**Evaluation of DTI Tractography at Long and Short Diffusion Times in *ex vivo* and *in vivo* Rhesus Macaques**

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**INTRODUCTION:** Diffusion-based tractography of neural pathways has been of prime importance in recent decades for non-invasive study of brain anatomy. Presently, tensor based techniques face a challenge in tracking thinner branching fibers. The estimated tensors depict the averaged direction of the diffusion tensor. Although newer techniques such as HARD [1], Q-ball [2] and GDTI [3] can successfully detect the presence of multiple fibers, tensor-based methods still remain popular due to their ease of implementation, and moderate gradient requirements. Here we show that the capabilities of diffusion tensor tractography can be improved by employing a very long diffusion time (tₐₐ₈), particularly at the cortical white (WM) and gray matter (GM) boundaries where diffusion anisotropy is low.

**METHODS:** Conventional Double Spin Echo sequence used for DTI is a T2 dependent sequence which suffers from a considerable signal loss at long tₐ₈. STEAM-based DTI sequence was used to enable DTI measurements at very long tₐ₈. Experiments were performed on formalin-fixed (n = 4) and *in vivo* (n = 4) rhesus brains. Ex vivo MRI parameters were: 16-shot EPI, TR = 3000 ms, TE = 80 ms, δ = 17 ms, Δ = 25 ms for short tₐ₈ (48 ms) and 169 ms for long tₐ₈ (192 ms), directions = 30, b = 1700 s/mm², resolution = 0.54x0.54x2 mm and number of averages = 34 for short tₐ₈, 70 for long tₐ₈. In vivo MRI parameters were: 4-shot EPI, TR = 3000 ms, TE = 63 ms, δ = 17 ms, Δ = 31 ms for short tₐ₈ (50 ms) and 181 ms for long tₐ₈ (200 ms), directions = 60, b = 1200 s/mm², resolution = 1x1x1 mm and number of averages = 3 for short tₐ₈, 6 for long tₐ₈. The number of averages for short and long tₐ₈ acquisition was adjusted to achieve the same SNR for comparison. Geometric averaging was used to reduce the contribution of cross terms [4]. For analysis, fractional anisotropy index (FA) was calculated. Three-phase plots [5] were obtained using the linear, planar and spherical measures of anisotropy (CL, CP, CS). Fiber tracking was performed using DTI Studio v2.4.01 [6] using identical thresholds between long and short tₐ₈ for comparison. Fiber tracking was terminated if FA < 0.15 and the turn angle for the fiber was > 65°.

**RESULTS:** At long tₐ₈, FA increased 10.7 ± 0.8 % *ex vivo* and 5.2 ± 0.4 % *in vivo* compared to short tₐ₈. In the three-phase plots (data not shown), CL increased at long tₐ₈, further supporting the increased diffusion anisotropy at long tₐ₈. This trend in CL was stronger *ex vivo* than *in vivo.*

**Figure 1** depicts the tracking results from the *ex vivo* study. The traced fibers extended further into the regions of the WM-GM interfaces when long tₐ₈ is used. For example, the IC fibers extended further into the striatal regions at long tₐ₈ (white arrow) and the paracentral gyrus region was better tracked (green arrow). Similar results were obtained for the *in vivo* studies albeit at lower SNR and resolution due to time constraints (**Figure 2**). Fiber length in the CC at long tₐ₈ was 15 ± 7 % (*ex vivo*) and 11 ± 6 % (*in vivo*) longer compared to short tₐ₈. Similarly, at long tₐ₈, fiber length in the IC increased 16 ± 6 % *ex vivo* and 13 ± 4 % *in vivo.*

**DISCUSSION and CONCLUSION:** At long tₐ₈, FA increased, and fiber tracking revealed longer fiber connections in regions of low FA at the same statistical thresholds. These results together offer encouraging data that DTI at long diffusion time could improve the ability of DTI tractography to trace smaller fibers. This conclusion was valid for both *ex vivo* and *in vivo* conditions although the effects for the *ex vivo* studies were larger and more apparent. Possible explanations are SNR, spatial resolution and tissue fixation. In particular, the reduced diffusion space in fixed tissue, evident by the reduced ADC, could be affected more by the use of long tₐ₈. Although the exact mechanism for the improvement with long tₐ₈ remains to be investigated, it is likely that the tissue structure in these voxels is better sampled by diffusion measurement at long tₐ₈. This provides a dominant fiber direction in these voxels and thereby improves fiber tracking.