Diffusion Tensor Imaging detects and characterizes proliferative diabetic retinopathy in the murine retina

S. Chandra[1], A. Barmpoutis[2], and J. Forder[1,3]

[1]Biomedical Engineering, University of Florida, Gainesville, Florida, United States, [2]Computer and Information Science, University of Florida, Gainesville, Florida, United States, [3]Radiology, University of Florida, Gainesville, Florida, United States

Introduction: Diabetic retinopathy is the most severe of the various other ocular problems associated with diabetes and still remains one of the foremost causes of blindness in the world [1]. The phenomenon that initiates this condition is the breakdown of the blood-retinal barrier (BRB) and neovascularization or angiogenesis around the retina resulting from hypoxia which is triggered by vascular endothelial growth factor (VEGF) [2]. In our previous endeavors we have been able to successfully detect and quantify changes in the retina using diffusion tensor imaging.

The specific aim in this study is to prove that the alterations in the eye following damage (by laser damage and diabetic retinopathy) can be detected by DTI. This is done by comparing the fractional anisotropies in the retinas of controls versus diabetic eyes. Before using actual diabetic eyes, we used mouse eyes subjected to a laser injury as a model for diabetic eyes since the laser procedure alters the BRB permeability comparable to diabetic retinopathy. This was followed by imaging eyes with diabetic retinopathy. The mice had been subjected to Type I diabetes. Both sets of experiments were successful in determining significant differences between the fractional anisotropies of retinas in controls compared to the lasered as well as diabetic retinas. Future studies will involve *in vivo* imaging of mice with various stages of the disease. *In vivo* imaging of control mice has been accomplished previously. This will ultimately lead to early diagnosis of the disease using DTI.

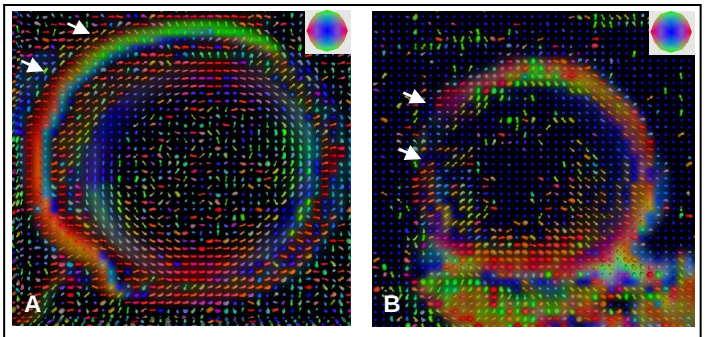


Fig 1.A: *Tensor map of control eye White arrows indicate the intact retina*

Fig 1.B: *Tensor map of lasered eye. Retinal disintegration detected by DTI*

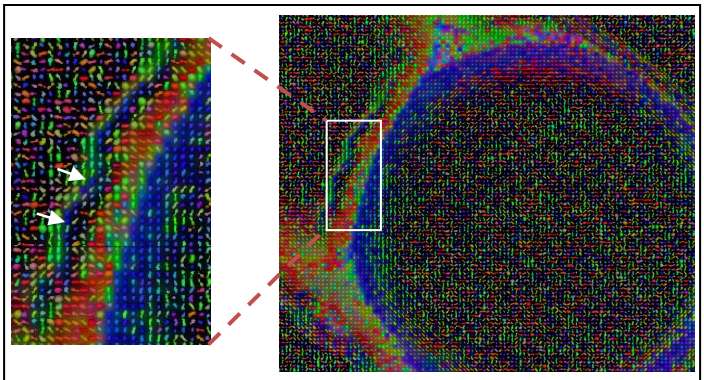
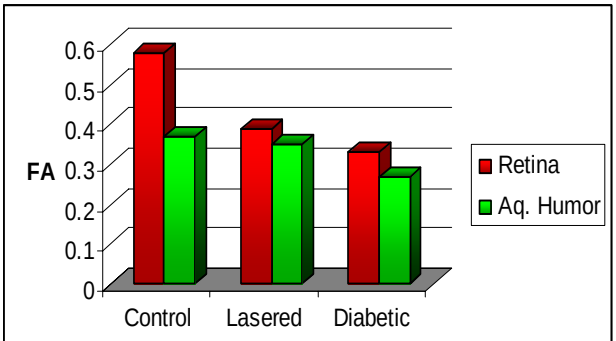


Fig 2: *Tensor map of Type I diabetic eye. White arrows indicate the area in the retina where BRB permeability has been compromised.*

Methods: The animal model used for the studies were C57 black mice. All animal procedures were performed in accordance with guidelines approved by the University of Florida Institutional Animal Care and Use Committee. A 17.6 T, 750 MHz wide bore (89mm) NMR spectrometer (Bruker Instruments, Billerica, MA) was used for our experiments. The *in vitro* studies were performed on eyes fixed in 3% w/v formalin solution. These were then washed in PBS and suspended in Fluorinert™ for imaging. A focal lesion was created using a laser technique for the lasered eyes. The diabetic eyes used for the study were extracted from mice with Type I diabetes. This was induced using multiple low doses of streptozotocin (40mg/kg for 5 days). Streptozotocin is particularly toxic to the beta cells in the pancreas which are responsible for producing insulin [3]. The mouse subject was diabetic for at least 3 months and was not on insulin therapy. The glycemia was >200mg/dl. The DT images were obtained using a 21 direction high angular resolution diffusion imaging (HARDI 21) sequence. A gradient strength of 800 mT/m was used resulting in a b-value of 970, which was maintained constant for all the 21 directions. The parameters used were TR=2500 ms, TE=18.7 ms, SW=50 kHz, Δ=7 ms, δ=1.5 ms, NA=8, matrix size=128 x 128 and FOV=1.2 mm x 1.2 mm.

Results: Fig 1.A is a tensor map of a control eye. The white arrows in each figure indicate the location of the retina. The control retina is seen to be intact and the direction of diffusion in the retina can be observed to be between the outer and inner layers of the retina. The retina of the lasered eye (Fig 1.B) is visibly disintegrated which was accurately detected by DTI. Fig 2 is the DT image of a Type I diabetic eye. This is a more accurate representation of the BRB permeability loss caused due to diabetic retinopathy. Here we see that as compared to the control eye, the diabetic eye displays much lower FA in the retina, as was expected in our hypothesis. Fig.3 is a graphical representation of the difference in FA values between the retina and aqueous humor in the control, lasered and diabetic eyes respectively. FA means and standard deviation values are listed in the table below. As is observed, the difference in FA is considerably higher in the control eye, but is much lesser in the lasered and diabetic eyes.



	Control		Lasered		Diabetic	
	Mean FA	Std dev	Mean FA	Std dev	Mean FA	Std dev
Retina	0.58	0.12	0.39	0.12	0.33	0.13
Aq.Humor	0.37	0.11	0.35	0.15	0.27	0.19

Fig 3: *Chart and corresponding table comparing FA values of the retina and aqueous humor of Control, Lasered and Diabetic eyes.*

Conclusion: Our specific aim that DTI can detect differences between normal and diabetic eyes is accomplished. Future studies will entail *in vivo* studies in diabetic mice over a period of time (between a period of 0 to 6 months) so as to detect various stages of diabetic retinopathy. This is an important step in our efforts aimed towards optimizing this technique as a perfectly non invasive method to visualize the extent of diabetic retinopathy in an individual or animal model.

References: [1] Frank, R.N., *Diabetic Retinopathy.* New Eng Journal of Med, 2004. **350**: p. 48-58, [2] Ferrara, N. and T. Davis-Smyth, *The Biology of Vascular Endothelial Growth Factor.* Endocr Rev, 1997. **18**(1): p. 4-25, [3] Rossini, A.A., et al., *Studies of Streptozotocin-Induced Insulitis and Diabetes.* Proc. of Nat. Acad of Sc., 1977. 74(6): p. 2485-2489.

Acknowledgements: This work was supported by innovative grants from the Juvenile Diabetes Research Foundation International (JRF) and AMRIS, NHMFL. We would like to Mr. Dan Clark and Mr. David Peterson for the construction of our *in vivo* coil(s). We would also like to thank Dr. Maria Grant and Dr. Sergio Li Calzi for providing the mice for our studies and Mr. Xeve Silver and Dr. Stephen Blackband for their contributions.