Average Axon Diameter Mapping of Pig Spinal Cord Using d-PFG Filtered MRI

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Introduction: The diameter of myelinated axons is a crucial neurophysiological parameter that is linearly related to conduction velocity. In the spinal cord, axons are somatotopically organized into nerve bundles that perform specific functions and are characterized by different diameters and diameter distributions. The purpose of this study is to evaluate the use of double pulsed-field gradient (d-PFG) MRI[1] to measure and map the apparent mean diameters within different regions of spinal cord white matter.

Materials and Methods: Formalin-fixed pig spinal cord was rehydrated and placed in a 10mm Shigemi tube (Shigemi Inc.) with the axons aligned with the z-axis of a 14T vertical-bore Bruker AVANCE III MRI scanner. PFG NMR parameters were: δ=3.15ms, Δ=60ms, and G was between 0 and 664 mT/m^{-1}. MRI parameters were: TR/TE = 3500/6.54 ms, FOV=11 mm, matrix size=128x128, and slice thickness = 4mm. A model previously developed to predict the MR signal attenuation due to restricted diffusion within impermeable cylinders[2], was fit to the DW signal attenuation data. This model also included a free water compartment[3]. A fiber diameter map within the white matter regions of the spinal cord was computed pixel-by-pixel. A k-means segmentation was performed using the average fiber diameters. For histological studies a portion of the formalin-fixed spinal cord was placed in 3% glutaraldehyde, post-fixed in osmium tetroxide, and embedded in plastic[4-5]. One-micron thick sections were stained with toluidine blue. Regions-of-interest (ROI) were determined and sectioned for evaluation on a Zeiss 109 transmission electron microscope (TEM). Measurements of mean axon diameter were obtained using Image-J and compared with the average axon diameter measured using d-PFG filtered MRI.

Results: Fig 1a shows the average fiber diameter map calculated from the d-PFG filtered MRI experiments. Fiber diameter estimates range between 3 and 5 μm. Five distinct k-mean clusters were also identified. Measurements of the average axon diameter obtained from histology and d-PFG filtered MRI agree very well.

Discussion: The fiber diameters obtained from d-PFG MRI are in the expected range for such specimen[6]. Clusters produced from the experiment match histological findings. While FA maps obtained from DTI in fixed spinal cord white matter can appear homogeneous, d-PFG MRI analysis clearly identifies distinct anatomical regions. This increased specificity derives from the strong dependence on microanatomical features of d-PFG MRI data.

Conclusion:D-PFG filtered MRI is emerging as a powerful tool for mapping axon diameter, and potentially other microstructural features of tissues. Because of the relatively low gradient strengths required to implement this method, as compared to (Stejskal-Tanner) single PFG MRI experiments, d-PFG MRI has great potential for use in clinical MRI systems.

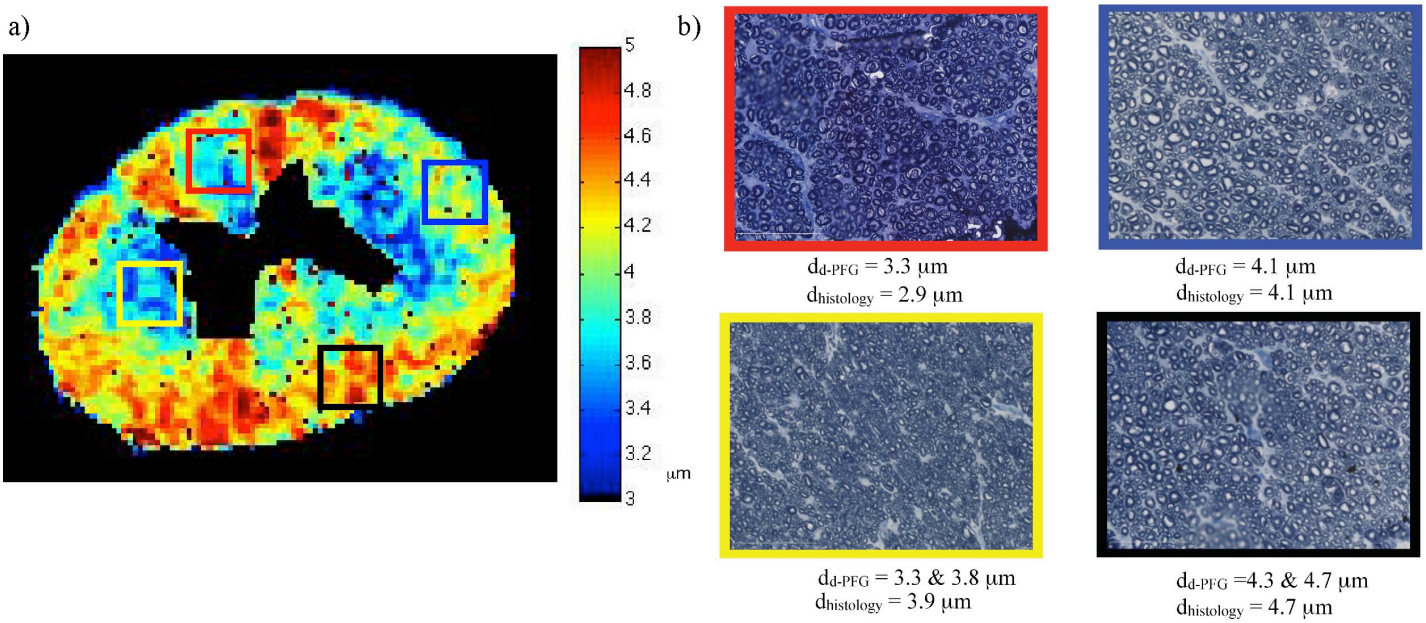

Figure 1. a) Average fiber diameter map computed from a d-PFG filtered MRI experiment. b) TEM images of Toluidine blue stained spinal cord sections used for histological studies corresponding to the ROIs in a). Average axon diameters are compared using d-PFG MRI and histology data.

References:

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