First report of Diffusion Tensor Imaging of the murine eye

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Introduction: High resolution Diffusion Tensor Imaging (DTI) has been applied to study neuronal connectivity in spinal cord [1], isolated brain preparations [2], and in the intact brain. However, to date this technique has not been applied to the study of the retina. This study involves DTI of excised mouse eyes. The main goals of this project were 1) to determine the feasibility of DTI methods in the eye for neuronal fiber orientation, and 2) to obtain extremely high resolution anatomical images of the mouse eye to distinguish individual cell layers within the retina. This work provides the foundation for our work on molecular imaging of stem cell migration to sites of vascular damage in the eye, and will aid in our interpretation of those results as well.

Materials & Methods: The eyes used for the study were extracted from C57 black (C57BL) mice. The eyes were either fixed in formalin, or freshly extracted. The fixed eyes were transferred into cold phosphate buffered saline (PBS) solution 24 hours prior to imaging to remove the fixative. On the day of the experiment, the eyes were transferred to a NMR tube and suspended in Fluorinert™ (FC-43; 3M Corp.) to remove extraneous signals from the bathing medium. A 750 MHz wide bore (89mm) NMR spectrometer (Bruker Instruments, Billerica, MA) was used for these experiments. The RF coil used was an Alderman-Grant coil, diameter = 4mm, length = 3.5 cm. The parameters used were TR = 3300 ms, TE = 15.9 ms, SW = 69 kHz, Δ= 6.55ms, δ= 2ms. Gradient strengths used were 200mT/m and 610mT/m resulting in b-values of 110 and 700, respectively. A total of 23 slices were obtained, each 140 microns thick, NA=10, matrix size=160x160 and FOV= 4mm x 4mm. A total of 7 Tensor directions were obtained. Diffusion tensors, average diffusivity and fractional anisotropy maps were generated using MAS™ (proprietary software, Dr. Mareci's Laboratory, McKnight Brain Institute, UF).

Results: High resolution images were obtained and diffusion tensors calculated. Fractional anisotropy (FA) and average diffusivity values were derived from the diffusion tensor data sets. The resolution achieved in the Gradient Echo image (Fig 1) enabled us to differentiate 6 distinct retinal layers as well as other significant ocular anatomy such as aqueous humor, ciliary bodies, and retinal vasculature. In Fig 2, the effect of using higher b-values is clearly visible as panel B (b-value = 700) shows significantly better contrast between the retina and aqueous humor compared to panel A (b-value =110), indicating a significant fraction of water is bound in the retina. The Average Diffusivity map (Fig3A) displays good anatomical detail as does the calculated S_0 map (Fig 3B). Fig3C shows the Fractional Anisotropy (FA) map. A marked difference was observed in the fractional anisotropies of the aqueous humor, retina and vasculature shown in Table 1. The FA in the retina is significantly more than that in the aqueous humor and vasculature. Fig 3D is Fiber orientation map displaying the color coded direction of the Eigen vectors. It is apparent from this data that diffusion occurs along the retinal layers rather than across layers, consistent with the tissue ultrastructure.

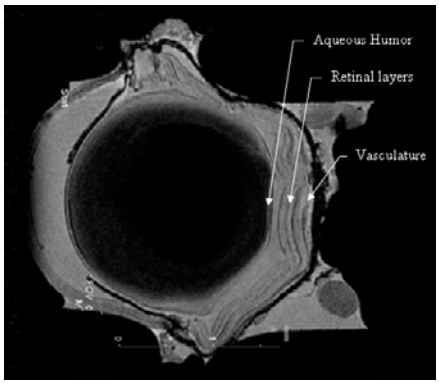

Fig. 1: *Gradient Echo 3D image*

Conclusions: The images obtained clearly show that DTI of the eye is feasible, and the 6 layers of the murine retina are clearly differentiated. We have observed anisotropic diffusion in the retina. The fact that diffusivity in the retina is restricted is likely due to orientation of nerve fibers, although to the best of our knowledge, the neuronal tissue within the retina is non-myelinated, in contrast to other neuronal tissues that have been studied (e.g., spinal cord and brain). Additional work is needed to determine the anatomical boundaries that could account for the anisotropic diffusion behavior. The findings of this study will aid future ocular studies, especially those involving retinal degeneration, such as macular degeneration, diabetes and glaucoma.

References: [1]. Chris Clark et al. NMR in Biomedicine (2002), Volume 15, Issue 7-8 , pg 578-586
[2] Zhang J et al. NeuroImage, Volume 15, Number 4, April 2002, pp. 892-901(10)

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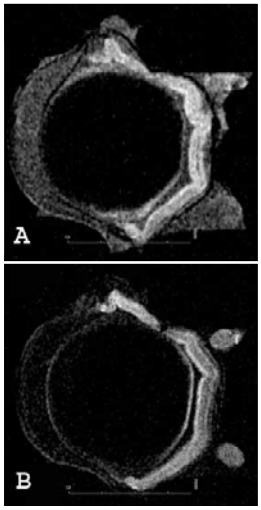

Fig.2: *DWI images*

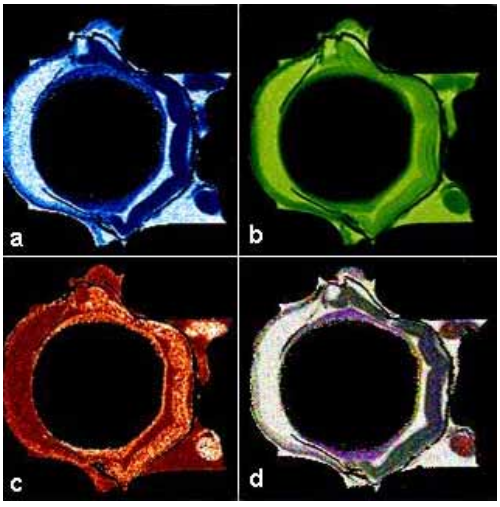

Fig. 3: *Tensor maps* ***a****: AD,* ***b****: S_0,* ***c****: FA,* ***d****: Fiber orientation*

ROI	Mean	Std Dev
Vasculature		
AD (μm^2/ms)	2.00	0.258
FA	0.358	0.111
Retina		
AD (μm^2/ms)	0.432	0.155
FA	0.692	0.188
Aqueous Humor		
AD (μm^2/ms)	1.64	0.472
FA	0.405	0.195

Table 1: *AD and FA values in various ROI*