Methods for comparing fiber orientation distribution (FOD) functions based on histology and diffusion MRI

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INTRODUCTION

In previous studies [1-4], we validated DTI-tractography-derived connectivity by comparing with the histological ground truth. Because DTI fiber orientation estimation is the basis of tractography, juxtaposing the DTI fiber orientation distribution (dFOD) and histological FOD (hFOD) is critical in validation studies. However, obtaining the 3D hFOD is challenging. This study introduces 1) two methods to estimate the hFOD from z-stack micrographs and 2) the procedure to compare dFOD with hFOD.

MATERIALS AND METHODS

Data acquisition: A bidirectional neural tracer biotinylated dextran amine (BDA) was injected into the left M1 cortex of the squirrel monkey. After two weeks, the monkey was sacrificed and the brain was immediately extracted, fixed and scanned (PGSE multishot spinwarp imaging, TR=4.6s, TE=42ms, number of gradient directions=31, b≈1020s/mm^2, voxel size=0.3mm×0.3mm×0.3mm, data matrix=128×128×192). Then the brain was frozen and sectioned coronally at 50μm thickness on a microtome. The block of frozen tissue was photographed for registration purposes. Every sixth section was reacted for BDA, which tagged the fibers connected with left M1. Each adjacent section was stained for myelin, which labeled all the myelinated fibers in the brain. Each BDA-tagged and myelin-stained section was photographed under a light microscope with 0.5X objective for registration purposes. Every z-stack (*i.e.*, a stack of micrographs taken with focal plane moving from top to bottom on the same field of view) at high resolution (>5X) on spot of interest was acquired for 3D hFOD extraction.

hFOD extraction: The image preprocessing steps included denoising, intensity normalization, deconvolution [5], fiber segmentation and spur removal. Two methods, skeletonization-initiated and Fourier filtering, were used on z-stack of fiber masks to extract hFODs.

The skeletonization-initiated method included the following steps: 1) repeatedly thinning the segmented fibers until the fiber skeletons were obtained; 2) detecting all the crossing points of the skeletons; 3) for skeletons without any crossing points, extracting the coordinates of the center of all the skeleton voxels for each fiber and then fitting those coordinates to a smooth curve; 4) for the skeleton with crossing point(s), dividing this skeleton into sub-skeletons using the crossing point as the cutoff point, and then fitting each sub-skeleton into a curve as in step 3); 5) estimating the 3D angle for each fitted curve and then adding up the number of voxels in fiber segments of the same orientation—this is interpreted as the value of the distribution at that orientation and voxel (we should notice that the calculated 3D angles are continuous variables with some uncertainty, so we divided the azimuthal angle range of 360° into 36 bins and the polar angle range of 180° into 36 bins to hold the orientations); 6) plotting the discrete hFOD using a spherical harmonic algorithm.

he other optional method employed a 3D extension of the 2D Fourier directional filtering method [6,7] and included the following steps: 1) converting the segmented data to the frequency domain by performing 3D Fourier transformation; 2) in the frequency domain, employing, respectively, a set of 3D directional filters with different angular orientations uniformly distributed on the unit sphere; 3) performing inverse Fourier transformation to each filtered image and summing up the number of voxels above the assigned threshold; 4) plotting the hFOD.

Comparison with dFOD: To compare the diffusion tensor to the hFOD in the same space, the tensor corresponding to each position in the micrograph space was calculated using partial volume interpolation of the original DW images. In order to preserve the tensor orientation in the micrograph space after registration, the tensor was rotated using the preservation of principal directions reorientation strategy [8]. The multi-step registration procedure [9] was performed to compute deformation field.

RESULTS

Fig.1 displays the process and results of extracting an hFOD from a simulated z-stack using skeletonization and Fourier filtering method. Fig.2 shows an example of the extracted BDA hFODs using the skeletonization-initiated and Fourier filtering methods. Fig. 3 displays an example of comparing dFOD with myelinated hFOD in micrograph space.

CONCLUSION AND DISCUSSION

Fig.1 revealed that the skeletionization-initiated and Fourier filtering methods roughly extracted the two orientations of the simulated fibers and the portions along each orientation. In real micrograph analysis, both hFOD extraction methods worked on high resolution BDA z-stack data, but the Fourier filtering method worked better on myelinated z-stack data because the myelinated fibers were too dense to be segmented individually. Fig.3 indicated that the diffusion isosurface was similar to the myelin-stained hFOD, both pointing primarily in the anterior-posterior direction, just underneath the left premotor cortex. Similar methods can be used to validate DTI/HARDI FOD to make quantitative comparisons.

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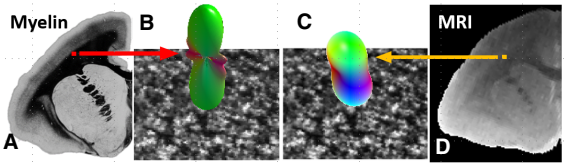

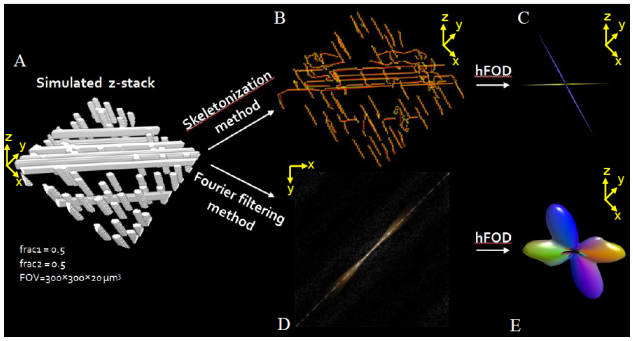

Fig.1. An example of extracting the hFOD from a simulated z-stack using the skeletonization and Fourier filtering methods.

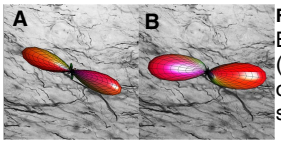

Fig.2. An example of the extracted BDA hFODs using the skeletonization (A) and Fourier filtering (B) methods, overlaid on one plane of the real BDA-stained z-stack micrographs.

Fig.3. An example of comparing myelin-stained hFOD (B) with diffusion isosurface (C) located in the red/yellow square underneath the left premotor cortex shown in myelin-stained (A) and MRI (D) slice. (Red indicates left/right; green indicates anterior/posterior and blue indicates superior/inferior). Similarity of FODs can be quantified using the angular correlation coefficient.