the diffusion b-value was 3,000 s/mm². The selection of directions was based on a downhill simplex method (Skare et al., 2000). The baseline images without diffusion gradient were acquired three times. The total scan duration was 13.1 hours.

The DTI data were processed using DTIFIT in FSL (Smith et al., 2004) and eigenvectors, eigenvalues, and fractional anisotropy (FA) maps were generated. To visualize white matter fiber orientation, each pixel was color-coded as red-green-blue (corresponding to x-y-z orientation respectively) based on the direction of its primary eigenvector, and intensity coded based on its FA value. To allow comparison with the T_2^* orientation map, a modified DTI map was generated. This was done by reducing the 3D tensor to 2D by excluding any y component in each voxel. From this 2D tensor, a 2D FA value was calculated. Then, the 2D FA value was multiplied by a primary eigenvector of the x and z directions. This created a 2D DTI fiber orientation map.

Results

The orientation dependent R_2^* variation is shown in Figure 2. The R_2^* curves clearly demonstrate orientation dependence, with maximum R_2^* values found when fibers are oriented perpendicular to the B_0 field. Significantly lower R_2^* values were found for parallel orientations. The mean ΔR_2^* from the four ROIs was 6.44 ± 0.15 Hz. In T_2^* , the mean ΔT_2^* was 2.91 ± 0.33 ms. The fitted curves for the isotropic model (dashed red lines) deviate significantly from the measurements. On the other hand, the anisotropic model (dotted blue lines) tightly matches the measurements, indicating the existence of a $\sin 4\theta$ component in the R_2^* angular dependency. The adjusted R^2 value for the isotropic model was 0.804 ± 0.029 . The value increased to 0.954 ± 0.010 for the anisotropic model (or 0.947 ± 0.019 for the phase coherence model), indicating significant improvement in fitting when a $\sin 4\theta$ component from susceptibility anisotropy is included in the model. The p-value from the F-test was 2.4×10^{-5} (or 7.2×10^{-6} for the phase coherence model) confirming the statistical significance of the additional regressors in the susceptibility anisotropy model.

Results of the anisotropic model fit are listed in Table 1. When the phases of $\sin 2\theta$ and $\sin 4\theta$ terms are compared, the positive peak of $\sin 2\theta$ coincides with the positive peak of $\sin 4\theta$. This phase coherence is clearly observed in Figure 3. This result, in addition to the high R^2 value mentioned above, suggest that the susceptibility perturber structure and susceptibility anisotropy structure are aligned in white matter.

Figure 4 shows the orientation dependent R_2^* curves for the phase coherence model. The shapes of the curves from the two samples exhibit great similarity. The parameter values of this model are listed in Table 2.

In Figure 5, T_2^* angle maps (5a and 5e), correlation coefficient maps (5b and 5f), ΔT_2^* maps (5c and 5g), and T_2^* orientation maps (5d and 5h) are shown for the central four slices in each sample. The angle and orientation map reveal the expected fiber orientation. For example, the corpus callosum shows fiber orientation roughly matching the macroscopic orientation, which is primarily in the left-right direction. Also, the areas of cortico-pontine

tract (CPT) and superior thalamic radiation (STR) show an up-down orientation. These orientation results can be further confirmed by the DTI results shown in Figure 6 and 7. The correlation coefficient maps (Fig. 5b and 5f) illustrate high correlation values in the areas of large FA values in DTI results (Fig. 6a and 6c). The ΔT_2^* maps (Fig. 5c and 5g) also show large ΔT_2^* in the areas of large FA values, suggesting that the ΔT_2^* maps may contain similar information as FA maps (see Fig. 7 for a comparison between the ΔT_2^* maps and the 2D FA maps.)

In the DTI images (Figure 6), the expected fiber orientations can be identified in areas containing large fiber bundles. Image distortion is visible, presumably due to B_0 inhomogeneity that is more prominent in the small bore system used for DTI. When the 2D DTI results are compared to the T_2^* orientation maps (Fig. 7 and Supplementary Fig. S1), the results demonstrate striking similarity between the two modalities although further investigation is necessary to compare the results more quantitatively. These results suggest that, in certain applications, T_2^* orientation maps may be an alternative to DTI for the study of white matter fiber orientation.

Discussion and Conclusions

In this study, significant effects of B_0 direction on R_2^* relaxation were observed in white matter of post-mortem human brain, confirming the finding of earlier works (Wiggins et al., 2008; Cherubini et al., 2009; Schäfer et al., 2009; Bender and Klose, 2010). Close inspection of the dependence of R_2^* on the orientation angle θ revealed not only a $\sin 2\theta$ component, expected from cylindrical field perturbers, but also a $\sin 4\theta$ component. This additional source could be explained by the effects of susceptibility anisotropy, a phenomenon that was recently observed in ex-vivo studies (Lee et al., 2010; Liu, 2010). Exploiting the observed angular dependency of R_2^* , we were able to reconstruct white matter fiber orientation maps that corresponded well to DTI-derived maps. We believe this method may have viable applications for high resolution ex-vivo fiber mapping.

The white matter angular dependence of T_2^* -weighted images has been suggested by Wiggins et al. in macaque (Wiggins et al., 2008) and by Schäfer et al. in human (Schäfer et al., 2009). Recently, Bender and Klose have demonstrated that T_2^* values also depend on the direction of B_0 relative to that of the white matter microstructure (Bender and Klose, 2010). On the other hand, no significant correlation between the T_2^* and fiber orientation was found in a similar experiment (Li et al., 2009a). One possible reason for the absence of significant correlation in that study is insufficient signal to noise ratio (SNR). When compared to the results from Bender and Klose, our findings agree on overall changes of T_2^* , revealing increased T_2^* values for fibers parallel to B_0 field. However, their data did not reveal a $\sin 4\theta$ component. This may be related to experimental differences such as the lower field strength (3 T) and a potentially SNR in that study.

Another possible source for angular dependencies additional to the $\sin 2\theta$ term is an orientation dependence of T_2 due to the so-called “magic-angle effect”, which has been observed in certain anisotropic structures (Fullerton et al., 1985; Henkelman et al., 1994; Chappell et al., 2004). For example, tendons, ligaments and cartilage have shown angular dependent of R_2 according to a $|3\cos^2\theta - 1|$ function which is equivalent to $|1.5\cos 2\theta + 0.5|$. A minimum R_2 (or maximum T_2) value was observed when the primary axis was angled by 54.7° relative to B_0 field. The phenomenon has been attributed to the dipole-dipole interaction of bound water molecules in collagen (Rubenstein et al., 1993). In highly myelinated nerve fibers, such dipole interactions could occur between myelin-bound water protons. Studies at a low field (1.5 T) have shown such effects in peripheral nerves (Chappell et al., 2004) but not in the central nervous system (Henkelman et al., 1994). When

the magic angle effect is assumed in conjunction with magnetic susceptibility (not including susceptibility anisotropy), the R_2^* can be modeled as $R_2^* = c_0 + c_1 \cdot |1.5 \cdot \cos(2\theta + \phi_0) + 0.5| + c_2 \cdot \sin(2\theta - \pi/2 + \phi_0)$. When this model is fitted to the ROIs in the samples, the resulting adjusted R^2 was 0.925 ± 0.023 and the p-value from the F-test for the magic angle regressor was $1.4 \cdot 10^{-4}$. The result shows somewhat lower adjusted R^2 value than those of the susceptibility anisotropy models, which were 0.954 ± 0.010 (anisotropic model) and 0.947 ± 0.019 (phase coherence model). Hence, the current study does not exclude the possibility of the magic angle effect in the measured R_2^* orientation. Magnetic susceptibility anisotropy and the magic angle effect may coexist in white matter, and further investigation possibly including measurements of T_2 effects on fiber orientation and their field dependence is necessary to confirm this.

The constituents of magnetic susceptibility may include myelin, iron and deoxyhemoglobin. The effects of myelin and iron on R_2^* have been suggested in several studies (Ogg et al., 1999; Haacke et al., 2005; Li et al., 2006; Li et al., 2009b; Fukunaga et al., 2010). The results from an iron extraction experiment demonstrated the effects of iron on R_2^* in gray matter (Fukunaga et al., 2010). For susceptibility anisotropy, however, myelin is likely to be the primary origin because of the highly ordered and anisotropic molecular structure of its phospholipid bilayer, which has been shown to exhibit susceptibility anisotropy in-vitro (Boroske and Helfrich, 1978; Scholz et al., 1984; Speyer et al., 1987). On the contrary, iron (either in ionic form or inside clusters such as ferritin), is not expected to show susceptibility anisotropy due to its spherical shape and sparse distribution (Connor et al., 1990; Connor and Menzies, 1996). Deoxyhemoglobin, which has been extensively studied for its effects on R_2^* in gray matter in fMRI, may have contributed little to the observed anisotropy due to its low concentration in WM (Weber et al., 2008).

When the phase coherent anisotropic model in Eq.8 is reorganized using trigonometry (ϕ_0 was set to $\pi/2$ to have the maximum R_2^* at $\pi/2$) and compared to Eq.5, the ratio of c_2/c_1 becomes $(\chi_{\perp} - \chi_{\parallel})/4(\chi_{\text{iso}} + \chi_{\parallel})$ when $\phi = \pi/2$. If we define $\Delta\chi_{\text{aniso}} = \chi_{\parallel} - \chi_{\perp}$ and $\chi_{\text{iso}}' = \chi_{\text{iso}} + \chi_{\parallel}$ then $\Delta\chi_{\text{aniso}}/\chi_{\text{iso}}' = -4c_2/c_1$. From Table 2, $c_1 = -3.048 \pm 0.124$ and $c_2 = 1.211 \pm 0.131$, and therefore, $\Delta\chi_{\text{aniso}}/\chi_{\text{iso}}' = 1.59$. A direct comparison of this ratio with the previous measurement (Lee et al., 2010) is not possible as the information of χ_{iso} (and χ_{iso}') relative to the susceptibility of the surrounding medium was not measured. The large fractional susceptibility anisotropy apparent in our data may be interpreted as follows: the isotropic susceptibility estimated from the orientation dependence of R_2^* may be lower than the individual contribution of myelin, as its (diamagnetic) effect may be partially canceled by that of (paramagnetic) ferritin. On the other hand, the anisotropic portion of susceptibility is likely to originate from myelin only as discussed earlier. Hence there is no cancellation for $\Delta\chi_{\text{aniso}}$, and thus the estimated ratio may appear larger than expected due to reduced χ_{iso} . Another way χ_{iso} may have been underestimated is through susceptibility anisotropy in the absence of perturber distribution effects (e.g. when myelin is not perfectly aligned along infinite parallel cylinders). Such a mechanism would generate a $\sin 2\theta$ dependency opposite to that resulting from perturber distribution effects alone, and lead to an underestimation of c_1 and thus χ_{iso} . An indication that the actual perturber distribution deviated from the cylindrical model comes from comparison of orientation dependence R_2' with the overall R_2' . For example, at 3 T, R_2^* of in-vivo white matter is 22.4 (24.0) Hz and R_2 is 13.5 (11.9) Hz for the frontal (occipital) area (Wansapura et al., 1999). The resulting R_2' from [Eq. 2] is 8.9 (12.1) Hz. These values are substantially higher than the orientation dependent R_2' component (0 to 2.68 Hz) found at 3T (Bender and Klose, 2010). Similarly, at 7 T, $R_2^* = 31.6$ Hz (Li et al., 2006) and $R_2 = 21.3$ Hz (Cox and Gowland, 2010), which leaves $R_2' = 10.3$ Hz. This is substantially higher than the orientation dependence of R_2' found in the current study (-2.2 to 4.3 Hz) although some of this effect may be explained by changes in relaxivity associated with the fixation process (Shepherd et al., 2009).

One limitation of the current study is that the angular resolution for the R_2^* angle map was limited to 10° . This resulted from the fact that the map was generated from the angle of highest correlation. Although this was adequate to demonstrate and characterize the orientation dependence, improved angular resolution may be obtained by least-square fitting of the phase. This will be particularly important when only a small number of orientations is available. In addition, the current study was limited by the fact that only in-plane rotation was performed, allowing the resolution of two orthogonal directions of fiber orientation. The expansion to full rotation space is straightforward using rotation in two different orientations. This would require a new container design as well as a new receiver coil design.

Recently, Liu has demonstrated the susceptibility tensor imaging (STI) based on susceptibility anisotropy in white matter (Liu, 2010). The method may share some of the same origins as the T_2^* orientation map. However, the STI results are based on image phase (Duyn et al., 2007) whereas T_2^* orientation is based on image magnitude. Further research is necessary to compare the advantages and disadvantages of the two methods.

One of the primary challenges of applying the current method to in vivo conditions is limited space and flexibility to orient a head inside of a scanner. Therefore, the method presented here is better suited for ex-vivo high resolution imaging of tissue samples. It has been known that fixation substantially reduces T_2 and T_2^* values and water diffusivity (Sun et al., 2003; Shepherd et al., 2009). This creates a unique challenge for ex-vivo high resolution DTI because diffusion gradients in a DTI sequence generally take several tens of milliseconds in a typical human scanner. Therefore, unless the size of sample is small enough to be scanned in an animal scanner where a high performance gradient system is available, acquiring an ex-vivo human whole brain DTI in a high resolution is challenging. Furthermore, the problem becomes more challenging at high fields due to reduced T_2 and T_2^* . On the other hand, in T_2^* orientation mapping, the reduced T_2^* may be compensated by increased contrast. Hence, increasing field strength may improve CNR more than linearly, benefitting from stronger susceptibility effects as well as increased proton polarization. As a result, the method introduced in this study may be useful in mapping details of white matter fiber orientation at high resolution that could be used as a template for other studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Demonstrate orientation dependent T_2^* in white matter of fixed human brain
2. Present a susceptibility anisotropy model with two sinusoidal components ($\sin 2\theta$ and $\sin 4\theta$) that closely matches to the observed T_2^* variation.
3. Present a novel white matter fiber orientation map based on T_2^* and compare it with a conventional fiber orientation map from DTI.

Figure 1.

(a) Tissue sample and container. The sample was rotated on the x-z plane (physical) such that the major fibers in corpus callosum changed the orientation relative to B_0 . (b) PVC shim sets were used to improve field homogeneity.

Figure 2.

The locations of ROIs in each sample (a and d) and the plots of R_2^* values over the angles in each ROI (b, and c for Sample 1; e and f for Sample 2). The fitted lines (dashed red for the isotropic susceptibility model; dotted blue for the anisotropic susceptibility model) clearly show that the isotropic model has poor fit whereas the anisotropic model tightly follows the measurement. The R_2^* values are the highest when the fiber orientation is perpendicular to B_0 field whereas the value becomes low when it is parallel to B_0 field.

Figure 3.

Fitted results of the anisotropic susceptibility model from Eq. 6 in Sample 1. The sinusoidal functions show coherence in their phases; the positive peaks of $\sin 4\theta$ components coincide with the peaks of $\sin 2\theta$.

Figure 4. The orientation dependent R_2^* curves from the phase coherence model from Eq. 8. The curves from each sample show great similarity.

Figure 5.

T_2^* angle maps (a and e), correlation coefficient maps (b and f), ΔT_2^* maps (c and g), and T_2^* orientation maps (d and h). The T_2^* angle map and orientation maps demonstrate the orientations of fiber bundles. In T_2^* angle map, dark blue and dark red represent approximately the same orientation therefore the color change from blue to red suggests smooth orientation variation. The ΔT_2^* maps as well as the correlation coefficient maps show similarity to FA maps shown in the DTI results (Fig. 6).

Figure 6. DTI using a high performance gradient MRI system. Red color indicates left and right orientation, blue color indicates up and down and green color is for through-plane orientation.

Figure 7.

Comparison between T_2^* and DTI orientation maps. The T_2^* orientation maps are the same as in Fig. 5d and 5h and the ΔT_2^* are the same as in Fig. 5c and 5g. The DTI images are the 2D DTI results multiplying 2D FA maps with 2D primary eigenvector maps.

Table 1

Model parameters for the susceptibility anisotropy model.

$$R_2^*(\theta)=c_0+c_1 \cdot \sin(2\theta+\phi_0)+c_2 \cdot \sin(4\theta+\phi_1)$$

		$c_1 \cdot \sin(2\theta + \phi_0)$			$c_2 \cdot \sin(4\theta + \phi_1)$			c_0 (Hz)
		c_1 (Hz)	ϕ_0 (°)	peak ^a (°)	c_2 (Hz)	ϕ_1 (°)	peak ^b (°)	
Sample 1	ROI1	-3.090	143.5	63.3	1.084	216.8	58.3; 148.3	46.9
	ROI2	-2.977	28.5	120.8	1.356	-40.0	32.5; 122.5	45.8
Sample 2	ROI1	-2.927	145.8	62.1	1.283	191.1	64.7 ; 154.7	52.8
	ROI2	-3.218	25.5	122.2	1.231	-8.7	24.7; 114.7	52.4

^a Peak is an angle ($\theta \in [0^\circ \text{ } 180^\circ]$) that $\sin(2\theta + \phi_0)$ becomes 1.

^b Peak is an angle ($\theta \in [0^\circ \text{ } 180^\circ]$) that $\sin(4\theta + \phi_1)$ becomes 1. A bolded number is the angle when both $\sin(2\theta + \phi_0)$ and $\sin(4\theta + \phi_1)$ become close to the positive peaks.

Table 2

Model parameters for the susceptibility anisotropy model with phase coherence.

$$R_2^*(\theta) = c_0 + c_1 \cdot \sin(2\theta + \phi_0) + c_2 \cdot \sin(4\theta + 2\phi_0) - \pi/2$$

		c_1 (Hz)	c_2 (Hz)	ϕ_0 (°)	c_0 (Hz)
Sample 1	ROI1	-3.085	1.054	146.7	46.9
	ROI2	-2.976	1.353	26.9	45.8
Sample 2	ROI1	-2.925	1.276	143.5	52.8
	ROI2	-3.204	1.160	30.9	52.4