

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 t_{diff} (192 ms) showed improved sensitivity to fiber tracking compared to short t_{diff} (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 t_{diff} , long t_{diff} increased white-matter FA by $6.0 \pm 0.5\%$, diffusion was more anisotropic, tensor orientations along major fiber tracts were more coherent, and tracked fibers were about $10.1 \pm 2.9\%$ longer in the corpus callosum and $7.3 \pm 2.8\%$ 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: DTI, Fiber tracking, MRI, Fractional anisotropy, Non-human primate, Fixed brain.

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 (t_{diff}) in the range of 30-50 ms. The root-mean-squared (rms) displacement $r = \sqrt{6Dt_{\text{diff}}}$ [9] is about $13.5 \mu\text{m}$ for a t_{diff} of 40 ms with a typical apparent diffusion coefficient (ADC) of $\sim 0.76 \times 10^{-3} \text{mm}^2/\text{s}$ 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 t_{diff} could potentially increase its sensitivity [12, 13] and provide more accurate tensors to improve nerve fiber tracking. Nair *et al.* [14] showed that fractional anisotropy (FA) at longer t_{diff} is more sensitive to changes in myelin-deficient mice. We recently reported that *ex vivo* brain DTI at long t_{diff} (192 ms) showed improved sensitivity to fiber tracking compared to short t_{diff} (48 ms) in 4% formalin-fixed non-human primate (NHP) brains [11]. FA increased at longer t_{diff} , and the tensor orientations in neighboring voxels along the fiber tract were more coherent [11]. The nerve fibers traced in the corpus callosum were $\sim 10\%$ longer and $\sim 20\%$ longer in the internal capsule at $t_{\text{diff}} = 192 \text{ms}$ compared to $t_{\text{diff}} = 48 \text{ms}$.

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 [11] 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_{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. 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 by increasing the mixing time, TM. 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_{diff} (48 ms) and 169 ms for long t_{diff} (192 ms), directions = 30, $b = 1700$ s/mm², resolution = 0.54 x 0.54 x 2 mm and number of averages = 34 for short t_{diff} , 70 for long t_{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_{diff} (50 ms) and 181 ms for long t_{diff} (200 ms), directions = 60, $b = 1200$ s/mm², resolution = 1x1x1 mm and number of averages = 3 for short t_{diff} , 6 for long t_{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_{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_{diff} . CL, CP and CS are measures of linear, planar and spherical anisotropy and are derived from

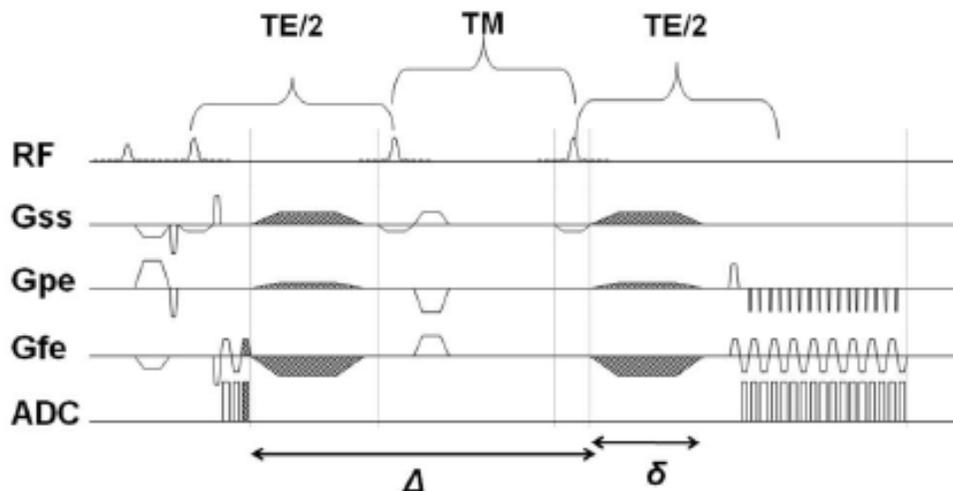


Fig. (1). Stimulated Echo Acquisition Mode Sequence with diffusion gradients. The mixing time, TM, can be increased, thereby increasing Δ (duration for which diffusion gradients are applied), and thereby achieve longer diffusion times without increase in TE.

the principle eigen values λ_1, λ_2 and λ_3 as follows [16]:

$$CS = \frac{\lambda_3}{3\langle\lambda\rangle}, CP = \frac{2(\lambda_2 - \lambda_3)}{3\langle\lambda\rangle}, CL = \frac{\lambda_1 - \lambda_2}{3\langle\lambda\rangle}, \text{ where } \langle\lambda\rangle = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}.$$

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

$$x = \frac{1 - CP + CL}{\sqrt{3}},$$

$$y = 1 - CP - CL \quad [1]$$

Changes in anisotropic diffusion in terms of CL and CP and isotropic diffusion in term of CS due to long t_{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_{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 were 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 were a database of numerous DTI scans is

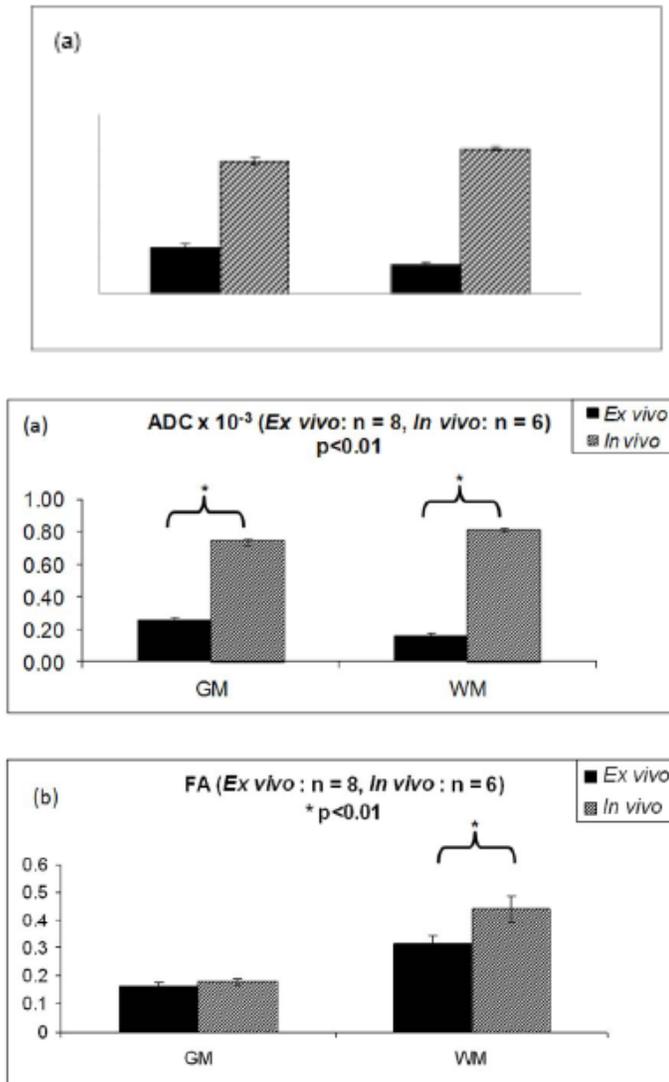


Fig. (2). (a) ADC in the GM and WM in the *in vivo* and *ex vivo* experiments. Compared to the *in vivo* values, ADC in the WM was reduced by almost by $61 \pm 5\%$ and ADC in the GM decreased by $39 \pm 8\%$ in the *ex vivo* samples. (b) Comparison of FA in the *in vivo* and *ex vivo* samples. FA reduced 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])

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

(A) Ex Vivo

t_{diff}	48 ms	192 ms	p-value
GM	0.15 \pm 0.02	0.16 \pm 0.04	0.6
WM	0.31 \pm 0.03	0.35 \pm 0.02	< 0.05

(B) In Vivo

STEAM50 vs. STEAM200			DSE vs. STEAM 200		
t_{diff} 50 ms	200 ms	p-value	DSE (48 ms)	200 ms	p-value
GM	0.17 \pm 0.01	0.18 \pm 0.01	0.8	0.18 \pm 0.01	0.7

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 was 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 [i.e., $2\lambda_1 / (\lambda_2 + \lambda_3)$] was 1.53 ± 0.08 for *ex vivo* WM and 2.10 ± 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_{diff} are summarized in Table 1. *Ex vivo* GM FA was unchanged, whereas *ex vivo* WM FA increased by $11.5 \pm 5.0\%$ ($p < 0.05$) for $t_{diff} = 196 \text{ ms}$ compared to $t_{diff} = 48 \text{ ms}$. Similarly, *in vivo* GM FA was unchanged, whereas *in vivo* WM FA increased by $6.03 \pm 0.52\%$ for $t_{diff} = 200 \text{ ms}$ compared to $t_{diff} = 50 \text{ ms}$ ($p < 0.01$). Further, FA estimated with STEAM50 and DSE in the *in vivo* studies were not significantly different. Similar results were observed for the *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 *in vivo* experiments at $t_{diff} = 50 \text{ ms}$ and $t_{diff} = 200 \text{ ms}$ (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 ($p < 0.05$)

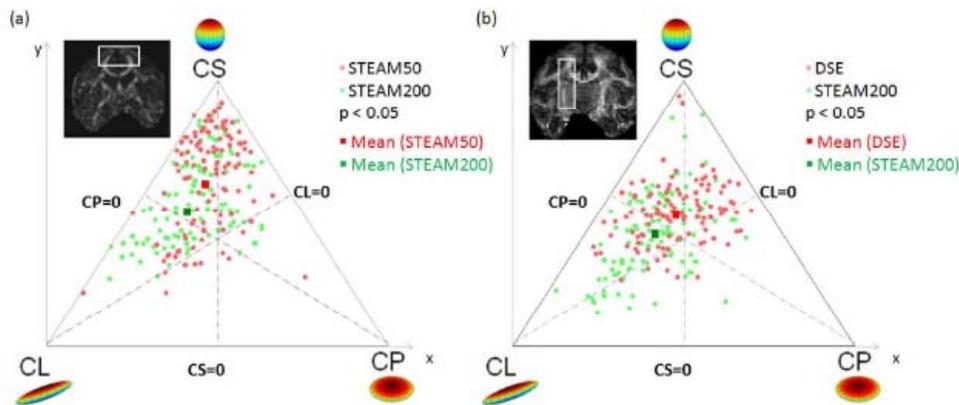


Fig. (3). Geometric measures of diffusion: CL, CP and CS are plotted as three phase plot. (a) Comparison of short and long t_{diff} by STEAM from the same animal. (b) Comparison of DSE and long t_{diff} by STEAM from the same animal. Light red dots indicate diffusion in voxels at short $t_{diff} = 50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

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

t_{diff}	STEAM50 vs. STEAM200		DSE vs. STEAM200	
	50 ms	200 ms	DSE (48 ms)	200 ms
Corpus Callosum	2.33 ± 0.51	1.93 ± 0.48	2.98 ± 0.49	2.60 ± 0.48
Cortico-spinal tract	2.00 ± 0.54	1.80 ± 0.52	2.46 ± 0.48	2.10 ± 0.60

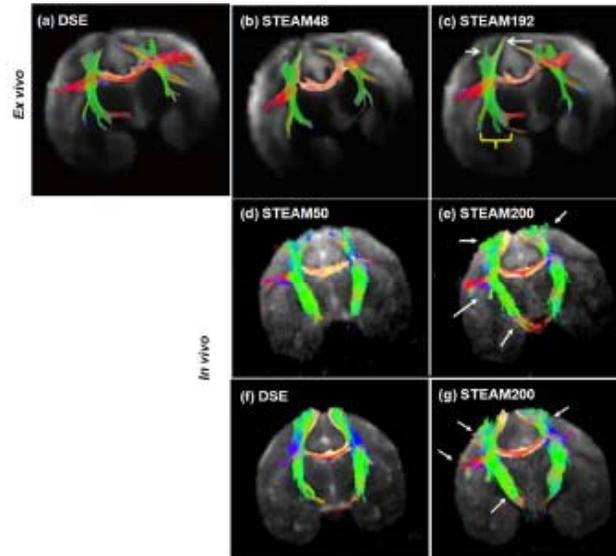


Fig. (4). DTI tractography: For the *ex vivo* study, fiber tracking using (a) DSE, (b) STEAM48 and (c) STEAM192 is shown in the same sample. White arrows and a yellow bracket mark the regions of improvement (longer fibers) using long t_{diff} . *In vivo* (d) short and (e) long t_{diff} tractography results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

at $t_{diff} = 200$ ms as opposed to $t_{diff} = 50$ ms. Similar results were also obtained with DSE sequence. Fig. (3b) compares the geometric measures of diffusion for DSE and STEAM200 in the cortico-spinal tract. DE along the same callosal fibers (Table 2) were reduced significantly ($p < 0.01$). Similar results were also obtained for the cortico-spinal tract. Thus the tensors along the nerve fiber pathway not only became more linearly anisotropic (i.e. directional) but also showed increased inter-voxel coherence. Comparison of the directional entropy revealed that DE decreased by $17 \pm 4\%$ in the callosum and by $10 \pm 2\%$ in the cortico-spinal tract at $t_{diff} = 200$ ms as opposed to $t_{diff} = 50$ ms ($p < 0.05$). Comparison of DE between DSE and STEAM200 showed a decrease of $12 \pm 2\%$ in callosum and of $15 \pm 3\%$ in the cortico-spinal tract in STEAM200 ($p < 0.05$).

Tractography outcome for *ex vivo* and *in vivo* experiments at short and long t_{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_{diff} are tabulated in Table 3 for the fiber tracts in the internal capsule and corpus callosum. In the *ex vivo* experiments, the callosal fibers were $14.6 \pm 5.8\%$ longer for the long t_{diff} compared to short t_{diff} . In the *in vivo* experiments, the callosal fibers were $10.1 \pm 2.9\%$ longer. Similarly, the increase in fiber length in the

internal capsule was $24.8 \pm 6.9\%$ for the *ex vivo* experiments and was $7.3 \pm 2.8\%$ for the *in vivo* experiments for the long t_{diff} compared to short t_{diff} . In comparison with the conventional sequence, the increase in fiber length in STEAM200 with respect to DSE was $6.2 \pm 1.5\%$ in the corpus callosum and about $11.5 \pm 2.9\%$ in the internal capsule. Overall, in the acquired brain volume, the 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_{diff} increased WM FA, linear anisotropy, tensor magnitude and tensor coherence amongst adjacent voxels, and tractography outcome compared to DTI at short t_{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_{diff} compared to short t_{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 $0.54 \times 0.54 \times 2 \text{ mm}^3$ acquired over ~ 20

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} .

	Corpus Callosum	Internal Capsule	Total Brain Volume
<i>Ex Vivo</i>			
STEAM48 vs. STEAM192	14.6 ± 5.8	24.8 ± 6.9	20.4 ± 9.1
<i>In Vivo</i>			
STEAM50 vs. STEAM200	10.1 ± 2.9	7.3 ± 2.8	8.5 ± 3.6
DSE vs. STEAM200	6.2 ± 1.5	11.4 ± 4.6	6.3 ± 2.2

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 weigh more on the measured signal intensity. However, DTI parameters such as FA and ADC were not significantly different between STEAM50 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 with STEAM acquisitions compared to *ex vivo*.

Fixation decreases *ex vivo* ADC. The *in vivo* brain ADC was $\sim 0.8 \times 10^{-3} \text{ mm}^2/\text{s}$ and a b value of $1200 \text{ mm}^2/\text{s}$ produced e-fold attenuation which produced optimal diffusion weighting. The fixed brain ADC was $0.16 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s}$ and a higher b value of $1700 \text{ mm}^2/\text{s}$ was used. Ideally, the b value for the tissue sample with an ADC of $0.16 \pm 0.02 \times 10^{-3} \text{ mm}^2/\text{s}$ would be $\sim 4700 \text{ mm}^2/\text{s}$. 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 $6 \mu\text{m}$, whereas the root-mean-squared (RMS) displacement *in vivo* is $15 \mu\text{m}$. At a t_{diff} of 196 ms in the *ex vivo* studies, this RMS displacement is approximately $14 \mu\text{m}$ and about $30 \mu\text{m}$ 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. This discrepancy could be due to different degree of tissue degeneration after post mortem [29,32]. 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*. 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 $t_{diff} = 200 \text{ ms}$ when compared with tractography outcome at $t_{diff} = 50 \text{ ms}$. 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 $t_{diff} = 200 \text{ ms}$ (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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CONFLICT OF INTEREST

None declared.

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