Structure Tensor Analysis of Histological Images to Examine Brain Microstructure

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Introduction

The mammalian central nervous system (CNS) exhibits structural connectivity and complexity on both the cellular (microscopic) and systems (macroscopic) levels. Diffusion tensor imaging (DTI) and advanced diffusion MRI methods effectively probe tissue microstructure at a macroscopic level, but accurate and comprehensive validation of such techniques has been notoriously difficult. Herein, we demonstrate the application of structure tensor (ST) analysis[1,2] to digital histological images to visualize and quantify brain microstructure on different scales and compare the results to those obtained from DTI.

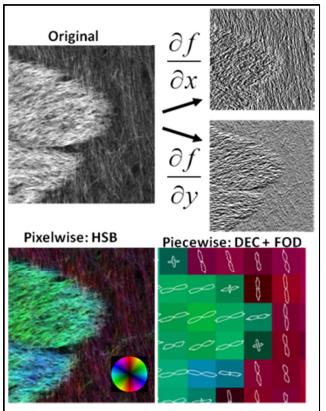

Figure 1. ST analysis employs matrix representation of image spatial derivatives (top) to derive orientation and anisotropy. Both pixelwise and piecewise analyses were performed.

Materials and Methods

Ex vivo rat brains underwent DTI on a 7T Bruker Biospec with 30 diffusion weighted (b=1000 mm^2/s) and 5 unweighted images (b=0) at an in-plane resolution of 156 µm^2 and thickness of 500 µm, and DTI parameter maps were computed. The brain was cryosectioned (8 µm thickness), stained with DiI[3], and imaged with fluorescence microscopy at a resolution of 0.65 µm^2. The composite images (250 Megapixels) were subjected to custom ST analysis[1] routines written in Matlab (Fig. 1). Pixelwise analysis allowed visualization at the native microscopic image resolution using Hue-Saturation-Brightness (HSB) images to depict orientation, anisotropy, and intensity, respectively[2]. Piecewise analysis allowed quantification of fiber orientation distributions (FOD) on a scale comparable to the DTI resolution. FODs were fit to a single or mixture of two von Mises distributions. Regions with multiple fiber populations ("crossing fibers") were identified as pixels with an improved goodness-of-fit (Adjusted-R^2 statistic) between the two fitting methods as well as pixels with a large angular difference (>30°) between the two distributions (Δθ) in the two-term fit.

Results

ST analysis allowed visualization and quantification of fiber orientations derived from histological sections. Despite apparent macroscopic uniformity, white matter regions such as the external capsule (Fig. 2b) had a greater microscopic heterogeneity compared to the corpus callosum (Fig. 2a), for example, as evidenced by the differently colored, interwoven fibers on the HSB images as well as the increased dispersion in the FODs. Anisotropy derived from single-tensor DTI and histology was highly correlated (Fig. 3A-C). FODs fit to a single or a mixture to two von Mises distributions (Fig. 3D-F) identified putative crossing fiber regions as evidenced by the increased adjusted-R^2 and angular difference between the two fiber distributions.

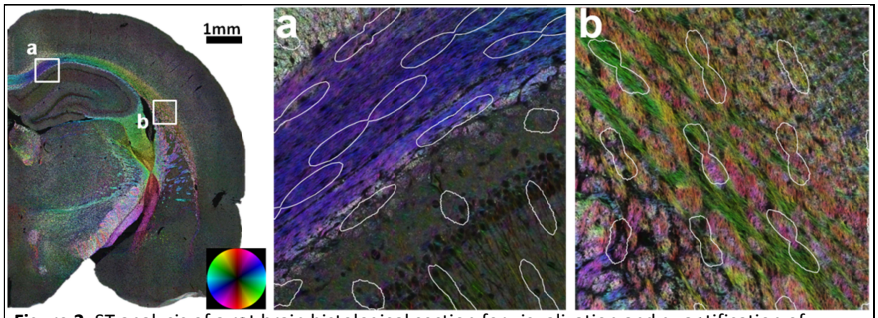

Figure 2. ST analysis of a rat brain histological section for visualization and quantification of microscopic fiber orientations (HSB images with FOD overlays).

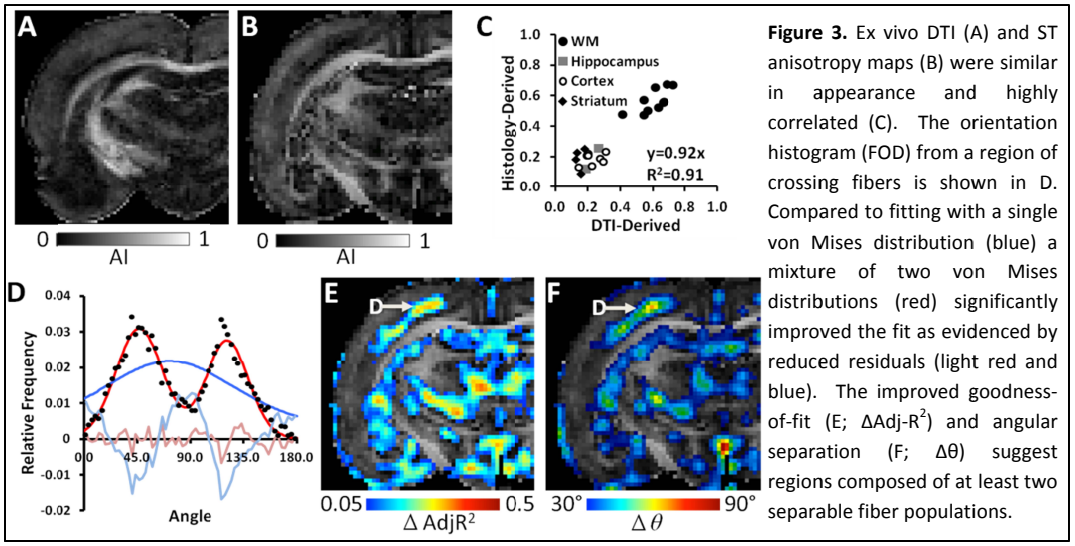

Figure 3. Ex vivo DTI (A) and ST anisotropy maps (B) were similar in appearance and highly correlated (C). The orientation histogram (FOD) from a region of crossing fibers is shown in D. Compared to fitting with a single von Mises distribution (blue) a mixture of two von Mises distributions (red) significantly improved the fit as evidenced by reduced residuals (light red and blue). The improved goodness-of-fit (E; ΔAdj-R^2) and angular separation (F; Δθ) suggest regions composed of at least two separable fiber populations.

Discussion and Conclusions

A method is presented to visualize and quantify brain structure on both micro- and macroscopic scales, albeit it is limited to 2D. It was motivated by the lack of validation techniques for DTI and advanced diffusion MRI results. Extension of the approach to 3D microscopic imaging would allow accurate validation of tractography findings and is the focus of future studies.

References

[1]Rezakhaniha, et al. *Biomech Mod. Mech* 2011; [2]Bigun, et al. *IEEE Trans Pat Anal Mach Intell* 2004; [3]Budde, et al. *Brain* 2011