Assessment of axon diameter distribution in mouse spinal cord with q-space imaging

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Introduction

Axon morphology is closely related to neural function, anatomy, and pathology. For example, axon diameter is directly proportional to conduction velocity[1] and diameter distribution is observed to vary with location[2] and disease[3]. Axon morphology is typically assessed destructively with histology using metrics such as mean axon diameter (MAD), intracellular volume fraction (ICF), and axon diameter distribution (ADD). Q-space imaging[4] (QSI) offers potential for indirect assessment of white matter (WM) architecture by exploiting the regularity of molecular diffusion barriers from axon membranes and myelin sheaths[5]. The Fourier transform of the QSI signal is known as the displacement probability density function (d-PDF), and contains information on axon morphology such as MAD and ICF[6,7]. In this work, we show, using healthy mouse spinal cords (SC), that ADD can also be inferred from the d-PDF. Similar to the AxCaliber method[8,9], by first separating the intra- and extra-cellular (ICS and ECS) signals, the ADD is calculated from fitting the overall signal by the individual contributions of axons of varying diameter weighted by the diameter distribution. We note that while the AxCaliber method operates in q-space, the method here operates in displacement space.

Methods

Previously published data[6,7] was reanalyzed here and a brief summary of the methodology is given below. Five SC sections were dissected from perfusion-fixed adult female C57 BL/6 mice. Experiments were performed with a custom-built 50T/m z-gradient/RF coil set interfaced to a 9.4T micro-imaging system (Bruker DMX 400). A diffusion-weighted stimulated-echo sequence was used: 64×64, FOV/THK=4/1*mm*, and TE/Δ/δ=17.4/10/0.4*ms*. The diffusion gradient was applied perpendicular to the SC long axis in 64 increments of q (q_{max}=0.82 μm^{-1}). Q-space decay curves were obtained for each pixel and averaged over ROIs selected within seven WM tracts (Fig. 1) and d-PDFs were calculated. ICS and ECS d-PDFs were separated using a two-compartment model[7]. This model assumes no exchange between ECS and ICS, which given the short diffusion time, Δ, is a reasonable assumption. MAD is estimated from the full-width-at-half-maximum (FWHM) of the ICS d-PDF, which was shown to agree well with MAD measured from histology[7]. Histology was performed on toluidine blue stained SC sections within the MRI slice and images were obtained from all WM tracts. The images were segmented into extra- and intra-axonal, and myelin regions with a custom Matlab algorithm and the ADD was computed.

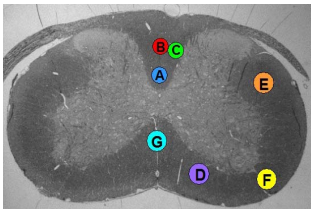

Figure 1. Optical image of SC section showing WM tract locations: A) dorsal corticospinal (dCST), B) gracilis (FG), C) cuneatus (FC), D) rubrospinal (RST), E) spinothalamic (STT), F) reticulospinal (ReST), G) vestibulospinal (VST).

Measuring ADD with QSI starts with calculating the d-PDF from a single axon with radius r (PDF_r), which is the auto-correlation function of the axon spin density[4]. By assuming the axon cross-section to be circular, PDF_r can be analytically computed. Therefore, ICS d-PDF = $\sum ADD(r) \times PDF_r$ (Eq. 1) where the summation is over all axon radii. Based on empirical observation[6-9], the ADD can be modeled as a gamma distribution: $ADD(r|\alpha,\beta) = (r^{\alpha-1}e^{-r/\beta})/(\beta^{\alpha}\Gamma(\alpha))$ (Eq. 2) where $\Gamma(\alpha)$ is the gamma function. Eq. 2 is incorporated into Eq. 1 and the α and β parameters of the gamma distribution are fitted to the ICS d-PDF using a nonlinear least-squares Matlab algorithm. The α and β parameters were then optimized so the calculated ADD had the same MAD as measured from the ICS d-PDF using an unconstrained nonlinear optimization Matlab algorithm.

Results and Discussion

All fitted PDFs showed good agreement with the ICS d-PDF (R^2>0.8). The calculated ADD all have MADs matching values measured with QSI as described above. Figure 2 shows ADDs measured from histology and QSI averaged over 5 mice for all WM tracts. The WM tracts were ordered from smallest MAD (dCST, 0.81±0.06 μm) to largest (VST, 1.73±0.21 μm). From the histologic ADDs, it is clear that there is a gradual trend of increasing variation in axon diameter with increasing MAD. QSI-derived ADDs semi-quantitatively agree with histologic ADDs. Importantly, QSI-derived ADDs consistently show the relative differences in ADD between WM tracts. That this method was sensitive to the subtle increase in the spread of ADD with increasing MAD suggests its potential to detect changes in ADD.

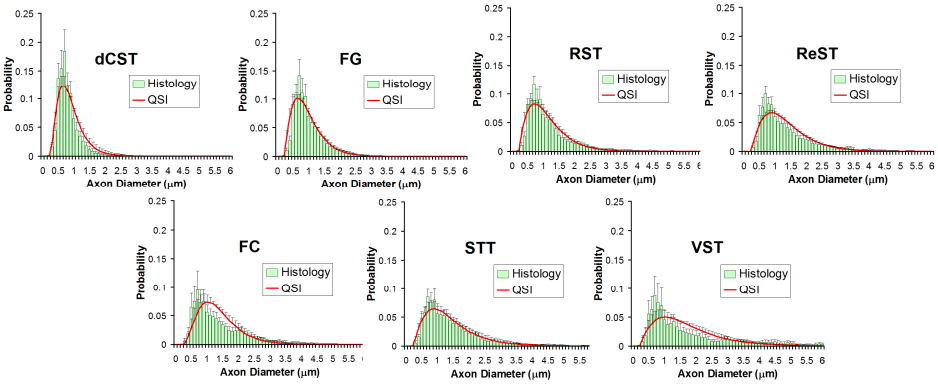

Figure 2. Plots of ADD measured with histology (green bars) and QSI (red line) for all WM tracts. All ADDs were averaged over 5 mice and the standard deviation bars are shown.

The QSI-derived ADDs systematically underestimates the ADD at diameters <1 μm. This may be the result of errors in the assumptions of this method, namely circular axon geometry and a gamma distributed ADD. In particular, the true ADD may not be gamma distributed, especially in the presence of pathology, and suggests the need for a non-parametric approach for estimating ADD[10]. Despite these limitations, the QSI method may still be able to detect relative changes in ADD and further work is needed.

Conclusion

This work demonstrates the feasibility of QSI to measure ADD in WM. The results show that this method is sensitive to gradual differences in ADD between WM tracts and suggests its potential to detect changes in ADD.

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