Comparison of In Vivo and Ex Vivo Diffusion Tensor Imaging in Rhesus Macaques at Short and Long Diffusion Times

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Abstract: Diffusion tensor imaging (DTI) is widely used to non-invasively study neural tissue micro-structure. While DTI tractography of large nerve fibers is well accepted, visualization of smaller fibers and resolution of branching fibers is challenging. Sensitivity of DTI to diffusion anisotropy can be further enhanced using long diffusion time that can provide a more accurate representation of the tissue micro-structure. We previously reported that ex vivo fixed brain DTI at long tₐff (192 ms) showed improved sensitivity to fiber tracking compared to short tₐff (48 ms) in 4% formalin-fixed non-human primate (NHP) brains. This study further tested the hypothesis that DTI at longer diffusion time improves DTI fiber tracking in the in vivo NHP brains on a clinical 3 Tesla MRI scanner. Compared to fixed brains, the in vivo ADC was larger by a factor of 5. Also, the white-matter FA was 28% higher in the in vivo study as compared to our ex vivo experiments. Compared to short tₐff, long tₐff increased white-matter FA by 6.0±0.5%, diffusion was more anisotropic, tensor orientations along major fiber tracts were more coherent, and tracked fibers were about 10.1±2.9% longer in the corpus callosum and 7.3±2.8% longer along the cortico-spinal tract. The overall improvements in tractography were, however, less pronounced in the in vivo brain than in fixed brains. Nonetheless, these in vivo findings reinforce that DTI tractography at long diffusion time improves tracking of smaller fibers in regions of low fractional anisotropy.

Keywords: ?????????????????

INTRODUCTION:

Diffusion tensor imaging (DTI) is widely used to non-invasively study neural tissue micro-structure from the entire brain and related central nervous system pathologies [1-4]. Diffusion contrast arises from the non-uniform water motion in the brain tissue. Cell membrane constitutes a significant barrier to water motion. In white matter (WM), water motion is less restrictive along the axonal fibers than across axonal fibers, resulting in anisotropic water motion. In grey matter (GM) however, the motion of water is comparatively isotropic, thus generating diffusion contrast between gray and white matter. DTI can also estimate the direction of nerve fibers from the direction of maximum diffusivity of water along the axon bundles.

While DTI tractography of large nerve fibers is well accepted, visualization of smaller fibers and resolution of fiber branching is challenging. A few methods have been proposed to overcome these challenges. Multiple fiber orientations and fiber branching can be inferred using various high angular diffusion imaging (HARDI) methods such as Q-ball imaging [5, 6], generalized DTI [7], spherical harmonic transformation [8]. Some of these approaches require very high b-values, large number of directions, or are constrained by low signal-to-noise (SNR). Developing DTI techniques, to better visualize smaller fibers and resolve fiber crossings, remains an active area of research.

DTI data is acquired with a diffusion time (tₐff) in the range of 30-50 ms. The root-mean-squared (rms) displacement r = \sqrt{6D_t_{diff}} [9] is about 13.5 μm for a tₐff of 40 ms with a typical apparent diffusion coefficient (ADC) of \sim 0.76 x 10⁻³ mm²/s in the in vivo brain. This dimension is of the order of a typical brain cell size. At longer diffusion times, water molecules are likely to encounter more barriers and thus could more accurately sample tissue structure [10, 11]. DTI at longer tₐff could potentially increase its sensitivity [12, 13] and provide accurate tensors to improve nerve fiber tracking. Nair et al. [14] showed that fractional anisotropy (FA) at longer tₐff is more sensitive to changes in myelin-deficient mice. We recently reported that ex vivo brain DTI at long tₐff (192 ms) showed improved sensitivity to fiber tracking compared to short tₐff (48 ms) in 4% formalin-fixed non-human primate (NHP) brains [11]. FA increased at longer tₐff, and the tensor orientations in neighboring voxels along the fiber tract were more coherent [11]. The nerve fibers traced in the corpus callosum were ~10% longer and ~20% longer in the internal capsule at tₐff = 192 ms compared to tₐff = 48 ms.

This study further tested the hypothesis that DTI at longer diffusion time improves DTI fiber tracking in the in vivo NHP brains on a clinical 3 Tesla MRI scanner. Comparisons with previously published ex vivo DTI data on fixed NHP brains were made. In conventional spin-echo DTI

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sequences, longer diffusion time requires longer echo time resulting in significant signal loss due to T2 decay. In contrast, in Stimulated-Echo-Acquisition-Mode (STEAM) sequence [3, 15], the mixing time can be increased to achieve long diffusion time without increasing echo time, avoiding signal loss due to T2 decay (Fig. 1). DTI was performed with short (50 ms) and long (200 ms) diffusion times. DTI parameters such as FA, CL (linear anisotropy), CP (planar anisotropy) and CS (spherical anisotropy) [16], directional entropy (DE) [17] and deterministic fiber tractography using line propagation [18] were compared between long and short t\text{diff} with matched signal-to-noise ratio (SNR) and identical statistical thresholds. Comparison was also made with conventional DTI acquisition using double spin-echo acquisition (DSE) [19].

**MATERIALS AND METHODOLOGY**

**Ex vivo Preparation**

For the ex vivo studies, data were those from a previous study [11]. Eight rhesus monkey brains were perfusion-fixed with 4% formalin for 2-3 days as reported in this study. The sample was rinsed with water to remove formalin and immersed in water containing 0.018 mg/ml MION particles. Submersion in aqueous solution prevented susceptibility artifacts at the tissue-air interface. Acquired images suffered from Gibbs ringing artifact due to the strong signal that was detected if pure water or saline was used. Addition of MION particles reduced the T2* of the solution, thereby eliminating Gibbs ringing. MION particles were large enough to remain outside the brain tissue. The sample was immobilized in the container using plastic holders.

**In Vivo Preparation**

For the in vivo studies, six rhesus monkeys (2 male, 4 female) were anesthetized initially with telazol (3-5 mg/kg) and maintained with 1-1.5% isoflurane during MRI scans as per institutional approval. The eyes were coated with PuraLube to prevent dryness. Lactate ringer solution (10 ml/kg/hr) was administered via an i.v. line. The animals were placed in a holder using a mouth-piece and ear bars. Heart-rate, respiration, temperature and blood pressure were continuously monitored during the experiment. All procedures were performed with Institutional Animal Care and Use Committee approval.

**MRI**

All experiments were done on a Siemens® TRIO 3 Tesla (Siemens, Erlangen, Germany) human scanner. Stimulated Echo Acquisition Mode (STEAM) [20,21] sequence was used to allow an increase in diffusion. Mixing time, TM, is increased to increase the diffusion time. Geometric averaging was used for STEAM to reduce the contribution of cross terms [22, 23].

The ex vivo MRI parameters were: 16-shot STEAM EPI, TR/TE = 3000 ms/80 ms, \( \delta = 17 \) ms, \( \Delta = 21 \) ms for short t\text{diff} (48 ms) and 169 ms for long t\text{diff} (192 ms), directions = 30, b = 1700 s/mm\(^2\), resolution = 0.54 x 0.54 x 2 mm and number of averages = 34 for short t\text{diff}, 70 for long t\text{diff}. These sequences are referred to as STEAM48 and STEAM196 henceforth.

The in vivo MRI parameters were: 4-shot EPI, TR /TE = 3000/63 ms, \( \delta = 17 \) ms, \( \Delta = 31 \) ms for short t\text{diff} (50 ms) and 181 ms for long t\text{diff} (200 ms), directions = 60, b = 1200 s/mm\(^2\), resolution = 1x1x1 mm and number of averages = 3 for short t\text{diff}, 6 for long t\text{diff}. These in vivo sequences are referred to as STEAM50 and STEAM200.

DSE was also acquired with MRI parameters similar to the STEAM50 except TE = 91 ms which was the shortest TE possible. The numbers of averages among STEAM DTI acquisitions at short and long t\text{diff} and DSE were adjusted to match SNR for comparison.

**Geometric Measures of Anisotropy and Directional Entropy**

In addition to the commonly used DTI parameters such as ADC and FA, geometric measures of anisotropy – CL, CP and CS [11, 16] – were also analyzed to evaluate the diffusion sensitivity at long t\text{diff}. CL, CP and CS are measures of linear, planar and spherical anisotropy and are derived from the principle eigen values \( \lambda_1, \lambda_2 \) and \( \lambda_3 \) as follows [16]:

\[
CS = \frac{\lambda_1}{3(\lambda)}, \quad CP = \frac{2(\lambda_2 - \lambda_1)}{3(\lambda)}, \quad CL = \frac{\lambda_2 - \lambda_1}{3(\lambda)} , \quad \text{where} \quad \frac{1}{3}(\lambda) = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}.
\]

Geometric measures were plotted in the form of three phase plots [11]. To that effect, x and y co-ordinates [32] to represent anisotropy can be plotted using

![Fig. (1). Stimulated Echo Acquisition Mode Sequence with diffusion gradients. The mixing time, TM, can be increased, thereby increasing \( \Delta \) (duration for which diffusion gradients are applied), and thereby achieve longer diffusion times without increase in TE.](image-url)
\[ x = \frac{1 - CP + CL}{\sqrt{3}} \]
\[ y = 1 - CP - CL \]  

[1]

Changes in anisotropic diffusion in terms of CL and CP and isotropic diffusion in term of CS due to long \( t_{\text{diff}} \) can be depicted on these plots.

Further, to verify the coherence of tensor directionality along a fiber tract, directional entropy (DE) [17] was also measured. DE is inversely proportional to the inter-voxel tensor coherence along a nerve fiber. DTI at long \( t_{\text{diff}} \) would produce more coherent tensor orientations along a fiber tract and hence a reduced DE is expected.

**Tractography**

FA, ADC, parallel and perpendicular diffusivity were calculated. Nerve fiber tracks are tracked from the tensor data, using either line propagation techniques or probabilistic maps. While line propagation techniques such as Fiber Assignments by Continuous Tracking (FACT) [24] offer a comparatively simple approach to visualize various neural pathways, the technique relies heavily on the estimated tensor data and hence on image quality and SNR. Probabilistic approaches [25-27] compute a 3D connectivity map representing all the likely neural pathways that may exist at the selected seed in the brain volume. However, these techniques require a priori knowledge of the neural tracts in the brain region of interest. A database of numerous DTI scans is required to obtain comprehensive information about the similarities and differences in each nerve fiber tract across different animals of the same species. In this study, tractography is performed using the Diffusion TENSOR Visualizer [28], a software package that uses an advanced line propagation method.

**RESULTS**

**ADC and FA in Ex vivo and In Vivo Samples**

Fig. (2) shows the ADC and FA values in WM and GM for the ex vivo and in vivo studies using the typical DSE sequence. The ADC values in the fixed tissue were much lower than those in the in vivo data in the white matter WM (\( p < 0.01 \)) (Fig. 2a). ADC was also measured in the fixed tissue and found to be \( 0.27 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s} \) in the grey matter as opposed to \( 0.74 \pm 0.01 \times 10^{-3} \text{ mm}^2/\text{s} \) in vivo. The WM ADC in fixed tissue was \( 0.16 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s} \) and in vivo, it was \( 0.81 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s} \). Surprisingly, WM ADC was higher than GM ADC, which also has been reported previously in some WM regions [29]. In addition, the ratio of parallel diffusivity to perpendicular diffusivity \( [\text{i.e., } 2\lambda_1/(\lambda_2+\lambda_3)] \) was \( 1.53 \pm 0.08 \) for ex vivo WM and \( 2.10 \pm 0.08 \) for in vivo WM.

The FA values in the fixed tissue were also much lower than those in the in vivo data in the white matter WM (\( p < 0.01 \)) (Fig. 2b). FA decreased by 28% in fixed WM compared to in vivo WM. FA decrease in GM was much less (10%).

**DTI at Long Diffusion Time**

The ex vivo and in vivo WM and GM FA values at the two \( t_{\text{diff}} \) are summarized in Table 1. Ex vivo GM FA was

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Fig. (2). (a) ADC in the GM and WM in the in vivo and ex vivo experiments. Compared to the in vivo values, ADC in the WM was reduced by almost by 61 ± 5% and ADC in the GM decreased by 39 ± 8% in the ex vivo samples. (b) Comparison of FA in the in vivo and ex vivo samples. FA reduced by almost 28% in the WM in the ex vivo study while in the GM the decrease was less prominent at 10% compared to the in vivo FA. (Ex vivo data obtained from [11])
unchanged, whereas \textit{ex vivo} WM FA increased by 11.5 ± 5.0\% (p < 0.05) for \( t_{\text{diff}} = 196 \) ms compared to \( t_{\text{diff}} = 48 \) ms. Similarly, \textit{in vivo} GM FA was unchanged, whereas \textit{in vivo} WM FA increased by 6.03 ± 0.52\% for \( t_{\text{diff}} = 200 \) ms compared to \( t_{\text{diff}} = 50 \) ms (p<0.01). Further, FA estimated with STEAM50 and DSE in the \textit{in vivo} studies were not significantly different. Similar results were observed for the \textit{ex vivo} study, thereby confirming that the DTI parameters evaluated with STEAM and DSE sequences do not differ significantly. SNRs calculated for the STEAM50, STEAM200 and DSE sequence were 24.58, 22.87 and 28.45 respectively.

Three-phase plots were obtained for the \textit{in vivo} experiments at \( t_{\text{diff}} = 50 \) ms and \( t_{\text{diff}} = 200 \) ms (Fig. 3a). The three-phase plot shows voxels of the cortical terminations of the callosal fiber tract as shown in the inset. The tendency for linear anisotropic diffusion increased significantly (p < 0.05) at \( t_{\text{diff}} = 200 \) ms as opposed to \( t_{\text{diff}} = 50 \) ms (p<0.01). Further, FA estimated with STEAM50 and DSE in the \textit{in vivo} studies were not significantly different. Similar results were observed for the \textit{ex vivo} study, thereby confirming that the DTI parameters evaluated with STEAM and DSE sequences do not differ significantly. SNRs calculated for the STEAM50, STEAM200 and DSE sequence were 24.58, 22.87 and 28.45 respectively.

Table 1. Effect of \( t_{\text{diff}} \) on FA Values. Mean ± SD (\( n = 8 \) \textit{Ex vivo}, \( n = 6 \) \textit{In Vivo}) (DSE Results for \textit{Ex vivo} not Mentioned here – Available in our Previous Study [11])

\begin{tabular}{|l|c|c|c|}
\hline
(A) \textit{Ex vivo} & \multicolumn{3}{|c|}{\( t_{\text{diff}} \)}
\hline
 & 48 ms & 192 ms & \textit{p-value} \\
\hline
GM & 0.15 ± 0.02 & 0.16 ± 0.04 & 0.6 \\
WM & 0.31 ± 0.03 & 0.35 ± 0.02 & < 0.05 \\
\hline
\end{tabular}

\begin{tabular}{|l|c|c|c|}
\hline
(B) \textit{In vivo} & STEAM50 vs. STEAM200 & DSE vs. STEAM 200 & \\
\hline
\textit{t}_{\text{diff}} & 50 ms & 200 ms & \textit{p-value} \\
\hline
GM & 0.17 ± 0.01 & 0.18 ± 0.01 & 0.8 \\
WM & 0.45 ± 0.04 & 0.49 ± 0.04 & < 0.01 \\
\hline
\end{tabular}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Fig. (3). Geometric measures of diffusion: CL, CP and CS are plotted as three phase plot. (a) Comparison of short and long \( t_{\text{diff}} \) by STEAM from the same animal. (b) Comparison of DSE and long \( t_{\text{diff}} \) by STEAM from the same animal. Light red dots indicate diffusion in voxels at short \( t_{\text{diff}} = 50 \) ms, Green dots represent diffusion in the same voxels at \( t_{\text{diff}} = 200 \) ms. At long \( t_{\text{diff}} \), diffusion tends to be significantly (p < 0.05) more linearly anisotropic. The red and green squares represent the mean diffusivity at \( t_{\text{diff}} = 50 \) ms and 200 ms respectively.}
\end{figure}

showed increased inter-voxel coherence. Comparison of the directional entropy revealed that DE decreased by 17 ± 4\% in the callosum and by 10 ± 2\% in the cortico-spinal tract at \( t_{\text{diff}} = 200\)ms as opposed to \( t_{\text{diff}} = 50 \) ms (p < 0.05). Comparison of DE between DSE and STEAM200 showed a decrease of 12 ± 2\% in callosum and of 15 ± 3\% in the cortico-spinal tract in STEAM200 (p < 0.05).

Tractography outcome for \textit{ex vivo} and \textit{in vivo} experiments at short and long \( t_{\text{diff}} \) were compared (Fig. 4). The fibers traced at long diffusion time were much longer than those traced at short diffusion time. The length of fibers and the % increase in the length of fibers at long \( t_{\text{diff}} \) are tabulated in Table 3 for the fiber tracts in the internal capsule and corpus callosum. In the \textit{ex vivo} experiments, the callosal fibers were 14.6 ± 5.8\% longer for the long \( t_{\text{diff}} \) compared to short \( t_{\text{diff}} \). In the \textit{in vivo} experiments, the callosal fibers were 10.1 ± 2.9\% longer. Similarly, the increase in fiber length in the internal capsule was 24.8 ± 6.9\% for the \textit{ex vivo} experiments and was 7.3 ± 2.8\% for the \textit{in vivo} experiments for the long \( t_{\text{diff}} \) compared to short \( t_{\text{diff}} \). In comparison with the conventional sequence, the increase in fiber length in STEAM200 with respect to DSE was 6.2 ± 1.5\% in the corpus callosum and about 11.5 ± 2.9\% in the internal capsule. Overall, in the acquired brain volume, the
Comparison of In Vivo and Ex Vivo Diffusion Tensor Imaging

The major findings of this study are: (1) FA and ADC of fixed tissue were markedly different from those of in vivo in our study. (2) DTI at longer $t_{\text{diff}}$ increased WM FA, linear anisotropy, tensor magnitude and tensor coherence amongst adjacent voxels, and tractography outcome compared to DTI at short $t_{\text{diff}}$. Specifically, nerve fibers tracked in the corpus callosum and cortico-spinal tracts in the in vivo rhesus brains were significantly longer at long $t_{\text{diff}}$ compared to short $t_{\text{diff}}$, consistent with our previous ex vivo study [11]. (3) The improvements in tractography and DTI characteristics at long diffusion time in the in vivo brains, however, were less pronounced than in the ex vivo brains reported earlier [11].

For the in vivo study, an isotropic resolution of 1 mm$^3$ was used, in contrast to the ex vivo studies with a high in-plane resolution of 0.54 x 0.54 x 2 mm$^3$ acquired over ~20 hours [11]. Lower resolution in the in vivo experiments causes larger partial volume effects which could affect DTI results. This is in part constrained by the duration for which the animals can be placed under anesthesia as well as physiological (respiratory and cardiac) motion.

The outcome of STEAM DTI at long diffusion time was compared with STEAM DTI at conventional diffusion time and also with the conventional spin echo DTI (DSE) sequence. T1 and T2 weightings for the WM and GM tissue with the STEAM and DSE acquisition are different. Signal intensity in the DSE sequence is affected more by protons with long T2 while in the STEAM sequence; protons with long T1 define the measured signal intensity. However, DTI parameters such as FA and ADC were not significantly different between STEAM and DSE, indicating that the tractography outcome is not significantly affected by different sequences.

Comparisons Between In Vivo and Ex Vivo Tissue Properties

Protein cross-linking, dehydration, and tissue degradation in formalin fixation post-mortem could alter MRI tissue properties [30, 31]. Fixation shortens tissue T1 and T2 relaxation. Since the T2 values of the brain tissue, in vivo, are much longer than those measured ex vivo, in vivo scans have an advantage of higher SNR per acquisition. In vivo brain T1 is also comparatively longer, translating into better SNR in the DTI acquisitions compared to ex vivo.

### Table 2. Directional Entropy ($p < 0.05$)

<table>
<thead>
<tr>
<th></th>
<th>STEAM50 vs STEAM200</th>
<th>DSE vs. STEAM200</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{\text{diff}}$</td>
<td>50 ms</td>
<td>200 ms</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>2.33 ± 0.51</td>
<td>1.93 ± 0.48</td>
</tr>
<tr>
<td>Cortico-spinal tract</td>
<td>2.00 ± 0.54</td>
<td>1.80 ± 0.52</td>
</tr>
</tbody>
</table>

Fig. (4). DTI tractography: For the ex vivo study, fiber tracking using (a) DSE, (b) STEAM48 and (c) STEAM192 is shown in the same sample. White arrows and a yellow bracket mark the regions of improvement (longer fibers) using long $t_{\text{diff}}$. In vivo (d) short and (e) long $t_{\text{diff}}$ tractography results by STEAM sequence were obtained from the same animal. In vivo (f) DSE and (g) long $t_{\text{diff}}$ by STEAM sequence were obtained from the same animal. Arrows mark the regions of improvement (longer fibers) using long diffusion time. (Ex vivo tractography obtained from experimental data in [11]).

Improvement in tractography was more significant in the ex vivo studies.

DISCUSSION

The major findings of this study are: (1) FA and ADC of fixed tissue were markedly different from those of in vivo in our study. (2) DTI at longer $t_{\text{diff}}$ increased WM FA, linear anisotropy, tensor magnitude and tensor coherence amongst adjacent voxels, and tractography outcome compared to DTI at short $t_{\text{diff}}$. Specifically, nerve fibers tracked in the corpus callosum and cortico-spinal tracts in the in vivo rhesus brains were significantly longer at long $t_{\text{diff}}$ compared to short $t_{\text{diff}}$, consistent with our previous ex vivo study [11]. (3) The improvements in tractography and DTI characteristics at long diffusion time in the in vivo brains, however, were less pronounced than in the ex vivo brains reported earlier [11].
Table 3. Percent Increases in Fiber Length and Brain Volume in the Ex vivo and In Vivo corpus callosum and internal capsule as a Result of Increased t_{diff}.

<table>
<thead>
<tr>
<th></th>
<th>Corpus Callosum</th>
<th>Internal Capsule</th>
<th>Total Brain Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEAM48 vs. TEAM192</td>
<td>14.6 ± 5.8</td>
<td>24.8 ± 6.9</td>
<td>20.4 ± 9.1</td>
</tr>
<tr>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEAM0 vs. STEAM200</td>
<td>10.1 ± 2.9</td>
<td>7.3 ± 2.8</td>
<td>8.5 ± 3.5</td>
</tr>
<tr>
<td>DSE vs. STEAM200</td>
<td>6.2 ± 1.5</td>
<td>11.4 ± 4.6</td>
<td>6.3 ± 2.2</td>
</tr>
</tbody>
</table>

Fixation decreases ex vivo ADC. The in vivo brain ADC was ~0.8 x 10^{-3} mm^2/s and a b value of 1200 mm^2/s produced e-fold attenuation which produced optimal diffusion weighting. The fixed brain ADC was 0.16 ± 0.02 x 10^{-3} mm^2/s and a higher b value of 1700 mm^2/s was used. Ideally, the b value for the tissue sample with an ADC of 0.16 ± 0.02 x 10^{-3} mm^2/s would be ~ 4700 mm^2/s. The optimal b value could not be achieved in the ex vivo experiments because it was constrained by SNR. Importantly, due to the reduced ADC in fixed tissue samples, the t_{diff} of 48ms results in a rms displacement of 6 μm, whereas the root-mean-squared (RMS) displacement in vivo is 15 μm. At a t_{diff} of 196 ms in the ex vivo studies, this RMS displacement is approximately 14 μm and about 30 μm for the in vivo studies. Thus, the sampling of the diffusion space is expected to yield more improvement with increasing t_{diff} for the fixed brain than for the in vivo brain as observed.

The study by D’Arceuil showed no significant change in FA values between in vivo and fixed samples at 7T [32]. However, fixation decreased FA 28% in WM and 10% in GM in our studies. Similar to the observation in the aforementioned study [32], the fiber bundles in our study were sparse in the fixed brain tissue compared to in vivo. This effect could be due to tissue deterioration during post mortem interval [29, 32], among other reasons (such as diffusion weighting where signals did not attenuate e-fold in fixed brain). Fixation also decreased fiber length, consistent with that reported previously.

**Geometric Measures of Anisotropy and Directional Entropy**

The geometric measures of diffusion anisotropy serve as excellent measures to determine whether the diffusion tensor in a voxel is linear, planar or spherical. Linear diffusion represents the presence of uni-directional nerve fibers, while planar diffusion represents the presence of multiple fiber directions. Spherical diffusion is equivalent to isotropic diffusion. This study showed that the diffusion along a fiber tract became more linear at long t_{diff} (200 ms), especially along the fiber terminations of the callosal tract in the cortical surface. Additionally, neighboring voxels showed more inter-voxel coherence of the tensor directions at long t_{diff}, consistent with the white matter anatomy in the cortical regions.

**Tractography:**

Tractography in the acquired brain volume revealed that the length of the tracked fibers increased significantly with t_{diff} = 200 ms when compared with tractography outcome at t_{diff} = 50 ms. The major nerve fiber pathways such as the corpus callosum and the cortico-spinal tract were tracked. The fiber branches of the callosal tract were much more abundant at t_{diff} = 200 ms (Fig. 3). Further, the fibers traced in the callosum and the internal capsule were much longer at long t_{diff} than at the conventional diffusion time. The improvements in tractography and DTI characteristics, although significant, were much less in the in vivo data which could be due to lower spatial resolution and fixation effect in the ex vivo study.

**CONCLUSION:**

In conclusion, this study demonstrates that in vivo DTI at long diffusion time significantly improves tractography outcome. Although these improvements were of a lesser magnitude than the ex vivo results, these in vivo experiments reinforce our findings that DTI tractography at long diffusion time significantly improves tracking of smaller fibers in regions of low fractional anisotropy. The use of long diffusion time increased FA values in the brain tissue, provided more coherent and directionally oriented tensors and resulted in tracking of longer fibers when compared to conventional tractography. Future studies will explore DTI at longer diffusion time and in human applications.

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