

## Using double diffusion encoded MRI to study tissue microstructure in diffuse axonal injury

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**Introduction:** Diffuse axonal injury (DAI) is a common pathology present in about 40%-50% of traumatic brain injuries (TBI)[1]. DAI ranges from mild to severe, causing microstructural changes (some on the scale of microns) within the brain[2, 3]. Subtle white matter changes associated with DAI are challenging to diagnose and characterize using conventional MRI methods and often can only be detected at autopsy[1]. Diffusion weighted MRI (DWI) based-methods such as diffusion tensor imaging (DTI) [4] can often detect these subtle changes in brain microstructure[5-7], however, the resulting metrics, such as fractional anisotropy (*FA*), lack sufficient biological specificity. Diffusion correlation methods, based upon double pulsed-field gradient (dPFG) or double diffusion encoding (DDE)[8-10], in which the signal is sensitized to the displacement correlations rather than the mean-squared displacement, may improve both sensitivity and specificity in detecting brain abnormalities following TBI. Here we use DDE MRI methods to study microstructural changes, such as the apparent axon diameter (AAD) in fixed mouse brain tissue following a closed-head impact model of engineered rotation acceleration (CHIMERA)[3].

**Materials and Methods:** Repeat CHIMERA injuries[3] were performed three times in a 24-hour intervals. Two energy levels were applied to the vertex of the head: 0J (sham), and 0.5J. One week after the initial injury mice were perfusion fixed with 4% formaldehyde in PBS and the brains extracted from the skulls and stored in fixative for 24-hours, then stored in PBS prior to MRI scanning. DWI data were acquired using a 3D EPI sequence with the following parameters: TE/TR=37/800ms, 8 segments, voxel resolution=100 $\mu\text{m}^3$ ; b-values=1700, 3800, 10000 s/mm<sup>2</sup> with 32 56 and 87 gradients orientations, respectively, and 6 gradients orientations with b-values=100, 200, 500, 1000, 10000 s/mm<sup>2</sup>; DDE filtered MRI data were acquired using a 3D EPI sequence with the following MRI parameters: TE/TR=23/700ms, 8 segments, voxel resolution=100 $\mu\text{m}^3$ ; DDE NMR parameters:  $\tau_m=0$ ,  $\delta=3$  ms,  $\Delta=20$  ms. Gradient orientations were uniformly distributed on a hemisphere;  $\phi$ , the angle between the two pulsed-field gradient (PFG) blocks, was applied twice  $-0^\circ$  and  $180^\circ$ -for each orientation[11]. DWI data were acquired in 3 shells, with  $q$  values of 84.8, 71.6, and 56.6 mm<sup>-1</sup>, each with 37, 16, and 7 orientations, respectively. Non-linear fitting was used to calculate the *FA* using the TORTOISE software package[12].The optic tract AAD was estimated from the DDE data using a three-dimensional implementation of the MCF method[13] that uses DTI data to determine the orientation of white matter.

**Results and Discussion:** The calculated mean values of the metrics obtained from DTI (*FA*) and DDE (AAD) show changes in the optic tract between the CHIMERA and sham mouse brains. ROI analysis of the dPFG MRI data show AADs of 8.6  $\mu\text{m}$  and 5.4  $\mu\text{m}$  for the right and left optic tracts of the CHIMERA and 4.8  $\mu\text{m}$  and 5.2  $\mu\text{m}$  of the right and left optic tract of the sham, respectively. The estimated AAD of the right CHIMERA brain is noticeably larger compared with the sham mouse. These findings suggest alterations in the tissue microstructure between the healthy and TBI brains, which may be due to axonal varicosities, gliosis and tissue loss.

**Conclusion:** DDE MRIs show promise in detecting cellular and microstructural alterations between DAI injured and healthy brains.

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