Diffusion Tensor Tractography of Individual Nerve Fibers in the Ventral Spinal Cord of the Rat With Histological Validation

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INTRODUCTION Diffusion tensor tractography (DTT) is a relatively new analysis technique that allows for long-distance mapping (over several imaging voxels) of white matter tracts in the nervous system. The method is based on data from diffusion tensor imaging (DTI) experiments from which the diffusion tensor, mean diffusivity, preferred diffusion direction (the primary eigenvector which is assumed to be parallel to the dominant fiber orientation in each voxel), and fractional anisotropy (FA) may be calculated (see e.g. [1] for review and references). Several schemes exist for performing tractography, but all algorithms employ either deterministic [2,3](e.g. the FACT algorithm employed here) or probabilistic [4] analysis based on the orientation of the primary eigenvector of the diffusion tensor to determine if connectivity exists between adjacent image voxels. Owing to their tissue microstructure—primarily long, unbranched, myelinated axons—white matter tracts exhibit high degrees of anisotropy caused by restriction of water perpendicular to the path of the axon. This property, combined with various historic problems associated with describing connectivity via serial histology, puts DTT analysis at the forefront of methodologies with the greatest likelihood of accurately describing connectivity in the central nervous system; however, because proper means of validating DTT data have been lacking, many questions still exist concerning its accuracy in elucidating fiber tracts. In this study, we offer a method of employing histological analysis combined with magnetic resonance microscopy (MRM) as a means of validating DTT analysis protocols. Because fiber tract orientation and inter-voxel connectivity can be verified by our histological method, no ambiguity exists as to the spatial positions of white mater tracts prior to the DTT analysis. It is our hope that this method will be employed when generating DTT analysis algorithms in order to yield ever more accurate tractography.

METHODS Imaging was performed on a 600MHz Bruker spectrometer interfaced with a 500µm microsurface coil developed by Bruker Instruments Inc [5] (Fig. 1). Perfusion-fixed (4% formaldehyde) spinal cord sections (50µm) were used. The tissue section was placed such that the boundary along the ventral horn between white and gray matter was contacting the coil face. The diagonalized diffusion tensor and primary eigenvector was determined with Matlab (The MathWorks Inc.) via non-linear least squares regression. We employed the full b-value matrix as given in the data log files for these calculations. The positions of alpha motor neurons in the mean diffusivity map were used to align the histology and the in-plane primary diffusion directions (in-plane coordinates of the primary eigenvector) obtained from the DTI data such that no quantitative structural information was transferred from the histology to the interpretation of the MR data. Several tissue samples were successfully analyzed using the methods described.

RESULTS Alpha motor neurons in the spinal cord gray matter are clearly visible in the histology (Fig. 2) and manifest as high intensity regions in the mean diffusivity map (not shown). Figure 2A shows the overlay produced by an affine transformation (translation, uniform scaling and rotation, no shear) of the diffusion orientation map (shortest vector components removed for clarity) onto the histology using the cell body positions in the histology and MR data as land marks. Figure 2B shows the result of DTT on the data using the FACT algorithm [2] as implemented in the software package DTI Tools (Freiburg University Hospital, Germany) [6]. The red dots indicate the coordinates of the cell bodies in the MR images (obtained from the mean diffusivity map).The correlation between DTI data, DTT, and histology, while not flawless, is clearly evident upon viewing the graphic overlays from these experiments.

DISCUSSION and CONCLUSIONS In this study, we have attempted to examine the utility of employing MR microscopy coupled with correlative histology as a means of validating current methods used for generating tensor and tractography data. The key to this technique lies in its ability to delineate axonal pathways via histology which serves as a template against which previously unverifiable DTI and DTT data may be compared. In this way, alternative algorithms which are now available may be compared and analyzed critically based on their relative success and new algorithms which produce data that better describes tissue structure can be devised.

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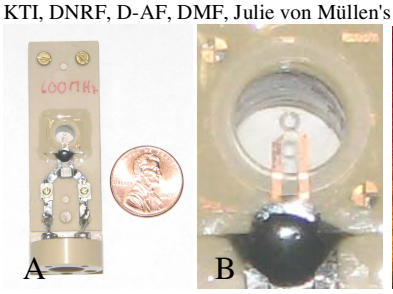

Figure 1. (A) Photograph of the 500µm surface microcoil developed by Bruker, Switzerland (Z76409). The four-turn coil sits inside a 5mm diameter, 500µm deep tissue well (B).

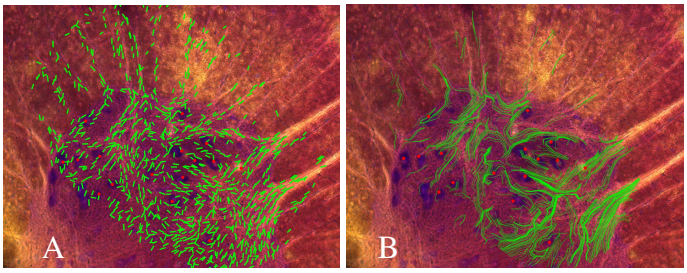

Figure 2. (A) Overlay of diffusion orientation map (green bars) onto correlative histology. Cell bodies of α-motor neurons (purple) are marked in the orientation map with dots (red) and were used to coregister the data. (B) Overlay of diffusion tensor tractography data calculated using the FACT algorithm. Images were coregistered in the same manner as panel A.