# Investigation of the diffusion tensor's primary eigenvector correspondence to tissue structure in MR microscopy of the human spinal cord with direct comparison to histology

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**INTRODUCTION** Diffusion tensor imaging (DTI) [1] and tractography (DTT) [2] are regularly employed in both the clinic and in research as means of investigating tissue structure and for delineating white matter tracts. Most current DTT techniques assume that the primary eigenvector of the diffusion tensor is co-linear with the dominant fiber orientation in the tissue contained in each imaging voxel. In a recent report we proposed a method for comparing DTT results to the histology of the actual tissue on which DTI experiments were performed [3]. Here we present new results obtained using these methods on samples of the ventral horn in human spinal cord. Specifically, we investigate the amount of information about tissue microstructure contained in the primary eigenvector by comparing results from DTI measurements at microscopic resolution to myelin-stained histology of the tissue samples employed.

**METHODS** Diffusion tensor microscopy (DTM) was performed (21 directions, resolution = 15.6µm in-plane) on a 600MHz Bruker imaging spectrometer interfaced with a 500µm microsurface coil developed by Bruker Biospin [4] (Fig. 1A). Immersion-fixed (4% formaldehyde) spinal cord sections (50µm) were used. Each tissue section was placed such that the white-gray matter boundary along the ventral horn was inside the coil's field of view. The diagonalized diffusion tensor and primary eigenvector was determined from the data with Matlab (R2009b, The MathWorks Inc.) via non-linear least squares regression. Following MR microscopy the tissue samples (5 in total) were removed from the coil and subjected to histological analysis using either Nissl stain (two samples) or the myelin stain Black-Gold II (Histo-Chem Inc., 1BGII) (three samples). Diffusion tensor tractography was performed using the FACT algorithm [2] as implemented in the Matlab-based DTI Tools software package (DTI Tools, Freiburg University Hospital, Germany).

**RESULTS** Figure 1C shows an example where the result of DTT is overlaid on the myelin-stained histology of the tissue sample. The overlay was produced by affine transformation (translation, uniform scaling and rotation, no shear) of the tractography. The DTT analysis is effectively two-dimensional using only the two in-plane components of the primary eigenvector. In order to investigate the remaining third component of the primary eigenvector (orthogonal to the tissue slice) we also show the variation in magnitude of this component (Fig 1D). The agreement between histology and the DTT analysis is evident and much of the same detail is seen in the map of the out-of-plane component. We have also developed a method for quantitative analysis of this agreement. The method is based on the co-registered myelin-stained histology which can be processed and down-sampled to match the MR resolution thereby producing maps of structural components in the tissue to be compared directly to the structural information obtained from the MR data. This quantitative analysis method predicts that on average 89% (±6%) of pixels predicted by DTI to contain structure are supported by histology.

**DISCUSSION and CONCLUSIONS** Here we report developments towards an improved understanding of the relation between the primary eigenvector obtained from DTM and the underlying tissue microstructure. The data (of which one example is given in Figure 1B-D) suggest a robust agreement between the primary eigenvector's direction and the structural components in the tissue at microscopic resolution. Our results confirm the primary eigenvector's role as a fundamental parameter with clear physical correlates and these results are therefore relevant to all techniques in which the primary eigenvector is employed in the investigation of tissue structure.

**REFERENCES and ACKNOWLEDGEMENTS [1]** Basser P.J. et al. Biophys J. 1994 66(1) 259-6. **[2]** Mori S. et al. Ann Neurol 1999; 45:265-9. **[3]** Flint J.J. et al. Neuroimage 2010 52(2) 556–561 **[4]** Massin C. et al. Sensors and Actuators A: Physical 2002 97:280-8. We thank Jesper Frandsen (CFIN) for helpful discussions and the AMRIS staff at UF's McKnight Brain Institute for technical support. Funding by the NIH (1R01EB012874), the NSF through the National High Magnetic Field Laboratory, and the Danish National Research Foundation (95093538- 2458, project 100297).

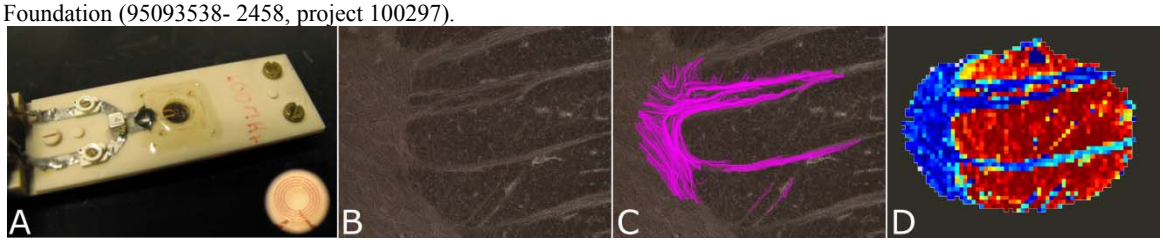


**Figure 1. A**) Photograph of the 500µm surface microcoil developed by Bruker BioSpin (B6370). The four-turn coil sits inside a 5mm diameter, 500µm deep tissue well (magnified coil inserted in lower right corner). **B**) Histology (Black-Gold II) of one of the tissue slices used in the MRM. **C**) Overlay of diffusion tensor tractography data calculated using the FACT algorithm (pink lines) onto the correlative histology shown in panel B. **D**) The variation in the out-of-plane component of the primary eigenvector: blue represents low values, warmer colors larger components. It can be seen that this component which is not used in the DTT in panel C also compares favorably with the histology.