# Probing cytoarchitectural heterogeneity in the human hippocampus with oscillating gradient diffusion tensor imaging

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**Purpose:** We investigate the effect of gradient frequency on diffusion tensor contrasts derived using oscillating-gradient diffusion MRI in the postmortem fixed human hippocampus. In brain tissue, water diffusion is restricted by microscopic barriers such as cell membranes, intra- and extra-cellular structures. Conventional pulsed gradient diffusion MR experiments are limited to sampling the diffusion spectrum at relatively low frequencies (or long diffusion times), owing to gradient hardware limitations. In systems of restricted diffusion, the diffusion spectrum displays frequency-dependent characteristics that are governed by the local tissue microstructure[1]. Therefore, additional insights into cellular microenvironments may be gained by diffusion MR measurements using rapidly modulating gradient waveforms, such as oscillating gradient spin echo (OGSE) sequences[2,3], which are beyond the regime of pulsed-gradient diffusion MRI. OGSE measurements can provide anatomically relevant contrasts in regions of the mouse brain[4]. Here, we investigate changes in DTI contrasts in the human hippocampus with varying modulation frequency of the diffusion-sensitizing gradients. Our results show, for the first time, distinct frequency-dependent contrasts in specific layers of the human hippocampus.

**Methods:** Ex vivo images of the human hippocampus were acquired on a 9.4T spectrometer (maximum gradient strength 1000 mT/m), using a diffusion-weighted multiple spin echo sequence (TE/TR= 47/1000 ms, 6 averages, 3 coronal slices with slice thickness= 1mm). One set of diffusion experiments was performed using conventional pulsed diffusion-sensitizing gradients (0 Hz, effective diffusion time $\tau_{eff}$ of 15 ms), and three additional datasets were acquired with the diffusion-encoding module modified with cosinusoidally-modulated oscillating gradient waveforms[4]. Data were acquired at three different gradient oscillation frequencies of 50, 100, and 150 Hz ($\tau_{eff}$ of 5, 2.5 and 1.67 ms, respectively). For each frequency, nine diffusion directions (b-value ~700 s/mm$^2$) and two non-diffusion weighted images were acquired at a resolution of 120 x 120 µm$^2$ and scan time of 9 min. **Table 1** lists the diffusion parameters for the PGSE and OGSE data. The diffusion tensor at each frequency was estimated by a Log-linear fitting method, and diagonalized to compute the fractional anisotropy (FA), parallel ($\lambda_{//}$) and perpendicular ($\lambda_{\perp}$) diffusivity indices, and direction-encoded color (DEC) maps. A voxel-wise linear least squares fitting of apparent diffusion coefficient (ADC) measurements versus gradient oscillation frequency was computed, to generate maps representing the rate of change of ADC with frequency (denoted as $\Delta_f$ADC)[4].

| *f (Hz)* | $\tau_{eff}$ *(ms)* | *G (mT/m)* | *b (s/mm$^2$)* |
|---|---|---|---|
| 0 | 15 | 217 | 702.3 |
| 50 | 5 | 235 | 702.1 |
| 100 | 2.5 | 454 | 701.8 |
| 150 | 1.67 | 674 | 701.9 |

**Table 1**: Diffusion parameters for PGSE and OGSE DTI.

**Results:** Oscillating gradient DTI revealed distinct frequency-dependent contrasts in the human hippocampus. **Fig. 1** shows coronal diffusion tensor images acquired with the pulsed gradient (f=0 Hz) and oscillating-gradient (f=150 Hz) sequences. The human hippocampus has a laminar anatomy comprising well-defined layers with distinct cytoarchitectural properties. Using oscillating diffusion gradients, enhanced contrasts were observed between hippocampal layers that appeared otherwise homogeneous in pulsed-gradient contrasts. Comparison of ADC maps revealed a drastic increase in ADC measurements with increasing gradient frequency in specific hippocampal layers: the stratum oriens (so), the stratum moleculare (sm), and the granule cell layer of the dentate gyrus (gcl) (**Fig. 1A-A'**). In comparison, adjacent regions including the pyramidal cell layer (py), showed only a moderate frequency-dependent increase of ADC. Maps generated by a linear fitting of ADC versus frequency ($\Delta_f$ADC) clearly delineated the so, sm, and gcl layers, which appeared significantly hyperintense compared to adjacent hippocampal layers (**Fig. 1C**). The profile of $\Delta_f$ADC measurements along a horizontal axis through the center of the map clearly shows three distinct peaks corresponding to the so, sm, and gcl layers (**Fig. 1D**). For the range of gradient frequencies used in our study, no apparent change in the primary orientation of diffusion anisotropy was observed in the DEC maps, although FA in white matter regions showed a progressive decrease with frequency (**Fig. 1B-B'**). Quantitative measurements (mean ± standard deviation) of ADC, $\lambda_{//}$, and $\lambda_{\perp}$ in the gcl, sm, and py layers are shown in **Fig. 2**. Mean ADC in the gcl layer was found to increase steadily from 0.42 to 0.79 µm$^2$/ms (~1.9 times increase) between 0 to 150 Hz. Pulsed-gradient ADC values in the zero frequency range did not differ significantly between the sm and py layers, but were significantly ($p<0.005$) different at higher frequencies (>50 Hz) (**Fig. 2**). Both $\lambda_{//}$ and $\lambda_{\perp}$ increased with gradient frequency, while a greater percentage increase in $\lambda_{\perp}$ compared to $\lambda_{//}$ resulted in an overall decrease in FA with frequency. Comparison of the relative rates of increase in diffusivity measurements in the different hippocampal layers clearly shows the progressive contrast enhancement with increasing gradient frequency.

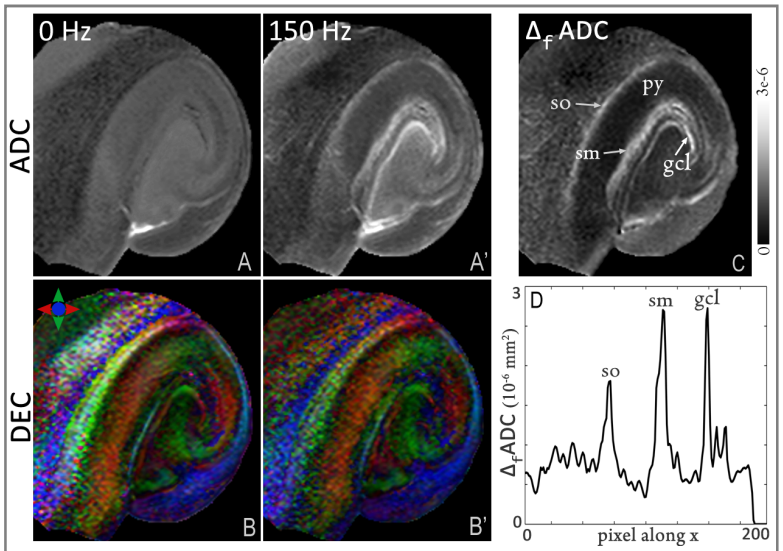


**Fig 1**: Frequency-dependent DTI contrasts in the hippocampus. A-B) Comparison of ADC and DEC maps at 0 Hz and 150 Hz. ADC at 150 Hz revealed enhanced contrasts between specific hippocampal layers (right), not seen with pulsed-gradient DTI (left). C) Linear fit of ADC versus frequency ($\Delta_f$ADC) shows the highlighted layers (arrows). so: stratum oriens, sm: stratum moleculare, gcl: granule cell layer. D) $\Delta_f$ADC profile along the image center shows distinct peaks corresponding to the highlighted layers.

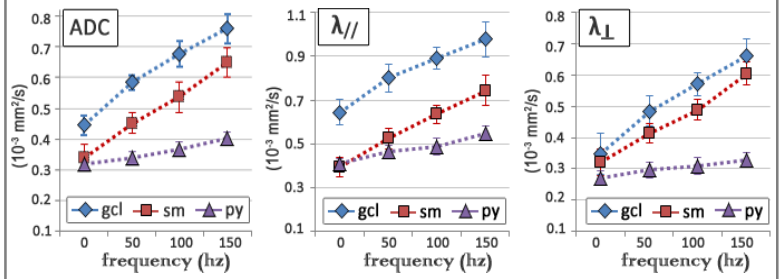


**Fig 2**: Relative rates of change in ADC, $\lambda_{//}$, and $\lambda_{\perp}$ measurements in the gcl, sm, and py hippocampal layers show the increasing contrast enhancement with gradient frequency.

**Discussion & Conclusion:** The range of gradient frequencies used in our study corresponds to free water root-mean-square displacements of ~3.0 to 9.2 µm at 37°C. Therefore, the observed frequency-dependent changes in ADC reflect the restrictive effects of underlying structural barriers over this spatial range. The gcl layer consists of very densely packed granule cells, while the so and sm layers consist of densely packed coherent axonal projections and neuropil, respectively. Interestingly, unlike findings in the mouse brain[4], the py layer in the human hippocampus does not show significant ADC increase with frequency, which may be attributed to the sparse packing density of py cells in the human hippocampus. These results suggest that the relative rates of frequency-dependent ADC increase in hippocampal layers reflect their distinct cytoarchitectural properties. Our findings presented here show that selective sampling of the diffusion spectrum with oscillating gradient DTI can generate unique contrasts in the human hippocampus that are sensitive to the cytoarchitectural heterogeneity of different hippocampal layers.

**References:** [**1**] Stepišnik, *Physica B* 183, 1993 [**2**] Does *et al*, *Mag Res Med* 49, 2003 [**3**] Gore *et al*, *NMR Biomed* 23, 2010 [**4**] Aggarwal *et al, Mag Res Med* 67, 2012.

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