Measuring three-dimensional morphological features of beaded axons using MRI

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Introduction: The shape and pore size distribution strongly influence macroscopic functional properties of tissue. Axon diameter is known to be correlated with the conduction velocity of nervous impulses, and the axon diameter distribution (ADD) has been shown to be a determinant of the amount of information that can propagate along a bundle of axons. Quantification of the compartment shape eccentricity, in addition to its size, is particularly valuable as a means to assess injured axons, as it is known to change following mechanical, chemical, or metabolic insults. This local variation in eccentricity is usually referred to as "beaded" axonal morphology, and its noninvasive characterization is of great potential diagnostic value in assessing the functional status of nervous tissue. Nonparametric estimation of the pore size distribution was first suggested using single pulsed-field gradient (s-PFG) experiments, and later by employing a double-PFG (d-PFG) sequence, which was shown to stabilize the reconstructed distribution. When axons are capped, their shape distribution must now be described by a bivariate distribution instead of a monovariate one. Specifically, a finite (capped) cylinder would be characterized by a 2-D joint radius-length distribution function (*R-L* distribution), with an associated marginal radius distribution (MRD) and a marginal length distribution (MLD). In this work we present an experimental design and analytical framework to measure the nonparametric joint *R-L* distribution of an ensemble of parallel finite cylindrical pores, and more generally, the eccentricity distribution of anisotropic pores as a first step to develop a microstructural MR pipeline for assessing axonal injury *in vivo*.

Methods: Rat sciatic nerves were excised and subjected to axial tension sufficient to induce beading or minimal tension to straighten out their macroscopic undulation (control). These nerves were immediately immersed in fixative and rehydrated in phosphate buffered saline (PBS) prior to acquiring MR data. Owing to the separation of position variables in both the parallel and perpendicular directions of the capped cylinder, we suggest that the direction of the diffusion encoding can be used in the same manner to obtain a complete microstructural description by a two-step experiment—first independently finding the MRD and MLD, and then estimating the joint R-L distribution. The first step involves preforming a set of d-PFG experiments with gradients encoding the orthogonal perpendicular and parallel directions of the cylinder. Then, to estimate the joint distribution, a d-PFG experiment with gradients encoding the *x-z* plane (*R-L* plane of symmetry) is performed, thus correlating the two orthogonal axes. The marginal distributions are then used as equality constraints for the estimation of the joint distribution. Using these marginal distributions as constraints allows the joint distribution to be reconstructed, even for an underdetermined system (i.e., more unknown variables than equations), which can reduce the number of MR acquisitions to a feasible number. Along with the experimental data using a 7T vertical-bore Bruker AVANCE III MR microimager, a representative joint distribution phantom corrupted by Gaussian white noise ($\sigma = 0.005$) was reconstructed to demonstrate the acquisition and processing pipeline.

Results and Discussion: The MRD and MLD were estimated from *x-y* plane and *z*-direction d-PFG experiments, respectively, both from beaded sciatic nerves and from simulations. To provide clear evidence that the use of the MRD and MLD as equality constraints in the joint distribution estimation improves the results, the estimation was performed twice – without and with using the information provided by the marginal distributions. From these simulations, it is clear that without the use of the additional information contained in the marginal distributions, the joint distribution reconstruction is inaccurate, as two out of the five peaks are missing, while the remainders of the estimated peaks are much broader than were prescribed.

Conclusion: The determination of the joint *R-L* distribution permits direct quantification of the compartment shape anisotropy (CSA). Nonparametric measurement of the CSA of injured (beaded) axons may help to characterize and quantify the amount of tissue damage and shed light on the injury mechanism and the possible microstructural changes that may occur following the injury—information which does not appear to be obtainable using other means.