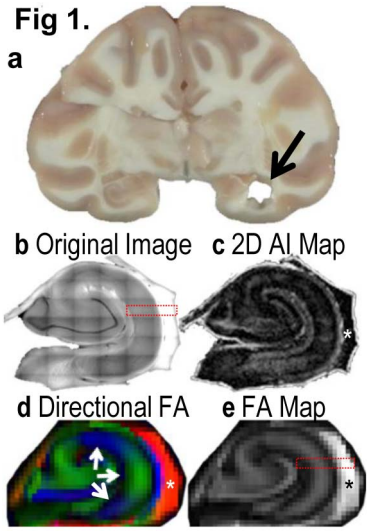
3D structure tensor analysis of light microscopy data for validating diffusion MRI

Ahmad Raza Khan[1,2], Lindsey A Leigland[3], Steven G Kohama[1], Anda Cornea[1], Sune Nørhøj Jespersen[4], and Christopher D Kroenke[1,2]

[1]*Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, Oregon, United States,* [2]*Advanced Imaging Research Center, Oregon Health & Science University, Portland, Oregon, United States,* [3]*Advanced Imaging Research Center, Oregon Health & Science University, Beaverton, Oregon, United States,* [4]*Center of Functionally Integrative Neuroscience, Aarhus University Hospital, Aarhus, Denmark*

TARGET AUDIENCE Clinician and basic research scientists who utilize diffusion as a form of MRI contrast.

PURPOSE For biophysical modeling of diffusion MRI data to facilitate accurate interpretation of neuroimaging measurements in terms of microstructural characteristics of tissue, experimental validation strategies are necessary. Budde et al. [1] recently applied structure tensor (ST) analysis to light microscopy images to validate diffusion anisotropy measurements performed on tissue prior to sectioning. In contrast to other microscopy-based validation strategies [2,3], the simplicity of ST analyses enable them to be implemented in a high-throughput manner, and hence they are suitable for group-comparison studies. However, previously, ST-based validation of diffusion MRI data was restricted to 2D analyses. As a result, it was necessary to perform ST analyses on tissue sectioned in a direction orthogonal to the direction of interest, and this strategy failed to characterize through-plane anisotropy. Here we show it is possible to extend ST analyses to 3D using serial image "stacks" acquired with confocal microscopy.

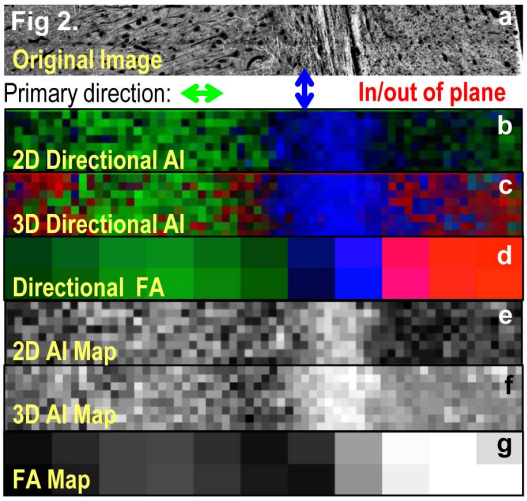


METHODS A 5 mm-thick coronal section of the right hippocampus and surrounding white matter was dissected from the perfusion paraformaldehyde-fixed brain of an adult rhesus macaque (arrow, Fig 1a). Ex vivo diffusion MRI (73 directions, b=2.5 ms/μm^2) was performed, using an 11.7 T small-animal MRI system as previously described [4]. Following MRI, the tissue was further subsectioned at 200 μm on vibratome, mounted on glass slides, and stained with the fluorescent lipophilic dye DiI as described [1,4]. A 2D montage of an entire tissue section was constructed at 10x magnification (Fig 1b), and for a subsection (red rectangles, Fig. 1), a 3D montage was digitized at 0.25 μm-sided isotropic resolution using a 63x objective and confocal microscope. A slice from the 3D stack is shown in Fig. 2a.

RESULTS As has been described previously [5], radially-oriented diffusion anisotropy is present throughout the gray matter (GM) of the cornu ammonis fields (Fig 1d, arrowheads). Additionally, more extreme diffusion anisotropy is observed in neighboring cerebral white matter (Fig 1 asterisks). The anisotropy index (AI) is a parameter obtained from ST analysis, that reflects anisotropy in image structures. This quantity is analogous, but not quantitatively related to fractional anisotropy (FA) in water diffusion. Notably, although the pattern of AI derived from 2D ST analysis is similar to FA in hippocampal GM, where "in-plane" anisotropy is present, the 2D AI differs from FA in the WM, where anisotropy is oriented orthogonal to the coronal plane.

A 3D ST analysis was carried out by generalizing the 2D expressions described by Budde et al. [1] for the region delineated with yellow rectangles in Fig 1. As shown in Fig 2, the region of high diffusion anisotropy arising from WM fibers oriented in/out of the plane (red, Fig 2d) is characterized by low AI in the 2D analysis (Fig 2e) but not in the 3D analysis (Fig 2f). Further, the 3D structure tensor and diffusion tensor directions coincide in regions of through-plane anisotropy, whereas the 2D ST analysis is insensitive to organization (red voxels, Figs 2b-d).

CONCLUSION Here we demonstrate the ability to perform 3D ST analyses on confocal light microscopy data of DiI-stained primate hippocmpal tissue. The advantage of 3D ST analysis relative to 2D derives from the ability to characterize through-plane anisotropy, in a manner that is similar to MRI-based diffusion anisotropy measurements. It is envisioned this analysis will extend the utility of ST-based validation analyses by removing the need to section tissue in a direction orthogonal to the primary direction(s) of diffusion anisotropy, and by enabling the analysis of tissue in which primary diffusion directions of interest are oblique to a single 2D plane.



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