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The influence of molecular order and microstructure on the $R2^*$ and the magnetic susceptibility tensor

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Abstract

In this work, we demonstrate that in the presence of ordered sub-voxel structure such as tubular organization, biomaterials with molecular isotropy exhibits only apparent $R2^*$ anisotropy, while biomaterials with molecular anisotropy exhibit both apparent $R2^*$ and susceptibility anisotropy by mean of susceptibility tensor imaging (STI). To this end, $R2^*$ and STI from gradient echo magnitude and phase data were examined in phantoms made from carbon fiber and Gadolinium (Gd) solutions with and without intrinsic molecular order and sub-voxel structure as well as in the *in vivo* brain. Confidence in the tensor reconstructions was evaluated with a wild bootstrap analysis. Carbon fiber showed both apparent anisotropy in $R2^*$ and anisotropy in STI, while the Gd filled capillary tubes only showed apparent anisotropy on $R2^*$. Similarly, white matter showed anisotropic $R2^*$ and magnetic susceptibility with higher confidence, while the cerebral veins displayed only strong apparent $R2^*$ tensor anisotropy. Ordered sub-voxel tissue microstructure leads to apparent $R2^*$ anisotropy, which can be found in both white matter tracts and cerebral veins. However, additional molecular anisotropy is required for magnetic susceptibility anisotropy, which can be found in white matter tracts but not in cerebral veins.

Keywords

Susceptibility tensor imaging; diffusion tensor imaging; white matter; anisotropy; $R2^*$ relaxation; wild bootstrap analysis

INTRODUCTION

There has been substantial interest in measuring the sub-voxel structure (microstructure) of the brain from the macroscopic MRI voxel signal. The $R2^*$ in white matter is dependent on fiber orientation with respect to the applied field and has been used to examine white matter

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fiber architecture [1–9]. The orientation dependence of $R2^*$ arises from the field inhomogeneity induced by the composition and arrangement of susceptibility sources [4,10–14]. Both local sources of magnetization within and outside a measured voxel can affect the field inhomogeneity within that voxel, making the interpretation of $R2^*$ in MRI non-trivial. Because anisotropy is usually used in relationship to a single material, we describe here the orientation dependence of $R2^*$ as “apparent” $R2^*$ anisotropy [15]. $R2^*$ may be approximated as the rate of T2 relaxation plus an inhomogeneity term proportional to TE times the field variance in that voxel, where sometimes the latter dominates $R2^*$ [16,17]. As the field measured from MRI signal phase depends on object orientation relative to the main field B_0 , $R2^*$ will be orientation dependent as well.

Recently, magnetic Susceptibility Tensor Imaging (STI) was introduced to provide a more direct way to examine the orientation dependent magnetization of tissues with molecular order such as myelin [18–20]. The highly organized structure of myelin [21] in white matter of the brain has been studied with susceptibility tensor reconstructions in vivo and in vitro [14,18–20,22–25]. These studies demonstrate significant anisotropy in susceptibility similar to that obtained with diffusion tensor imaging (DTI) [26–28].

We investigate both $R2^*$ and susceptibility tensors simultaneously for their dependence on microstructure and molecular properties using specifically designed phantoms. Examples of the effects observed in the phantom studies are then examined in human brain data in white matter fiber tracts and deep periventricular veins. Because of the increased sensitivity to noise of the $R2^*$ and susceptibility tensor anisotropy [4,18], the tensor eigenvectors are compared instead, using a wild bootstrap analysis to measure the confidence in the measured eigenvectors. This allows us to quantify and compare the stability of the principal axes of the tensors. We find significant correlation of the eigenvectors in major white matter tracts between susceptibility and $R2^*$ measurements, but not in deep periventricular veins, consistent with the presence (or lack) of molecular order and microstructure.

METHODS

Phantoms with specific sub-voxel microstructure and molecular anisotropy

The phantoms below were constructed to represent each of four categories depending on the presence (or absence) of molecular anisotropy and microstructural organization (Figure 1):

- Category I Anisotropic molecules present in an organized microstructure.
- Category II Isotropic molecules present in an organized microstructure.
- Category III Anisotropic molecules in a disorganized microstructure.
- Category IV Isotropic molecules in a disorganized microstructure.

Here, molecular order (isotropic or anisotropic) refers to the intrinsic magnetic susceptibility properties of the material, such as the anisotropy of the magnetic susceptibility of white matter molecules or the isotropic magnetic susceptibility of venous plasma water molecules. Microstructure (organized or disorganized) refers to the specific arrangement of the molecules within the voxel. An example of organized microstructure is the fiber structure in

which brain white matter is organized, while an example of disorganized structure is flowing venous plasma.

Two phantoms were constructed, of which T2* weighted images of the relevant sections are displayed in Figure 2. The first phantom represented category II. Glass capillary tubes were used to construct a cylindrical sub-voxel arrangement of isotropic susceptibility sources. Fifty-nine unheparinized capillary tubes (Fischer scientific, Pittsburgh, PA, USA), each 8.5cm long, were included. The capillary tubes contained a 5mM gadolinium solution (Magnevist, Berlex Laboratories) corresponding to a susceptibility of 1.62ppm [29,30]. The capillary tubes were filled with the Gd solution through capillary action and surface tension upon submersion in the solution. Despite careful handling, a few air bubbles remained inside the capillary tubes. Next, they were stretched using heat from their original dimensions (inner diameter (ID): 1.15mm, wall thickness of 0.2mm) to an ID of approximately 0.34mm and wall thickness of 0.05mm. The ID and thickness values after stretching were estimated from the amount of stretching, while assuming that the volume of the glass and Gd solution remained constant and that the stretching was uniform. By then assuming a diamagnetic susceptibility for the glass of -1.95ppm [31] (after referencing to water) and 1.62ppm for Gd, this led to an estimate of $0.09 \pm 0.02\text{ ppm}$ for the total susceptibility of the Gd filled capillary tubes. No breaking of the capillary tubes was observed during stretching and subsequent handling and inclusion in the phantom. Sections of approximately 8.5cm length were cut and waterproof silicone sealant was used to seal and bind their ends. The tubes were contained within a cylindrical phantom, 10 cm in diameter and 6 cm in height, filled with 1% agarose gel.

A second phantom was constructed containing sources belonging to categories I, III and IV. A cylindrical phantom with the same size as above contained: 1) a straight bar of 12K carbon fiber tow, which was a bundle of 12,000 carbon fiber strands 5.2 μm in diameter (ACP Composites, Livermore CA) (category I). 2) a ring of carbon fiber tow around a 50ml centrifuge tube with radius 3 cm (category I), 3) a mixture of finely cut, <1mm in length, but unaligned carbon fiber sections mixed with agar (category III), and 4) two balloons, nearly spherical in shape with maximum diameter of 1.5 and 1.2 cm, containing gadolinium solutions (Magnevist) with concentrations, 2.5mM and 5 mM, corresponding to susceptibilities of 0.81ppm (Balloon 1) and 1.62ppm (Balloon 2) respectively (category IV) [29,30]. All carbon fiber components were constructed from the same 12K carbon fiber tow. All were included in a cylindrical phantom, 10 cm in diameter and 6.5 cm in height, filled with 1% agarose gel. The centrifuge tube was used only in the construction of the ring source: once the carbon fiber was secured in a ring with agar the centrifuge tube was removed, after which the rest of the phantom was filled with agar.

Data acquisition of phantom and human subjects at multiple orientations

All data were acquired on a General Electric (GE) 3T MRI scanner (HDx, GE Healthcare, Waukesha, WI, USA) using an eight channel head coil and a Styrofoam ball sample holder to reproducibly rotate the phantom. A total of 16 orientations uniformly spaced over a unit sphere were acquired for both phantoms. Imaging parameters were as follows: 3D multi echo gradient echo (MEGRE) acquisition, 12 echoes, TR 104.3ms, first TE 3.4ms, echo

spacing 3.2 ms, flip angle 15°, bandwidth of 62.5kHz, field of view 13cm and at 1mm³ resolution. The carbon fiber phantom used the same imaging parameters, except for the number of echoes (10) and the TR (88.4 ms).

In the volunteer study, approved by our institutional review board, images were acquired with 11 echoes, 2.66ms first echo, 2.64ms echo spacing, 46.9ms TR, 15° flip angle, 24cm field of view, 62.5kHz bandwidth, 160×160 acquisition matrix and 1.5mm³ resolution with the 3D MEGRE sequence used in the phantom studies. The transmit-receive birdcage head coil was used in the volunteer study to permit a greater degree of rotation. The head tilts performed by the volunteer included a combination of forward, backward, left and right leaning tilts in both supine and prone positions for a total of 12 acquired head orientations. Diffusion data was acquired with a product 2D EPI dual spin echo diffusion weighted sequence, with parameters: 2×2×2.4mm³ resolution, 33 directions, 1000 s/mm² b-value, 22 cm field of view, 110×110 acquisition matrix, 2.4 mm slice thickness, 85.3ms echo time, 17s TR and 1953.12Hz bandwidth per pixel.

Data analysis

Susceptibility and R2* tensor reconstructions—Affine rigid image registration of orientations was performed with FSL [32–34]. $\mathbf{B}_0$ distortion of the diffusion-weighted images was corrected using FUGUE [32,33]. Diffusion tensor data was reconstructed using FSL [35]. The background field was removed from the field measured in STI in each orientation using the Projection unto Dipole Fields method [36]. A conjugate gradient solver was used to fit the multiple orientation phase data to estimate the STI [20]:

$$\mathbf{X}(\mathbf{k}) = \underset{\mathbf{X}(\mathbf{k})}{\operatorname{argmin}} \sum_p \left\| w_p \left(F^T T^{-1} (\Delta_p(\mathbf{k})) - F^T T^{-1} \left(\frac{\hat{\mathbf{b}}_p \cdot (\mathbf{X} \cdot \hat{\mathbf{b}}_p)}{3} - \hat{\mathbf{b}}_p \cdot \mathbf{k} \frac{\mathbf{k} \cdot (\mathbf{X} \cdot \hat{\mathbf{b}}_p)}{k^2} \right) \right) \right\|_2^2 \quad (1)$$

where $\Delta_p(\mathbf{k})$ is the field in k-space acquired at the p -th orientation; $\hat{\mathbf{b}}_p$ is the unit direction of the main field, $\mathbf{B}_0/|\mathbf{B}_0|$, for the p -th orientation; w_p is the inverse of the magnitude of the T2*w image of the p -th orientation, which reflects differences in SNR in the subject as it is rotated within the coil through orientations; $\mathbf{X}(\mathbf{k})$ is the Fourier transform of the susceptibility tensor χ .

The elements of the $\widehat{\mathfrak{R}2^*}$ tensor (R2T) was estimated from the apparent decay rate $R2_p^*$ at each orientation p using a conjugate gradient solver:

$$\widehat{\mathfrak{R}2^*} = \underset{\mathfrak{R}2^*}{\operatorname{argmin}} \sum_p \left\| w_p \left(R2_p^* - \hat{\mathbf{b}}_p^t \cdot (\widehat{\mathfrak{R}2^*} \cdot \hat{\mathbf{b}}_p) \right) \right\|_2, \quad (2)$$

Here, $R2_p^*$ was corrected for the effect of the variations of the background field, following closely the implementation in [37], which was determined in quantitative susceptibility mapping [17,36]. This correction was particularly important near air tissue interfaces.

Tensor anisotropy analysis—Singular value decomposition was performed to examine reconstructed eigenvectors and eigenvalues from tensor reconstructions. Denoting the tensor

eigenvalues as λ_i , $i = 1, 2, 3$ (in descending order), we defined the mean tensor as $1/3(\lambda_1 + \lambda_2 + \lambda_3)$ and the tensor anisotropy (TA) as $TA = \lambda_1 - \lambda_3$. For display purposes only, regions of strong susceptibility were emphasized by multiplying the phantom vectors by the trace of the $R2^*$ tensor (defined as the sum of its three eigenvalues). Regions of interest (ROI) of size between 326 and 690 voxels were placed manually on the magnitude images for phantoms and volunteer studies. For the capillary tubes phantom, the ROI was placed in the middle of the tubes, thus avoiding the silicone sealant material that was present only on either end. In the volunteer studies, ROIs were drawn in the splenium of the corpus callosum (SCC), optic radiations (OR) and peripheral white matter adjacent to the OR (PWM). Periventricular veins (PVVs) were measured to represent regions in the human body with cylindrical structure, but without molecular order. Their width is at or below the voxel size and they are a close in vivo analog to the capillary tube phantom for which a direction can be clearly defined. Mean and standard deviation of ROI measurements were recorded.

The correlation of eigenvectors was defined as $C = |\mathbf{V}_a \cdot \mathbf{V}_b|$, where $\mathbf{V}_a$ and $\mathbf{V}_b$ are the normalized principal eigenvectors estimated from STI, DTI or R2T. In both phantom and human data $\mathbf{V}_{STI}$ is the maximum eigenvector (EV1) and $\mathbf{V}_{R2^*}$ is the minimum eigenvector (EV3) of the reconstructed tensors. In this context, the maximum and minimum eigenvector were defined as the eigenvector corresponding to the maximum and minimum eigenvalue, respectively. When measuring the correlation in eigenvectors within PVVs the observed orientation of veins was estimated manually from a straight segment of the vein visible on SWI [38]. To identify the location of tissues of interest in color maps, $\mathbf{V}_{STI}$ and $\mathbf{V}_{R2^*}$ are weighted by the fractional anisotropy from DTI or R2* from a single orientation to highlight white matter and veins respectively.

In order to compute the voxel by voxel uncertainty of the STI and R2T estimates, we used the bootstrap method [39]. The bootstrap is a general method for determining the uncertainty of fitting parameters obtained from fitting of noisy data. As applied in MRI, the noisy data corresponds to the MRI voxel data ($T2^*$ weighted, diffusion weighted or susceptibility weighted, etc.). The bootstrap is used when analytical expressions for the error propagations are intractable. This is especially the case for nonlinear operations such as the computation of eigenvalues and eigenvectors. When the bootstrap method was applied to DTI [40] it relied on the measurement of multiple measurements for each direction, a time consuming process. The introduction of the so-called wild bootstrap method [41] allowed its use to scans where each direction was acquired only once. The application of the bootstrap method to MRI is not limited to diffusion, it has, for instance, been used to estimate uncertainty of $T1$ estimates [42]. In this study, a wild bootstrap analysis was performed to compute the 95% confidence intervals (CI) for the estimated tensor eigenvectors for both R2T and STI in phantom and human data (see Appendix for details). In order to simplify the display of the variability of the eigenvectors, the angle between the eigenvector for each bootstrap iteration and the mean of that eigenvector across the bootstrap iterations was computed. We then defined the confidence interval for each eigenvector as the width (in degrees) of the central 95 percent of these angles. 1000 iterations were computed for phantoms and human R2T and 350 iterations for the human STI reconstruction.

RESULTS

Phantom experiments

Table 1 lists the R2T and STI tensor eigenvalue properties measured in the phantoms. The mean susceptibility of the capillary tubes was paramagnetic, 0.15 ± 0.10 ppm (Table 1), consistent with the susceptibility of the Gadolinium solution. A higher correlation between R2T and STI was found for the molecularly ordered carbon fiber phantoms (Bar: 0.98 ± 0.03 , Ring: 0.96 ± 0.07) than for the capillary tube (0.29 ± 0.25). Low correlation was found for the gadolinium balloons (Balloon 1: 0.54 ± 0.30 , Balloon 2: 0.50 ± 0.29 , Table 1). Figure 3 shows this effect in $|V_{R2*} \cdot V_{STI}|$ with high correlation in eigenvectors within the carbon fiber ring and the subsequent decrease in alignment in the remaining cases, capillary tubes (II), carbon fiber bits (III) and gadolinium balloons (IV). For the Gd capillary tubes, there is high confidence in the direction of the R2T only, fourth row Figure 3. Lastly, there is low confidence in the vectors of both STI and R2T in the Gd balloons, column IV Figure 3.

Figure 4 shows the confidence for all eigenvectors for cases I – IV. For intact carbon fiber sources (Ring and Bar) the confidence in the direction of the fiber axis in R2T and STI is greater (narrower confidence interval) than the remaining eigenvectors. For the capillary tubes, the confidence interval of the capillary tube direction is only consistently narrow for R2T. Only low confidence (wide confidence intervals) were observed for both STI and R2T in both the gadolinium balloons and the disordered carbon fiber bits.

Human Study

Figure 5 demonstrates the sensitivity of R2* and susceptibility measurements to white matter fiber architecture, shown in the SCC and OR, and the presence of periventricular veins. Figure 5 includes a drawing of the ROIs chosen for analysis (left column, first row) as well as an SWI image for reference (left column, second row). Areas with high correlation between DTI (Figure 5, left column, third row), STI (Figure 5, left column, fourth row) and R2T (Figure 5, left column, fifth row) eigenvectors were found, particularly within the major white matter tracts. Table 2a lists the correlations between the eigenvectors of the DTI, STI and R2*, showing high correlation between them for the SCC and the OR. Voxel by voxel correlation between STI and R2T eigenvectors is shown in Figure 5, right column, third row. In Figure 5, the confidence intervals for the principal STI (right column, first row) and R2T (right column, second row) eigenvectors are also shown. Table 2b lists the ROI averaged values. They are generally narrowest within major white matter tracts. Less confidence in V_{STI} and V_{R2*} was observed overall in the periphery of the brain (CI PWM V_{STI} : $62^\circ \pm 19^\circ$ V_{R2*} : $62^\circ \pm 26^\circ$). Values for (apparent) anisotropy were consistent with previous literature: R2T: SCC: 16 ± 5.6 Hz; OR: 14 ± 5.2 Hz and STI: SCC: 52 ± 23 ppb; OR: 54 ± 17 ppb [4,18].

Figure 5 also displays the contrast in V_{R2*} present between the veins and surrounding white matter, the presence of these veins are demonstrated with QSM (right column, fourth row) and R2* (right column, fifth row). The correlation between eigenvectors of 21 measured veins was 0.57 ± 0.18 between R2T and manual measurements of the vessel direction. In the larger A-P oriented veins, outlined in Figure 5, right column, the correlation was higher,

R2T versus the measured direction (V_M): left vein: 0.89 ± 0.1 , right: 0.86 ± 0.22 . Similarly, there was generally greater confidence in V_{R2^*} than in V_{STI} (Table 2c)

DISCUSSION

In this work, phantom experiments demonstrate that both molecular anisotropy and ordered microstructure (category I) results in both $R2^*$ and susceptibility tensor anisotropy, while ordered microstructure alone without molecular anisotropy (category II) leads to apparent $R2^*$ anisotropy only. Additionally disordered microstructure was shown to lead to apparent isotropic $R2^*$ and susceptibility, regardless of whether molecular anisotropy was present (category III) or not (category IV). The results regarding categories I & II were supported in vivo by examining the structure of major white matter tracts and periventricular veins.

The carbon fiber phantom represented the presence of both sub-voxel structure and intrinsic molecular organization (category I). $R2T$ and STI eigenvectors were highly correlated (Figure 3 (I) and Table 1). The bootstrap analysis indicated increased confidence in these tensor estimations, such that this observed correlation is likely correct. The reported measures of anisotropy were within the range of previous measurements of properties of carbon fiber [43]. In the gadolinium filled capillary tubes (category II), the $R2T$ and STI eigenvectors were much less correlated, Figure 3 (II) and Table 1; microstructure is present without molecular order. In both categories III (the carbon fiber bits) and IV (the gadolinium balloons), wider confidence intervals were observed for both STI and $R2T$, Figure 3. Correlation between eigenvectors clearly demonstrated the presence of apparent $R2T$ and STI in carbon fiber bar and ring and apparent $R2T$ but not STI anisotropy in the capillary tubes.

Estimated confidence intervals corroborated the measurements of correlation in the estimated fiber directions in phantom studies. The axis of the fiber was reconstructed with greater confidence compared to the remaining eigenvectors (Figure 4), whereas wider confidence intervals were observed for the minor tensor axes. This symmetry is consistent with the known cylindrical architecture of carbon fiber (category I) and the capillary tubes (category II). The STI reconstruction in the capillary tubes showed no clear symmetry, evidenced in the large overlap of the confidence intervals Figure 4. In contrast, $R2T$ showed a clear symmetry in the capillary tubes.

Applying the same analysis to the in vivo data, white matter shows increased correlation between $R2T$ and STI , similar to the correlation observed for STI or $R2T$ with DTI and consistent with both microstructure and molecular order (category I). The appearance of the PVVs (category II) is most obvious on V_{R2^*} , Figure 5, whereas there is very little contrast between the PVVs and the surrounding white matter in V_{STI} . More importantly, $R2T$ had much higher confidence than STI in the PVVs, which also correlated with the measured venous direction. Similar to what was observed in the phantom for structured materials, the confidence intervals in the major fiber tracts in STI and $R2T$ had the greatest confidence in the principal eigenvectors, but only in $R2T$ for the PVVs, Figure 5.

There are a number of challenges in computing the R2T and STI maps. First, only a limited number of orientations can be performed in vivo in a very motivated subject with a long scan time. Limited orientations induced error in the reconstructed tensors, as demonstrated by the wide confidence interval in the wild bootstrap method for certain fiber tracts such as the centrum semiovale (CS) along the superior inferior axis as previously observed [20]. Previous work has investigated the influence of acquisition noise, of the number of orientations and of the limit on the range of feasible orientations in vivo on the susceptibility tensor variability [20]. A second challenge is the potential for misregistration between volumes at different orientations. Changes in partial volume effects between different orientations can lead to artificial increases in the apparent anisotropy at tissue borders in R2T. The fitting of the tensor and computing its eigenvalues are highly nonlinear operations that amplify noise in the acquired data. In addition, the tensor anisotropy in this work was defined as the difference between the largest and the smallest tensor eigenvalue. This definition was adopted because eigenvalues can be negative for STI and to avoid having to choose a reference for susceptibility measurements. Therefore, any noise in the acquisition will bias the measure towards *strictly* positive values (sorting bias) [44,45]. This bias is especially present when the anisotropy is low or zero, as is the case with the Gd balloons. The bootstrap method is then used to establish the confidence intervals for the measured parameters. These are, for instance, fairly wide for the direction of the three tensor eigenvectors for the Gd Balloons in Figure 5. Another limitation of this work, was the availability of in vivo data in only a single subject. This was due to the fact that the acquisition of 12 different head positions was, as discussed above, both lengthy, required several separate scanning sessions, and, for some orientations, somewhat uncomfortable.

The results reported in this study support the use of R2T and STI, which are derived from the same GRE data, for the probing of sub-voxel structure and the magnetic properties of the molecules within this structure. This may allow for example the direct probing of myelin content in the context of neurological diseases such as multiple sclerosis that affect the formation of myelin and lipid metabolism. Myelination processes could potentially be monitored by measuring changes in the R2T and STI anisotropy of white matter.

CONCLUSION

Observation in phantom and in vivo demonstrated that voxels containing microstructure and anisotropic molecules have both apparent R2* anisotropy and magnetic susceptibility anisotropy. Voxels containing only microstructure but no anisotropic molecules display only apparent R2* anisotropy but not susceptibility anisotropy. Lastly, voxels containing no microstructure, regardless of molecular anisotropy, are isotropic in both R2* (apparent) and susceptibility.

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APPENDIX A: FORMULATION OF WILD BOOTSTRAP ITERATIONS

Wild bootstrapping is a non-parametric statistical analysis method that permits inference on model parameters from data with multiple measurements; DTI studies have used this to assess confidence in estimates of tensor parameters, anisotropy and reconstructed eigenvectors [40,41]. This analysis was chosen for its ability to make inferences on systems with heteroscedastic noise [46], since the noise in phase and apparent $R2^*$ is not uniform across the imaged volume or the acquired orientations due to variations in signal sensitivity across the volume and orientations. The formulation of the bootstrap iteration is simple for the two systems under investigation in this work, Eq. 2 and 8 For STI and R2T respectively. In the R2T the observed $R2^*$ rate from each orientation is considered to be an unbiased estimate of the $R2^*$ decay rate after the correction for Rician noise in the magnitude data. In the wild bootstrap analysis, the data is resampled by constructing N bootstrap samples $R2_{ip}^*$ based on the fitted R2T, $\widehat{\mathbf{R}2^*}$:

$$R2_{ip}^* = \hat{\mathbf{b}}_p \cdot (\widehat{\mathbf{R}2^*} \cdot \hat{\mathbf{b}}_p) + \varepsilon_i \quad (\text{A.1})$$

Here, ε_i is defined as $\varepsilon_i = a\mathbf{u}\mathbf{F}_i$, where a is the number of degrees of freedom ($a = \sqrt{n/(n-k)}$, n is the number of observations and k is the number of parameters), $\mathbf{F}_i$ is a random vector defined as:

$$\mathbf{F}_i = \begin{cases} -1, & P < 0.5 \\ 1, & P \geq 0.5 \end{cases} \quad (\text{A.2})$$

where P is drawn from a uniform distribution on the unit interval $[0,1]$, and the vector $\mathbf{u}$ is the residuals of the fit of the system with the acquired data,

$$\hat{u}_p = R2_p - \hat{\mathbf{b}}_p \cdot (\widehat{\mathbf{R}2^*} \cdot \hat{\mathbf{b}}_p). \quad (\text{A.3})$$

For every bootstrap sample, $\mathbf{R}2_i^*$, a new tensor $\widehat{\mathbf{R}2_i^*}$ is estimated. Similarly, the definition of the STI bootstrap sample is:

$$\Delta_i = \mathbf{D}\mathbf{X} + \varepsilon_i \quad (\text{A.4})$$

Here ε_i has the same formal definition as for the R2T problem where $\mathbf{u}$ is now the residual of the fit of the local phase data with the susceptibility tensor model.

Confidence intervals were constructed from the distribution of estimated parameters from the bootstrapped iterations, 95% confidence intervals were constructed from the 2.5 and 97.5 percentiles. Statistical evaluation of eigenvectors requires additional considerations due to the inherent ambiguity in the definition of the principal eigenvector, where both an eigenvector and its anti-parallel equivalent describes the axis of the tensor equally well and the arithmetic mean is not applicable. Robust statistics of the mean dyadic tensor of a region of interest have been demonstrated in analysis of diffusion tensor imaging previously and defined as follows [40,47]. The mean dyadic tensor is:

$$\langle \varepsilon_l^i \varepsilon_l^{iT} \rangle = \left\langle \begin{bmatrix} (\varepsilon_{lX}^i)^2 & \varepsilon_{lX}^i \varepsilon_{lY}^i & \varepsilon_{lX}^i \varepsilon_{lZ}^i \\ \varepsilon_{lX}^i \varepsilon_{lY}^i & (\varepsilon_{lY}^i)^2 & \varepsilon_{lY}^i \varepsilon_{lZ}^i \\ \varepsilon_{lX}^i \varepsilon_{lZ}^i & \varepsilon_{lY}^i \varepsilon_{lZ}^i & (\varepsilon_{lZ}^i)^2 \end{bmatrix} \right\rangle = \frac{1}{N} \sum_{i=1}^N \varepsilon_l^i \varepsilon_l^{iT}, \quad (\text{A.5})$$

where ε_l^i is the l^{th} eigenvector from the l^{th} iteration, from a total of N bootstrap iterations. The 95th percentile of the angle between the principal eigenvector of the mean dyadic tensor and the eigenvector for each bootstrap iteration defines the half angle of the cone of the 95% confidence interval surrounding the mean principal direction of the tensor. The 95% confidence interval was calculated for both phantom and human data.

		Microstructural Properties	
		Organized	Disorganized
Molecular Properties	Anisotropic	I Carbon Fiber Bar/ Ring (white matter) R2* and STI anisotropy	III Carbon Fiber Bits R2* and STI isotropy
	Isotropic	II Capillary Tubes (cerebral veins) R2* anisotropy only	IV Gadolinium Balloons R2* and STI isotropy

Figure 1.

Definition of categories I–IV according to the presence or absence of molecular anisotropy and/or microstructural order. Each category is represented in the phantom using carbon fiber and Gadolinium structures. Categories I & II are investigated in white matter tracts and cerebral veins, respectively, in the *in vivo* brain.

Figure 2.

T2* weighted images of one section of phantom 1 and two sections of phantom 2 used in this study. Shown are the various compartments: capillary tubes (phantom 1), Gd balloons, carbon fiber ring, bar and bits (phantom 2). Details of the phantoms are described in the text.

Figure 3.

Phantom results demonstrating the four categories in each column. Eigenvectors from STI and R2T weighted by the mean R2T (first two rows), the 95% confidence interval from STI and R2T (rows 3 and 4), the correlation between V_{STI} and V_{R2^*} (row 5, $W = 0-1$) and the mean STI and R2T (row 6 ($W = -0.4-0.4$ ppm (columns I-III) and $W = -1.5-1.5$ ppm (column IV) and row 7 $W = 0-70$ Hz).

Figure 4.

Confidence intervals of all eigenvectors for STI and R2T from the phantom data. Cylindrical symmetry is observed in the confidence intervals from the carbon fiber bar and ring sources and R2T of the capillary tubes.

Figure 5.

In vivo results in two axial slices. Left column: depiction of the ROIs used for analysis (row 1), SWI image obtained from a single orientation of the STI data (row 2), principal eigenvectors V_{DTI} (row 3), V_{STI} (row 4) and V_{R2T} (row 5) within the SCC and OR (left sub-column) and periventricular veins (right sub-column). Right column: the confidence interval from STI (row 1) and R2T (rows 2), correlation between V_{STI} and V_{R2*} (row 3, $W = 0-1$) and the mean STI (row 4: $W = -70-70$ ppb) and mean R2T (row 5: $W = 0-40$ Hz). V_{STI} and V_{R2*} are weighted by DTI fractional anisotropy (FA) for the SCC and OR images to highlight white matter and by $R2^*$ from a single orientation to highlight veins on the vein images.

Tensor eigenvalue properties (TM, tensor mean and TA, tensor anisotropy, see text) and correlation of STI and R2T in phantom sources (CF = Carbon Fiber, Gd = Gadolinium)

Table 1

	STI				R2*	
	$ V_{R2*} \cdot V_{STI} $	Mean (ppm)	Anisotropy (ppm)	Mean (Hz)	Anisotropy (Hz)	
CF Bar (I)	0.98±0.03	-0.26±0.05	0.55±0.17	68±7.5	101±14.0	
CF Ring (I)	0.96±0.07	-0.12±0.05	0.34±0.08	43±14.4	85±30.4	
Gd Tubes(II)	0.29±0.25	0.15±0.10	0.44±0.19	58±13.2	98±28.0	
CF Bits (III)	0.45±0.28	0.08±0.07	0.18±0.07	46±16.1	29±21.1	
Gd Balloon 1 (IV)	0.54±0.30	0.71±0.03	0.22±0.08	11±1.5	10±7.1	
Gd Balloon 2 (IV)	0.50±0.29	1.33±0.06	0.50±0.18	25±3.2	13±7.7	

Table 2

a) Correlation between eigenvectors from R2*, STI, and DTI in phantom and b) the confidence interval (degrees) of their principal eigenvectors, and c) confidence intervals for the STI and R2T eigenvectors for the left and right A-P oriented vein (Figure 5).

a)		
	Correlation	
	SCC	OR
$ V_{R2^*} \cdot V_{STI} $	0.73 ± 0.26	0.63 ± 0.31
$ V_{R2^*} \cdot V_{DTI} $	0.78 ± 0.22	0.83 ± 0.19
$ V_{STI} \cdot V_{DTI} $	0.68 ± 0.23	0.68 ± 0.20

b)		
	95% Confidence (°)	
	SCC	OR
V_{R2^*}	32 ± 26	67 ± 23
V_{STI}	49 ± 20	26 ± 14

c)		
	95% Confidence (°)	
	A-P vein left	A-P vein right
$EV1_{R2^*}$	65 ± 18	74 ± 22
$EV2_{R2^*}$	65 ± 18	75 ± 20
$EV3_{R2^*}$	18 ± 11	32 ± 14
$EV1_{STI}$	41 ± 11	64 ± 16
$EV2_{STI}$	47 ± 13	69 ± 16
$EV3_{STI}$	35 ± 12	47 ± 24