Validation of orientation distribution functions in 3D using confocal microscopy

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PURPOSE Diffusion MRI (dMRI) is commonly used in neuroimaging studies to assess white matter "integrity", reconstruct major fiber bundles, and quantify connectivity between brain regions. Because the fiber orientation estimated from dMRI is the basis of all fiber tractography algorithms, it is necessary to compare the diffusion-based estimates directly to histological ground truth. Previous validation studies have been limited to 2D analysis of histological sections[1,2], typically with light microscopy, and only recently have 3D methods been proposed for tissue characterization[3]. Here we use 3D structure tensor analysis of confocal z-stacks to compare the histological fiber orientation distribution (FOD) with those of various dMRI signal models – including DTI, Q-ball, and DSI. The purpose of this study is to (1) determine to what extent the dMRI signal is consistent with the histological FODs, and (2) establish how well different dMRI models predict the ground truth FODs.

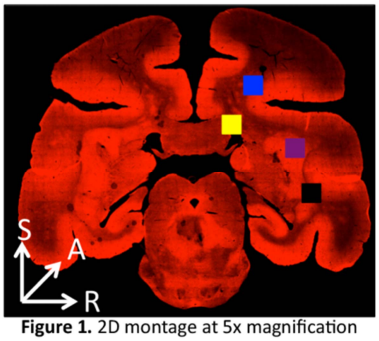

Figure 1. 2D montage at 5x magnification

METHODS *CONFOCAL IMAGING*: A paraformaldehyde fixed squirrel monkey brain was frozen and sectioned coronally at 50um thickness. Sections were stained with the fluorescent lipophilic dye, DiI, as previously described[1]. A 2D montage of the entire tissue was created at 5x magnification (Figure 1) to be used for registration to MRI data. Serial image stacks (FOV=300um*300um*50um) were acquired on a Zeiss LSM confocal microscope at 63x magnification. *DIFFUSION MRI*: Ex vivo diffusion MRI was performed on a 9.4 T Varian scanner using three acquisition schemes for different diffusion models: DTI (30 directions; b=2000s/mm^2), Q-ball (90 ; b=7000s/mm^2), and DSI (258 volumes, max b=10,000s/mm^2), all acquired at 300um isotropic resolution. *FOD/ODF COMPARISON*: A multi-step registration procedure was performed to align diffusion and histological FOD/ODFs[2]. In order to preserve proper orientation after registration, spatial normalization was performed on the dMRI data as described in [4].

RESULTS Figure 2 illustrates the ability of the 3D structure tensor to extract the histological FOD in 3D, as well as resolve crossing fibers (location shown as black box in Fig. 1). The histological FOD (Fig. 2A,B) is shown along with the ODF derived from Q-ball (2C), DSI (2D), and the diffusion tensor (2E). Three regions of interest (outlined in blue, yellow, and purple), each the size of four MRI voxels, are shown in Figure 3A (location shown as blue, yellow, and purple boxes in Fig. 1, respectively). The top row of each is also shown in Fig. 3B, rotated to visualize the "through-plane" orientation. The primary fiber orientation derived from histology was compared to that of dMRI, and the angular difference calculated was 4.9°±3.8° for Q-ball, 6.7°±5.3° for DSI, and 10.2°±5.1° for DTI.

CONCLUSION Here we demonstrate the ability to extract the 3D FOD from confocal z-stacks. This provides a gold standard for dMRI validation studies, which have previously been limited to 2D analysis. The data show good agreement between histological and dMRI estimates of fiber orientation in voxels containing nearly parallel fibers. In cases of crossing fibers, QBI and DSI are able to resolve the component fibers with good (5-7°) accuracy.

REFERENCES [1] Budde et al. 2012, *Neuroimage* 63:1-10. [2] Choe et al. 2012. *NMR Biomed.* 25, 900-908. [3] Khan et al. 2014, *ISMRM* proceedings, abstract 4426. [4] Hong et al. 2009, *Magn Reson Med.* 61(6): 1520-1527.

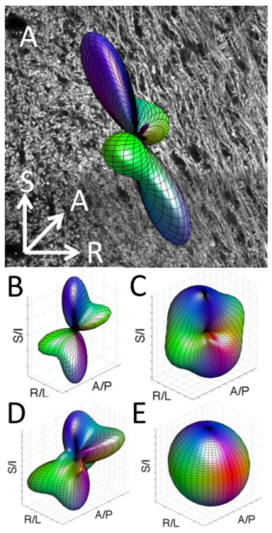

Figure 2. 3D histological FOD (A,B) in a crossing fiber region, with corresponding ODF from Q-ball (C), DSI (D), and DTI (E).

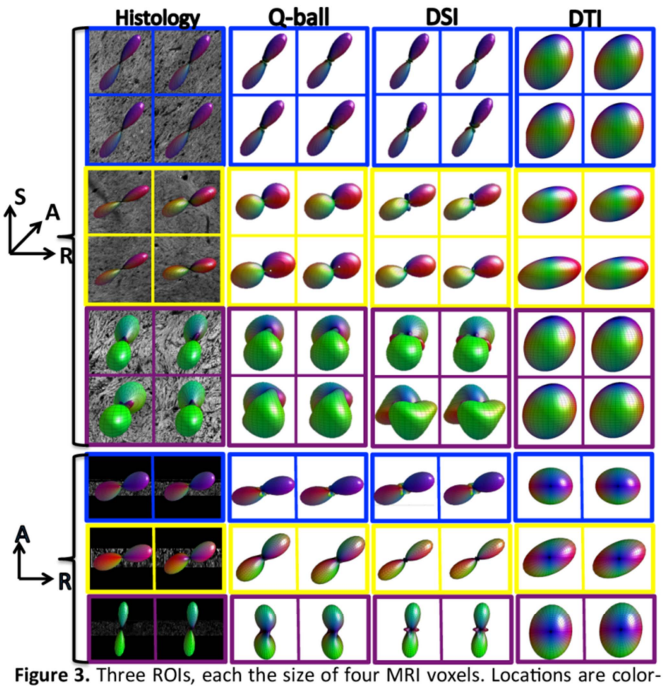

Figure 3. Three ROIs, each the size of four MRI voxels. Locations are color-coded based on Fig. 1. From left to right: histological FOD, Q-ball ODF, DSI ODF, and Diffusion Tensor. Shown in the orientation acquired from confocal microscope (A), and rotated to show "through-plane" orientation (B).