Axon diameter distribution (ADD) mapping of porcine spinal cord using d-PFG MRI

Dan Benjamini[1,2], Michal E Komlosh[1,3], Uri Nevo[2], and Peter J Basser[1]
[1]NIH, Bethesda, MD, United States, [2]Tel Aviv University, Tel Aviv, Israel, [3]USUHS, Bethesda, MD, United States

Introduction: Noninvasive characterization of the axon diameter distribution (ADD) provides insights about the transmission of information along the nerves, and is altered in several diseases, such as amyotrophic lateral sclerosis [1] and multiple sclerosis [2, 3]. Parametric ADD estimation of white matter tissue was previously demonstrated using data from a single pulsed-filed gradient (s-PFG) experiment with varying diffusion periods [4], while assuming a γ-distribution for the ADD. Obtaining the ADD using a non-parametric distribution (i.e., one with no *a priori* assumptions) would provide comprehensive sub-voxel microstructural information without introducing the bias involved in assuming its shape. Estimation of such an empirical pore size distribution has been demonstrated with s-PFG NMR in conjunction with solving an inverse linear problem [5]. Recently this linear estimation approach was extended to include a second dimension in the parameter space using a double pulsed-field gradient (d-PFG) experiment [6]. This 2-D method was shown to improve the stability and reliability of the estimated empirical size distribution [6]. Previously, the 2-D NMR method was performed on calibrated microcapillary size distribution phantoms [7], and then migrated to MRI using a glass capillary array phantom [8], both resulting in accurate size distribution estimates.

Materials and Methods: A porcine spinal cord was excised and put immediately in a 4% formalin solution. Prior to performing MRI experiments the sample was rehydrated with phosphate buffered saline (PBS). The rehydrated spine was then immersed in perfluoropolyether (Fomblin LC/8, Solvay Solexis, Italy) and inserted into a 10 mm Shigemi tube (Shigemi Inc., Japan) with a plunger matched to the susceptibility of water. The tube was placed in a 7T vertical-bore Bruker AVANCE III MR microimager. Diffusion tensor MRI (DTI) was performed prior to d-PFG NMR to verify nerve fiber orientation. d-PFG NMR parameters were: $\delta = 3.15$ ms, $\Delta = 30$ ms, 5 diffusion gradient amplitudes G = 370-664 mTm^{-1}. The angle, φ between the two consecutive PFG blocks was varied between 0 and 2π in the plane perpendicular to the axon's axis of symmetry. MRI parameters were: TR/TE = 3000/6.4 ms, and spatial resolution = 0.105 x 0.105 x 4 mm^3. The acquired data was then fit, pixel-by-pixel, to the 2-D linear size distribution model according to [6, 7]. The empirical ADD is discrete, comprising 30 bins with a 0.22 μm resolution. The analysis provided spatially resolved maps of the ADD, intra- and extra-cellular diffusion coefficients and relative volumetric fractions. Distinct domains in white matter, where the ADDs are similar, were found by clustering with an iterative K-means algorithm (K=6) with spatial constraints.

Results and Discussion: The ADD map is presented in Fig. 1, where different colors are used to mark the ADD clusters. The average ADDs of the clusters are shown in Fig. 2. Since the ADDs are not continuous functions, variation within neighboring pixels can be expected. As a result, the clustering algorithm cannot always resolve the ADDs with uniform and continuous borders. A spatially constrained clustering algorithm was used to overcome this issue. Symmetry of the clusters is evident in the ADD map in Fig. 1, which is expected from knowledge of the underlying neuroanatomy and somatotopic organization of white matter pathways in the spine. Although the average axon diameter of each cluster is very similar (0.89, 1.68, 1.26, 1.10, 1.32, 1.00 μm), the ADDs reveal underlying axonal microstructure, and emphasize different structural regions.

Conclusion: While a parametric ADD may well-describe a healthy, normal neural tissue, a non-parametric ADD provides an objective description of any white matter tissue, regardless of its viability. As function and anatomy are strongly linked in the nervous system, ADD estimation could prove to have clinical MRI applications owing to its critical functional role in the central and peripheral nervous systems, in normal and abnormal tissue.

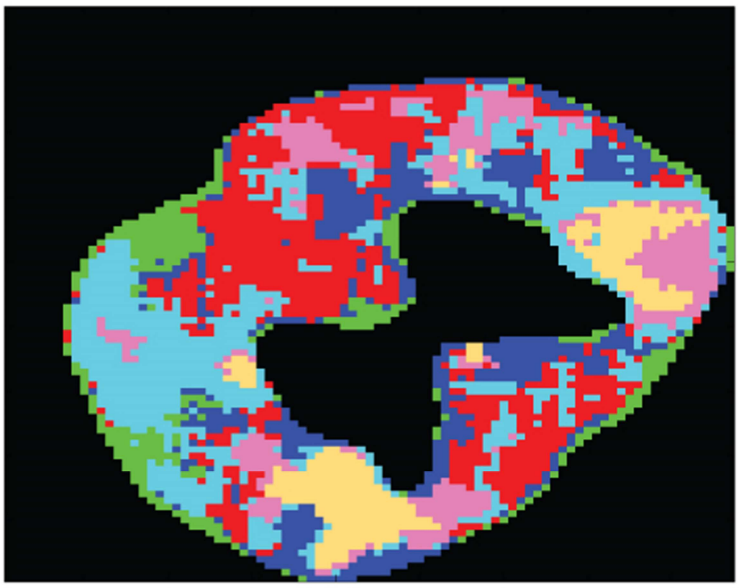

Figure 1: The ADD clustered map of the spinal cord. Different clusters and letters are used to mark the different clusters.

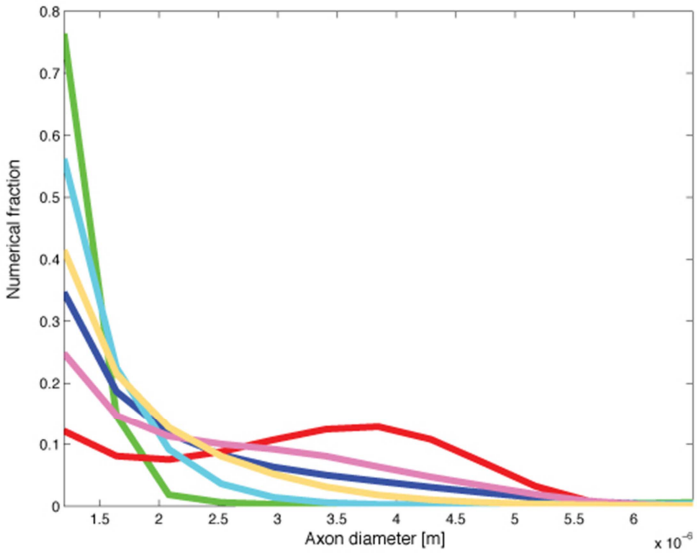

Figure 2: ADDs from the different clusters. Each ADDs color corresponds the color of the cluster in Fig. 1.

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