ActiveAx using dictionary learning with electron microscopy validation

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Target audience: This abstract is intended for quantitative and microstructural imaging communities.

Purpose: Diffusion Weighted Imaging (DWI) is an extremely important tool for *in vivo* and non-invasive axonal morphometry[1]. ActiveAx[2] is a DWI model-based technique that provides orientationally invariant indices of axon diameter and density. In such parametric approaches, the fitting procedure is a limitation, because various independent parameters have a similar effect on signal decay and the initial conditions become critical. To tackle this hurdle, we propose a dictionary learning approach (here referred to ActiveAx DL). We generate a dictionary for each voxel directly from the data, so that some characteristics of the tissue and signal, such as fibre orientation and SNR, are pre-specified, allowing the dictionary to reflect mainly the parameters of interest. We tested ActiveAx DL on the mouse corpus callosum, and compared estimated axon diameter indices with values obtained with the conventional technique and with electron microscopy (EM). Two PGSE protocols, one with G_{max} of 300 mT/m [3], and one with G_{max} of 1.3 T/m (maximum *b*-value of 105,000 s/mm^2) were optimized[2] to gain high sensitivity to callosal axons in the mouse.

Methods: *Preparation and DWI:* Here, we provide the results of ActiveAx in sub-regions of the corpus callosum of an *ex vivo* mouse brain. An adult mouse (C57BL/6J) was fixed and imaged on a 16.4 T Bruker scanner (Bruker Biospin, Germany) using a 15 mm SAW coil (M2M Imaging, USA). Three mid-sagittal slices were scanned with in-plane resolution of 100×100 µm and slice thickness of 300 µm (only the most central slice was used). For ActiveAx with G_{max} of 300 mT/m: 3 shells, each 120 directions (using Camino [4]), G = {300, 220, 300} mT/m, δ = {5.6, 7.0, 10.5} ms, Δ = {12.1, 20.4, 16.9} ms, TE/TR of 35/750 ms was used. For ActiveAx with G_{max} of 1.3 T/m: 5 shells, 4×60 and 120 directions for the high *b*-value, G = {1107, 1227, 464, 509, 1350} mT/m, δ = {1.1, 2.3, 6.3, 5.6, 8.6} ms, Δ = {28.4, 7.0, 23.0, 23.7, 13.6} ms, TE/TR of 35/750 ms was used, where *b*-value = $(2\pi q)^2(\Delta - \delta/3)$ (s/m^2), where $q = (2\pi)^{-1}\gamma\delta G$ (m^{-1}).

EM imaging: Following imaging, the brain was sectioned sagittally at 50µm thickness using a vibratome. On the mid-sagittal section, the corpus callosum was isolated and samples of the genu, body and splenium were separated. Sample preparation was carried out according to the methods of [5]. Fig. 1 illustrates a representative sub-image from each region. Sections were cut on a UC6 ultra-microtome (ultracut S, Reichert, Leica, Sweden) at 60nm, and imaged at ×5000 in a transmission electron microscope at 80 kV (JEM 1011, Jeol, Japan). Images were captured with an Olympus Morada digital camera. A total of ~20,000 axons was manually segmented (7,680, 5,260, and 7,188 in the genu, body and splenium, respectively). Axon diameter indices were then calculated from the axon diameter distribution as described in [2] (Fig. 1A).

ActiveAx and ActiveAx DL: To obtain axon diameter indices from DWI, the MMWMD model[2] was fitted to each voxel of the data, using the Camino software package. For ActiveAx DL and for each voxel of the corpus callosum, we performed the following steps:

- SNR of each shell was calculated. Fiber orientation was also obtained by fitting one shell of the data to the DTI model,
- Volume fraction of stationary water was obtained by fitting all shells to a Ball-Stick (with Watson distribution)-Dot model [6]
- A dictionary was generated using a Zeppelin-Cylinder-Dot model, with the following inputs:
  1) Scan protocol (G, Δ, δ, TE, gradient directions), 2) SNR, fibre orientation, and stationary volume fraction from above,
  3) Axon diameters of 0.1 – 6 µm, with 0.1 µm steps, 4) Axon volume fraction of 0.3 – 0.9, with 0.05 steps,
  5) Parallel diffusivity of 0.6 mm^2/ms, tortuosity model of similar to [2] and no exchange rate was considered.
  Resulting in a dictionary of M×N (M configuration and N gradient directions).
- The dictionary was trained with axon diameter values of the simulation step as labels.
- The axon diameter of the given DW voxel was then obtained by finding the best match (nearest neighbour) in the dictionary.

Results: Mean axon diameter values from EM were ~0.55 µm in sub-regions of the mouse corpus callosum. ActiveAx correlates more closely with the volume weighted mean diameter[2], which we refer to here as the *axon diameter index*; these were slightly different across sub-regions (0.8 – 0.93 µm). Values obtain from DWI (Fig. 1B), even with G_{max} of 1.3 T/m, were higher than those from EM values. For ActiveAx 300 mT/m, the axon diameter indices obtained from ActiveAx DL were significantly lower than those obtained with conventional ActiveAx ($p < 0.00001$ using paired t-test). In addition, the variance of ActiveAx DL values were lower compared with the conventional ActiveAx. In contrast, for ActiveAx 1.3 T/m, values from the conventional ActiveAx were slightly lower, with lower variances (not significant). Surprisingly, axon diameter values obtained from ActiveAx DL 300 mT/m were lower than that from ActiveAx DL 1.3 T/m.

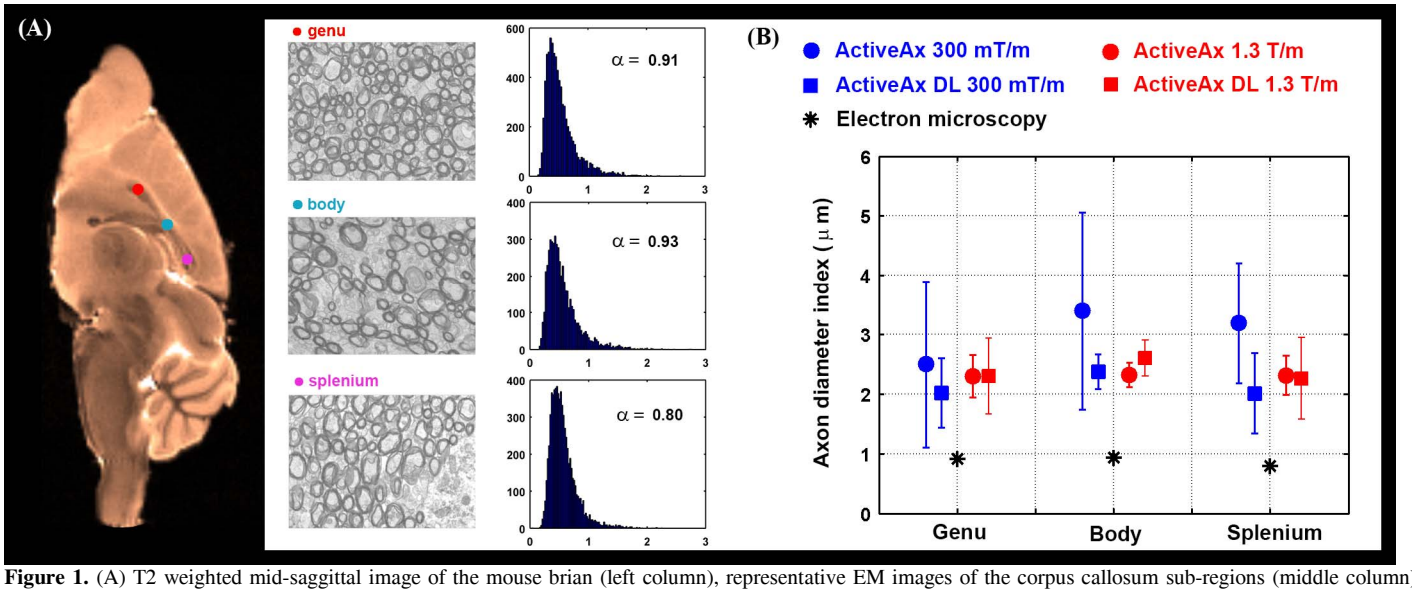

Figure 1. (A) T2 weighted mid-saggittal image of the mouse brian (left column), representative EM images of the corpus callosum sub-regions (middle column), axon diameter distribution and axon diameter indices (α) of each region (right column). (B) Axon diameter indices obtained from differen techniques.

Discussion: In this work, we proposed and evaluated a dictionary learning approach to obtain the axon diameter index using the ActiveAx framework. We found that, firstly, for the protocol with G_{max} of 300 mT/m, the dictionary learning approach is more sensitive and stable than conventional ActiveAx. In the protocol with G_{max} of 1.3 T/m, estimated values of the dictionary learning approach were lower than expected. This may be due to the relatively simple model that was used in our method. There is likely to be significant fibre dispersion and undulation, which the model does not account for. Dictionary learning, as being used here, performs best when the model can fully explain the system. Our secondary observation was that employing G_{max} of 1.3 T/m yielded higher precision and accuracy than G_{max} of 300 mT/m; however estimated axon diameter values were limited to values higher than 2µm. Simulations suggested that this was due to the physical limitation of the PGSE (not presented here).

References:

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