Validation of MRI microstructure measurements with Coherent Anti-Stokes Raman Scattering (CARS)

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Purpose. Validation of MRI microstructure measurements, such as AxCaliber [1], is typically performed using optical [2,3] or electron microscopy[1,4]. These methods are limited in that only small portion of the tissue can be imaged (typical FOV on the order of 100 µm). Moreover, large-scale histology is time-consuming and susceptible to artifacts caused by fixation, staining, embedding, and cutting deformations. Coherent Anti-Stokes Raman Scattering (CARS) microscopy is a feasible alternative to conventional histology, which can be used to perform large-scale microscopy images of tissue with a high optical resolution (350nm) [5] (see Figure 1). Another advantage of CARS is that fresh tissue can be imaged without the need for staining because myelin can be used as an endogenous image contrast by setting the laser frequencies appropriately. The objective of this study was to perform a demonstration about the use of CARS imaging for validating MRI microstructure measurements.

Methods. *Acquisition*. An *ex vivo* thoracic segment of rat spinal cord (perfusion and post-fixed fixed with paraformaldehyde 4%) was scanned on a 7T preclinical scanner (Agilent) equipped with 600 mT/m gradients. The tissue was inserted into a small glass tube filled with buffered water. A custom-made solenoid coil was used for transmission and signal reception (S_{11} ~ -30dB). A single shot EPI sequence was used: four axial slices, matrix 64x64, BW=24kHz, TR=1s, TE=70ms, δ=3/3/8/15ms, Δ = 10/40/40/40 ms, Gmax = [0 .. 600] mT/m (200 increments). Resolution was 0.1x0.2x2 mm^3. Total acquisition time was 5 min. *Model*. The extra-cellular diffusion was modeled as Gaussian and intra-cellular diffusion was modeled as restricted in cylinders using Gaussian phase distribution (GPD) [6]. The fitting parameters were (i) a single axon diameter (as in ActiveAx [7]), (ii) fraction of restricted water (fr), (iii) Apparent diffusion coefficient for the hindered compartment. We assumed a diffusion of 1.4µm^2/ms along the spinal cord. The fitting procedure used the non-linear least-square optimizer (Levenberg Marquardt) implemented in Matlab. *CARS imaging:* After MRI acquisition, the sample was isolated and 350 µm thick transverse sections were cut with a vibratome (Leica, VT 1000). An entire transverse section was imaged on a custom video-rate laser-scanning microscope in four hours. The field of view of the CARS microscope was (FOV) 159.5 µm by 113.5 µm (208x227 nm/pixel) with a 60× objective lens (UPLSAPO 1.2 NA w, Olympus). A stitching procedure was applied on recorded images to reconstruct a thoracic section of the rat spinal cord (1847 µm by 2661µm). *CARS image analysis.* Eight regions of interest (182x182µm) were segmented automatically using a custom-made Matlab software based on extended-minima transform and active contour method with regularizations for size and circular smoothness. *Statistics:* The eight regions of interest were manually drawn on MRI volumes following co-registration of both modalities. We corrected the mean axon diameter (d') from histology by taking into account the difference of water volume between larger and smaller axons: $d' = \sum_d \left(\frac{\pi d^2}{\sum_a \pi a^2}\right) d$.

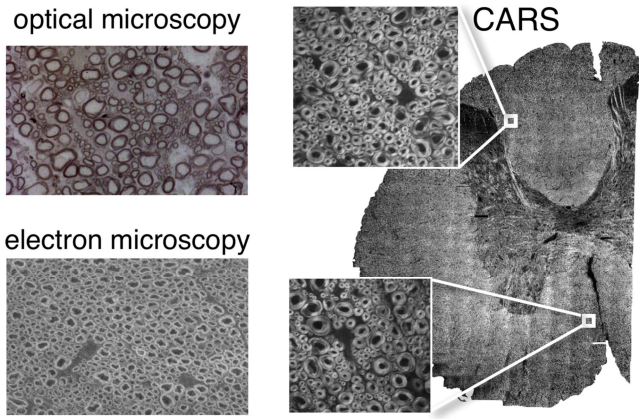

Fig 1. *Illustration of optical microscopy (NA=0.95, 40x), scanning electro microscopy (1000x) and CARS imaging for imaging myelinated axons.*

Results. Axon diameters measured with CARS and MRI were significantly correlated (r=0.87, p=0.0046). Figure 2a shows a map of axon diameter estimated with MRI, Fig 2b shows the ROIs for MRI vs. CARS comparison, Fig 2c shows an example of GPD fitting and Fig 2d shows the axon diameter measures for CARS and MRI. A global overestimation of axon diameter (~2µm) was found for MRI data compared to CARS.

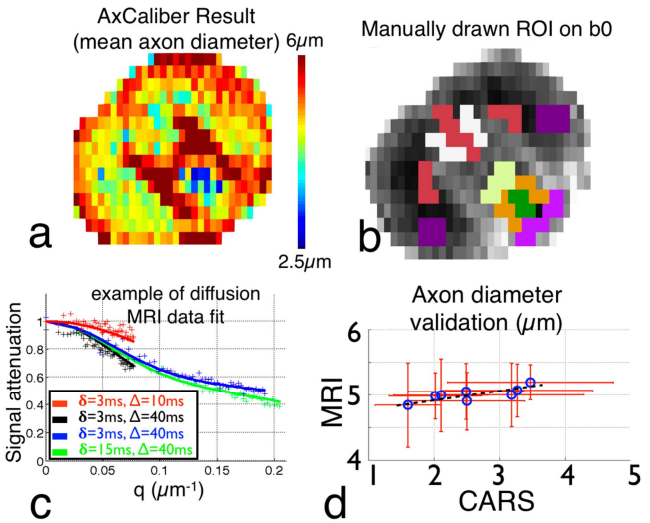

Fig 2. *Mapping of axon diameter from MRI (a). Manual ROIs used for comparison (b). Example of MRI qspace fit (c). Comparison between MRI (with standard deviation across voxels) and CARS (with std of axon diameter distribution) (d)*

Discussion. In this work, we demonstrated the benefits of staining-free CARS microscopy for the validation of MR-based microstructure measurements. This high optical resolution myelin imaging technique provided a map of a local axons diameters distribution throughout the tissue. The overestimation of the axon diameter with MRI (~2 µm) is likely due to the low contribution of diffusion-related signal attenuation in small axons (1-2 µm) relative to larger axons (>3µm). This effect has been revealed in simulations [8]. In conclusion, CARS microscopy enables large-scale myelin imaging at sub-micrometer resolution without the need for staining.

References. [1] Y. Assaf et al., *Magn Reson Med*, vol. 59, no. 6, pp. 1347–1354, Jun. 2008. **[2]** H. H. Ong et al., *Neuroimage*, vol. 40, no. 4, pp. 1619–1632, 2008. **[3]** A. N. Dula et al., *Magn Reson Med*, vol. 63, no. 4, pp. 902–909, Apr. 2010. **[4]** D. Barazany et al., *Brain*, vol. 132, no. Pt 5, pp. 1210–1220, May 2009. **[5]** E. Bélanger et al., *Opt. Express*, vol. 17, no. 21, p. 18419, Oct. 2009. **[6]** L. Z. Wang et al., *J Magn Reson A*, vol. 117, no. 2, pp. 209–219, Dec. 1995. **[7]** D. C. Alexander et al., *Magn Reson Med*, vol. 60, no. 2, pp. 439–448, Aug. 2008. **[8]** D. C. Alexander et al., *Neuroimage*, vol. 52, no. 4, pp. 1374–1389, 2010.

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