Quantification of 3D Microscopic Tissue Features in CLARITY Data for Comparison with Diffusion MRI

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Target Audience: Physicists, neuroscientists and clinicians who utilize diffusion MRI.

Introduction: Diffusion MRI has many variations and can provide information about a wide variety of different tissue microstructure features. Unfortunately, most diffusion MRI methods currently lack ground-truth comparisons to validate results and hone techniques. A new tissue clearing technique called CLARITY[1] may represent a step towards the long-sought after gold-standard that diffusion MRI has traditionally lacked. The CLARITY hydrogel and tissue clearing process[1] renders cuboids of tissue optically transparent and permeable to macromolecules by washing lipids and other biomolecules out of the tissue. The tissue cuboid is kept intact by building a hydrogel-based infrastructure from within the tissue prior to the washing process. In this way CLARITY enables 3D microscopic visualization of cellular and fiber structures within an intact whole, fixed, animal brain or small samples of human brain. Compared to conventional histology, CLARITY has the advantage of keeping the brain intact and maintaining a global perspective. Clarified tissue samples can also be used for multiple rounds of whole-tissue molecular phenotyping on the same brain specimen without structural damage or degraded antigenicity. However, the mechanisms by which diffusion MRI and CLARITY probe tissue microstructures are distinctly different (the attenuation of the diffusion MRI signal due to diffusion patterns of water in tissue versus histological staining (e.g. for neurofilaments)). The spatial resolution of diffusion MRI and CLARITY is also dramatically different with CLARITY labeling individual neurons at sub-micron resolution (e.g. ~0.6μm×0.6μm×5.0 μm in the presented experiment) versus diffusion MRI voxels capturing the net diffusion information in the range of ~55μm isotropic (for *ex vivo* mouse)[2] to ~2mm isotropic (for *in vivo* human) voxels. Therefore, the images from the two methods contain substantially distinct information and their integration is challenging. Towards the goal of working across scales and leveraging the two human brain mapping methods (CLARITY and diffusion MRI), we demonstrate how to extract 3D computational features such as the structure tensor (ST) from CLARITY data that could then be compared with diffusion MRI measurements such as the diffusion tensor.

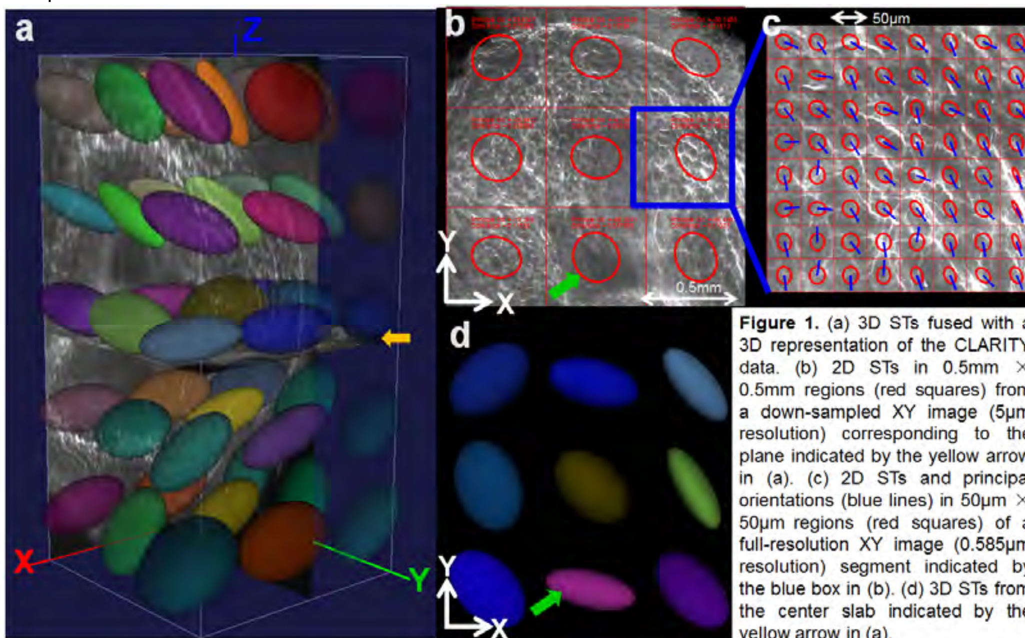

Figure 1. (a) 3D STs fused with a 3D representation of the CLARITY data. (b) 2D STs in 0.5mm × 0.5mm regions (red squares) from a down-sampled XY image (5μm resolution) corresponding to the plane indicated by the yellow arrow in (a). (c) 2D STs and principal orientations (blue lines) in 50μm × 50μm regions (red squares) of a full-resolution XY image (0.585μm resolution) segment indicated by the blue box in (b). (d) 3D STs from the center slab indicated by the yellow arrow in (a).

Methods: *Data Acquisition*. A mouse brain was cleared using the CLARITY protocol described in [3]. Phosphorylated neurofilament staining was utilized to label axons and a few dendrites. Light sheet fluorescence microscopy was used to acquire the images[3]. Ligh sheet fluorescence microscopy illuminates a thin slice of the sample perpendicular to the direction of observation, which determines the through-plane resolution. We refer to the direction of observation as the Z direction and the perpendicular plane as XY plane. A subset of the mouse brain data consisting of the right olfactory bulb ($1.5\times1.5\times2.5mm^3$) was used in the analysis presented here (Fig. 1a). This 3D dataset consists of a sequence of 500 images (2400x2400 pixels) with an in-plane resolution of 0.585 μm and slice thickness of 5 μm. *Data Analysis*. ST analysis[4-6] was applied to regions of the CLARITY data that are comparable to an MRI voxel size, (0.5mm×0.5mm and 50μm×50μm in 2D and 0.5mm×0.5mm×0.5mm in 3D). For 3D analysis, the CLARITY images were down-sampled in-plane by a factor of 8 using a bicubic filter in order to create isotropic resolution. The ST was calculated as the second-moment matrix of the image intensity gradients computed using the derivative of Gaussian kernel in each of the X, Y and Z directions. The ST summarizes the orientations of the underlying structure in the images based on the fact that the image intensity gradients are strongest orthogonal to the orientation of the structural components (fibers).

Results and Discussion: The 2D STs appear to faithfully capture the dominant orientation of the neurofilaments displayed in the CLARITY images (Fig. 1b, c). The 3D STs show some evidence of capturing 3D geometry that is missed by the 2D STs (same voxel comparison indicated by green arrow Fig. 1b, d). CLARITY imaging may represent a step towards the long-sought after gold-standard that diffusion MRI has traditionally lacked, however, the substantially distinct information and resolution contained within these imaging modalities makes their integration challenging. We have demonstrated here that 3D ST analysis of CLARITY images stained for neurofilaments provide a strong basis to begin comparisons between *ex vivo* diffusion MRI and CLARITY performed in the same sample. In the current abstract only the second-order ST model which summarizes fiber orientations within a given region of interest is presented. A higher order ST could also be used to capture more complicated fiber patterns.

Acknowledgments: Funding was provided by the Li Ka Shing-Oxford-Stanford Big Data in Human Health seed grant, Stanford Radiology Angel Funds, GE Healthcare, NIH: P41-EB015891, S10-RR026351.

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