

# Probing microscopic cellular architecture in the mouse brain by oscillating gradient diffusion tensor imaging

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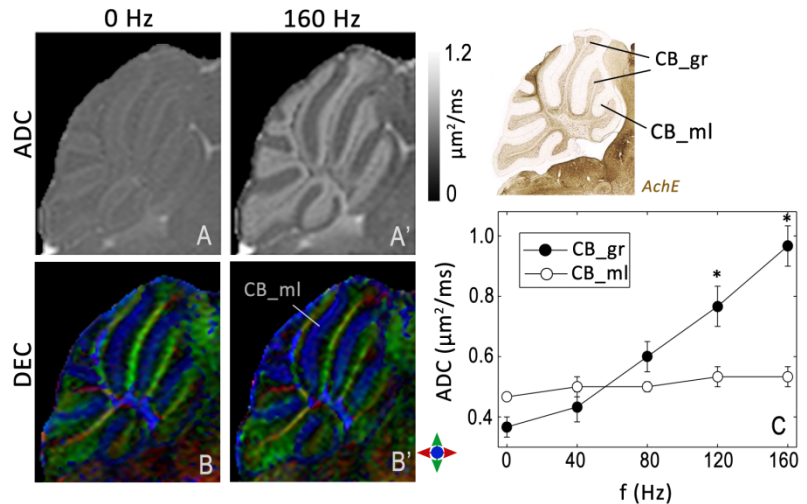
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**Introduction:** Diffusion MRI is a unique tool for studying tissue microstructure. Conventional pulsed gradient diffusion MRI experiments are sensitive to the combined effects of diffusion barriers at different spatial scales, such as intracellular and extracellular structures, but cannot distinguish between these spatial scales. The ability to examine diffusion patterns from cellular to tissue scales separately can potentially reveal more information on cell and tissue organization and heterogeneity, than available from conventional diffusion MRI. With oscillating diffusion gradient waveforms, it is possible to examine diffusion at separate spatial scales, by varying the modulation frequency of the oscillating gradients [1,2]. In this study, we implemented an oscillating diffusion gradient sequence for three dimensional diffusion tensor imaging of perfusion fixed mouse brain specimens. Diffusion-weighted images with oscillating diffusion-sensitizing gradients along six independent axes at multiple frequencies ( $f$ ) were fitted into a tensor model. The resulting diffusion tensor spectrum  $\mathbf{D}(f)$  revealed, for the first time, unique tissue contrasts in the mouse cerebellum and hippocampus.

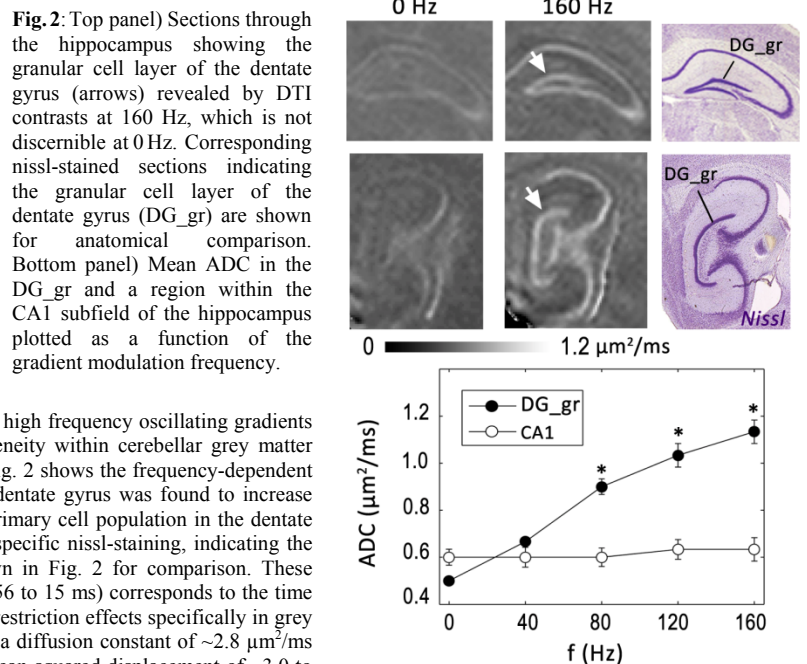
**Methods:** A 3D diffusion-weighted sequence with oscillating diffusion gradients was implemented for high-resolution imaging of the mouse brain. The diffusion-encoding module consisted of two cosine-modulated gradient waveforms of frequency  $f$  flanking a  $180^\circ$  RF pulse [2]. Acquisition was based on a gradient and spin echo (GRASE) scheme with navigator echo phase correction, previously developed by our group for high-field DTI [3]. Ex vivo images of adult C57BL/6J mouse brains ( $n=5$ ) were acquired on an 11.7 T spectrometer ( $N_{\text{eff}}=4$ ,  $TE/TR = 66/700$  ms,  $NA=4$ , diffusion gradient duration = 25 ms), using a pulsed gradient sequence (0 Hz, effective diffusion time  $\Delta_{\text{eff}}$  of 15 ms), and the oscillating diffusion gradient sequence with four different gradient modulation frequencies of 40, 80, 120 and 160 Hz, giving  $\Delta_{\text{eff}}$  of 6.25, 3.125, 2 and 1.56 ms respectively. For each frequency, six diffusion directions ( $b$ -value  $\sim 700$  s/mm<sup>2</sup>) and two non-diffusion weighted images were acquired at a resolution of  $100 \times 100 \times 100 \mu\text{m}^3$  and scan time of 50 min. The diffusion tensor at each frequency was estimated by a Log-linear fitting method, and diagonalized to compute the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) indices. Direction-encoded color (DEC) maps were computed from the primary eigenvector and FA maps.

**Results & Discussion:** Using oscillating diffusion gradients, unique frequency-dependent contrasts were observed in the mouse cerebellum and hippocampus. Fig. 1 shows the mid-sagittal diffusion tensor images of the mouse cerebellum acquired with pulsed gradient ( $f=0$  Hz) and oscillating gradient ( $f=160$  Hz) sequences. The cerebellar cortex has a highly organized cytoarchitecture, with a granular cell layer consisting primarily of the cell bodies of granule cells, and an outer molecular layer containing the axonal parallel fibers of the granule cells. Comparison of ADC maps revealed enhanced contrasts between the molecular layer and granular cell layer in the cerebellum with increasing frequency. In the zero frequency range, the molecular layer (CB\_ml, bright blue in the DEC map) had a higher ADC than the granular cell layer (CB\_gr), however at frequencies higher than 80 Hz ( $\Delta_{\text{eff}} < 3.125$  ms), this ADC contrast between CB\_ml and CB\_gr was reversed (the plot in Fig. 1). The mean ADC in the granular cell layer was found to increase steadily from  $0.37 \mu\text{m}^2/\text{ms}$  to  $0.97 \mu\text{m}^2/\text{ms}$  ( $\sim 2.6$  times increase) between 0 to 160 Hz. Mean FA in the CB\_gr also decreased with increasing frequency, mainly due to increases in the secondary and tertiary eigenvalues. This reduction in FA was confined to the granular cell layer, while the molecular layer and cerebellar white matter that are regions of high diffusion anisotropy, did not show any significant changes in FA at shorter diffusion times included in this study (DEC maps in Fig. 1). These results indicate that DTI measured using relatively high frequency oscillating gradients can provide enhanced contrasts sensitive to the microstructural heterogeneity within cerebellar grey matter regions, which are not discernible in conventional pulsed gradient DTI. Fig. 2 shows the frequency-dependent DTI contrast observed within the hippocampus. The mean ADC in the dentate gyrus was found to increase from  $0.50 \mu\text{m}^2/\text{ms}$  to  $1.13 \mu\text{m}^2/\text{ms}$  from 0 to 160 Hz. Interestingly, the primary cell population in the dentate gyrus also consists of granule cells. Corresponding sections with nuclei-specific nissl-staining, indicating the stained granular cell layer of the dentate gyrus (DG\_gr) are also shown in Fig. 2 for comparison. These findings suggest that the range of frequencies used in this study ( $\Delta_{\text{eff}} = 1.56$  to 15 ms) corresponds to the time scale over which  $\mathbf{D}(f)$  measurements are sensitive to changes in diffusion restriction effects specifically in grey matter regions consisting of granule cell populations and their axons. For a diffusion constant of  $\sim 2.8 \mu\text{m}^2/\text{ms}$  (that of free water at  $37^\circ\text{C}$ ), this frequency range corresponds to a root mean squared displacement of  $\sim 3.0$  to  $9.2 \mu\text{m}$ , which is comparable to the diameter of granule cells measured in the mouse brain ( $5\text{--}6 \mu\text{m}$ ). The results indicate that DTI with oscillating gradients to sample diffusion at sub-cellular levels can reveal enhanced grey matter contrasts within the mouse brain. Sampling the diffusion spectrum  $\mathbf{D}(f)$  over different frequency domains can thus provide a contrast generation mechanism based on sensitivity to the spatial scale of structural barriers to water diffusion within the brain.

**References:** [1] Stepisnik, *Physica B* 183, 1992 [2] Does et al, *Mag Res Med* 49, 2003 [3] Aggarwal et al, *Mag Res Med* 64, 2010



**Fig. 1:** Frequency-dependent DTI contrasts in the mouse cerebellum. A-B) Comparison of ADC and DEC maps at 0 Hz (A,B) and 160 Hz (A',B'). ADC at 160 Hz revealed enhanced contrasts between the molecular and granular cell layers (right), not seen with pulsed gradient DTI (left). C) Measured ADC as a function of frequency in the cerebellar molecular (CB\_ml) and granular cell (CB\_gr) layers.



**Fig. 2:** Top panel) Sections through the hippocampus showing the granular cell layer of the dentate gyrus (arrows) revealed by DTI contrasts at 0 Hz, which is not discernible at 160 Hz. Corresponding nissl-stained sections indicating the granular cell layer of the dentate gyrus (DG\_gr) are shown for anatomical comparison. Bottom panel) Mean ADC in the DG\_gr and a region within the CA1 subfield of the hippocampus plotted as a function of the gradient modulation frequency.