Detailed laminar characteristics of the human neocortex revealed by NODDI and histology

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Introduction

Diffusion weighted imaging (DWI) has the potential to provide the richest noninvasive description of cortical cytoarchitecture. Previous work has shown that the diffusion properties of the primary visual cortex (V1) are layer-specific (Kleinnijenhuis et al., 2012). In particular, the stria of Gennari displays low diffusivity and anisotropy. Here, we extend these findings by fitting the recent NODDI tissue model (Zhang et al., 2012) to multi-shell DWI data to support a more fine-grained division of cortical layers and compare our results to histology to aid in interpretation and validation of our data.

Methods

Human V1 samples (1x1x1 cm) were investigated with *ex vivo* DWI. Prior to MR imaging, samples were fixed (>2 months), soaked in phosphate buffered saline (>72h) and mounted in a syringe with proton-free liquid (~24h). DWI was performed on a preclinical MR system (Bruker Biospec 94/20 USR with BGA12S HP gradient set [660 mT/m; 4570 T/m/s] equipped with a Bruker CryoProbe 1x2 coil array cooled to 20-30K). DWI was acquired in 768 unique directions with 0.2 mm isotropic resolution (read-out segmented SE-EPI with 4 segments; matrix=128x128; FOV=25.6x25.6 mm; 45 slices; TR/TE=6750/23.8 ms; b=[0,12000,4000,12000] mm^2s^{-1} in 768 directions; δ=7 ms; Δ=11.3 ms; TA=24h). DWI volumes were coregistered using FSLv5.0 and diffusion tensors and Fiber Orientation Distributions (FODs) were estimated using MRtrix v0.2.10. The neurite density (ICVF; volume fraction occupied by cylindrical structures) and orientation dispersion (κ, concentration around the dominant direction) were fitted using the multi-compartment tissue model NODDI (Zhang et al., 2012). Isotropic (ISO) and isotropic restriction compartment (IRVF; required for ex vivo samples [Alexander et al., 2010]) were included. Prior to histology, the samples were bisected in the axial imaging plane and embedded in parafin for sectioning at 5 µm. Histological stains included hematoxylin and eosin (H&E) for cell bodies, Bodian for axons and Luxol Fast Blue (LFB) for myelin. The sections were digitized using a Zeiss microscope equiped with an automated table operated by Neurolucida v10 software, creating seamless virtual slices at 20x magnification. The virtual slices were processed in Matlab using structure tensor analysis described by Budde et al., 2012, i.e. coding orientation, anisotropy and staining intensity as hue, saturation and brightness.

Results

In the mean diffusivity map (Fig.1a), three laminar subdivisions could be distinguished in V1 cortex: the superficial layers, the stria of Gennari and the deep layers. In V1, NODDI neurite density (Fig.2a) is distinct in at least four layers: the deep layers subdivide in an inner and outer layer; the stria of Gennari is characterized by high neurite density and the superficial layers have a low neurite density. The dispersion parameter κ (Figs.2d) correlates well with fractional anisotropy (Figs.1b), but κ appears to be a more sensitive measure of fiber coherence because more contrast is observed in WM as well as GM. Fig. 3 (Bodian) and Fig.4 (LFB) show that the reconstructed FODs in Fig 1c match reasonably well with the in-plane orientations in the histological slice. Within the cortex, histological results show that the prominent bright layer in the κ-map originates from increased coherence of radial fascicles of myelinated fibers penetrating layers V and IV (e.g. Fig.3f: blue fibers). The dark layer above the bright layer coincides with the stria of Gennari, as shown by the increased component of transverse fibers (e.g. Fig.4g).

Discussion

Additional detail on cortical architecture is obtained by fitting the NODDI multi-compartment model to multi-shell data when compared to diffusion-tensor metrics. The dispersion parameter κ is more sensitive than FA. As NODDI does not require extreme b-values and scan times *in vivo* (Zhang et al., 2012), these results suggest that NODDI has more potential for capturing cortical microstructure than compared to diffusion tensor metrics. The 2D FODs from the Bodian sections appear to more sensitive in detecting transverse neurite components as compared to the LFB sections.

Figure 1. DT metrics & FODs

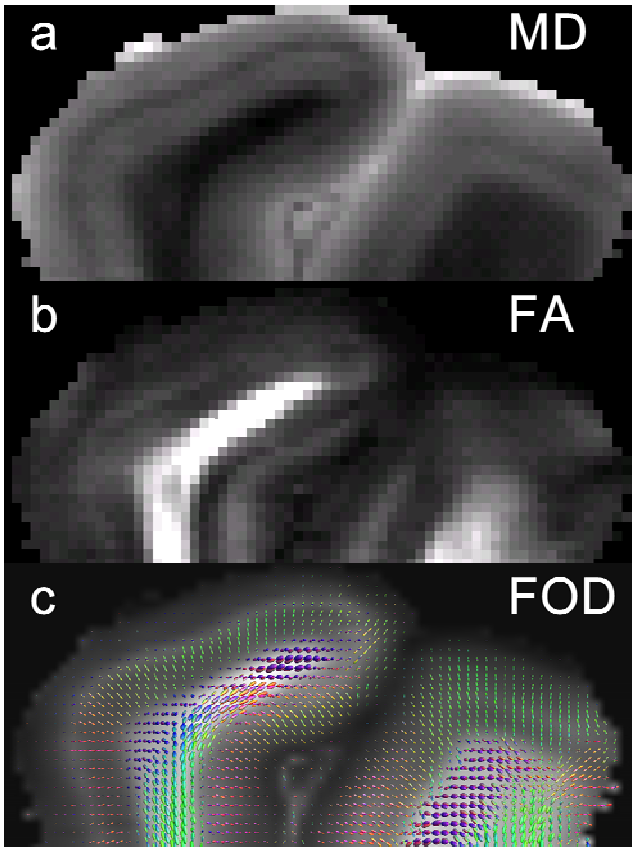

Figure 2. NODDI maps

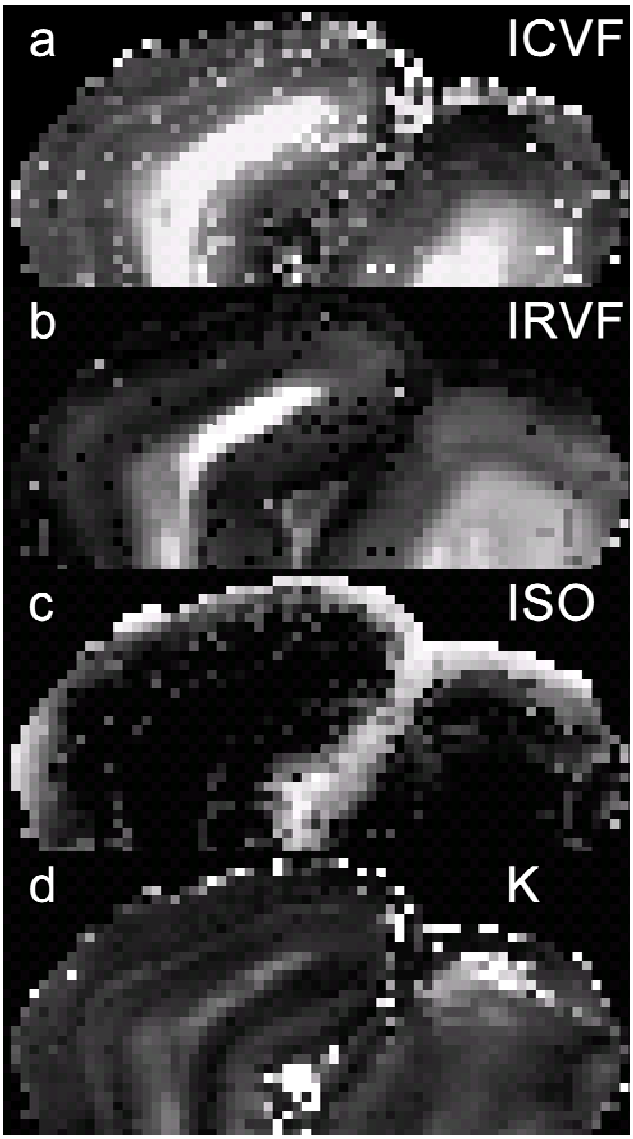

Figure 4. Bodian staining with HSVmap coding for orientation, anisotropy and staining intensity. 2D FOD maps are tilewise histograms (500x500 pixels) of pixelwise structure tensor orientation.

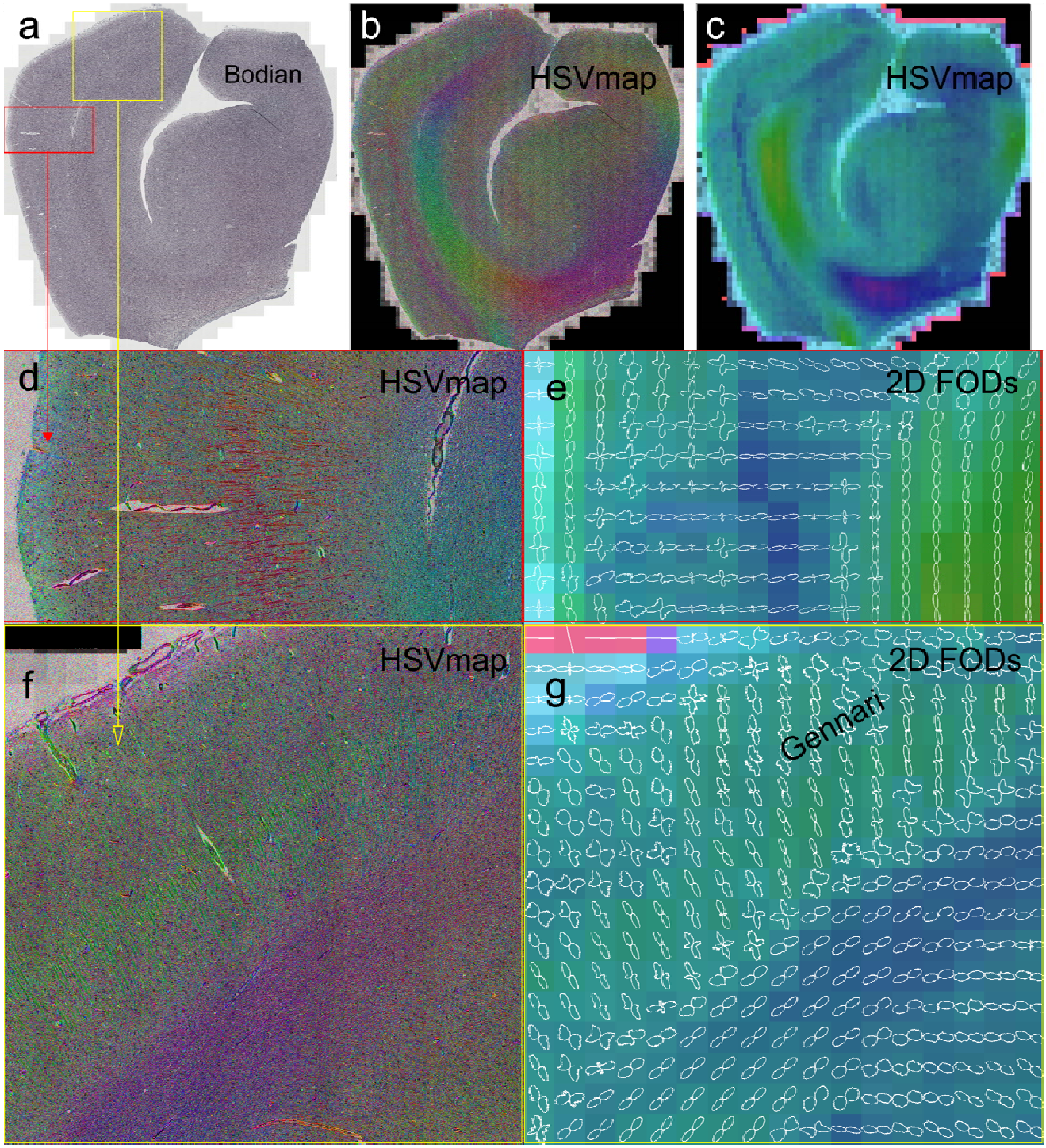

Figure 4. LFB + HE staining with HSVmap coding for orientation, anisotropy and staining intensity. 2D FOD maps are tilewise histograms (500x500 pixels) of pixelwise structure tensor orientation.