# Dendrite Density from Magnetic Resonance Diffusion Measurements: Comparison with Histology

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**Introduction:** Due to its inherent sensitivity to biophysical properties on the micrometer scale, diffusion imaging holds promise of revealing microstructural details of living tissue. However, in order to properly harness this power, accurate diffusion models are indispensable, and consequently there is intense current interest in developing such models. Recently, a promising model based on basic physical principles was introduced to describe diffusion in brain tissue [1]. One interesting aspect of this model is its ability to measure dendrite densities using diffusion weighted MRI, a tool which could be of substantial impact e.g. in the study of cortical plasticity. Here we present preliminary results of our efforts towards histological validation of this model.

**Theory:** In this model, it is assumed that, from the point of view of diffusion, the dendrites and axons of neurons can be described as a collection of long cylinders with longitudinal and transverse diffusion constants, $D_L$ and $D_T$ respectively [1, 3]. We assume that on the scale of the diffusion time the cylinders are impermeable to water, and the collection of cylinders in a given voxel is described by $f(\theta,\varphi)d\Omega$, the fraction of cylinders in the solid angle $d\Omega$, which is expanded in spherical harmonics. Water outside of the cylinders is modeled as isotropic diffusion with an effective diffusion constant $D_{\text{eff}}$. This amounts to assuming that during the course of the diffusion experiment, water molecules in this compartment can freely sample extracellular space as well as nonneuronal intracellular space, such as glial cells. Implementing this lead to an expression for the signal decay from a narrow pulse Stejskal-Tanner spin echo experiment, consisting of a contribution from compartments with cylindrical symmetry (dendrites and axons) proportional to $\nu$, and compartments with isotropic symmetry (cell bodies and nonneuronal tissue) proportional to $1-\nu$ [1]. As a result of the difference in b-factor and diffusion gradient direction dependence of the two terms, it is possible to determine the relative volume fraction $\nu$ of dendrites and axons, among other things [1].

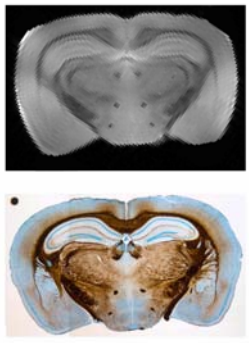

*Fig. 1. MR image and corresponding histological section.*

**Materials and methods:** An adult male mouse brain was removed by craniotomy and immersion fixed in 4% paraformaldehyde for several weeks. Before imaging, the brain was washed in phosphate buffered saline for 24 hours, after which it was immersed in a 10 mm NMR glass cylinder filled with Flourinert (3M), an MRI invisible liquid**.** 171 diffusion images over 30 slices were acquired using a standard spin echo diffusion weighted pulse sequence implemented on a 17 Tesla Bruker system, equipped with gradients capable of 300 G/cm for micro imaging. The imaging parameters were: 128 x 128 data matrix, field of view 12.8 mm x 12.8 mm, slice thickness 0.5 mm, TR/TE = 6 s/14 ms. The diffusion parameters were $\delta/\Delta =$ 2 ms/8 ms, and 18 b-factors ranging linearly from 0 to 15 ms/μm$^2$. For each nonzero b-factor, 9 different orientations were used according to a spherical 5-design [4], and in addition 18 b=0 images were acquired. Subsequently, the brain was immersed in 30% sucrose for one day before freezing and sectioning into 40 μm thick histological sections oriented according to the obtained coronal MR images. The histological slices were then stained for cell bodies using Nissl staining and myelin using a recently proposed autometallographic technique [5]. The MR diffusion images were fit to the model presented in [1] on a pixelwise basis using a nonlinear least squares routine implemented in Matlab.

**Results:** Two slices located in the anterior (slice 1) and mid part of the cerebrum (slice 2) were selected for further analysis, and three anatomically different regions of interest (ROIs) were drawn on each. Fig. 1 demonstrates the excellent correspondence of the obtained MRI images with the obtained histological slices. In order to compare the model predictions for the dendrite density, we show in Fig. 2 two slices of histology and dendrite density with the three ROI-outlines on each. We focus on the Nissl-stained slices, as the density of cell bodies is expected to correlate inversely with dendrite density in a first approximation, assuming low variability of the extracellular and capillary volume fractions across brain regions. Note the high degree of qualitative agreement in Fig. 2: blue areas on the histological sections (high cell density) coincide with black areas (low dendrite/axon density) on the $\nu$ parameter maps, and vice versa. In Fig. 3 we plot the mean ROI-values of the histology sections versus the dendrite density slices. The correlation is highly significant, $p = 0.0021$, and the best linear fit is shown also.

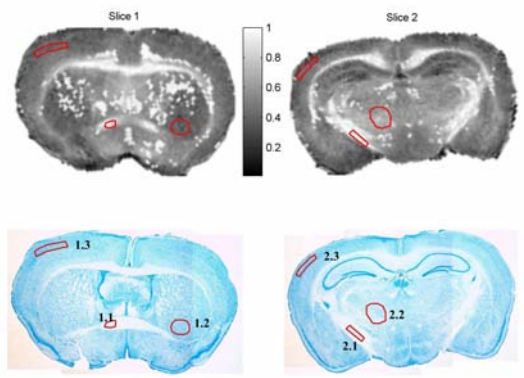


*Fig. 2. Density of the cylindrical compartment ν (top row) and corresponding histological sections (bottom row), along with six anatomical ROIs: Anterior Commisure (1.1), Striatum (1.2), Cortex (1.3), Internal Capsule (2.1), Thalamus (2.2), and Cortex (2.3).*

**Discussion and conclusion:** In the quest towards a better understanding of diffusion weighted MRI, it is not only important that theoretical models rest on plausible physical principles and fit the data well; their biophysical foundations should also be supported by independent modalities such as histology. We believe that the present work offers an advancement towards this goal, with encouraging results for the model presented in [1]. We have subsequently performed an optimization analysis based on Cramer-Rao lower bound theory, indicating that a significant decrease in the uncertainty of $\nu$ can be achieved using a slightly different b-factor scheme and pulse sequence parameters. This will allow us to make a more detailed comparison of the model with histology, and this is the focus of our current efforts.

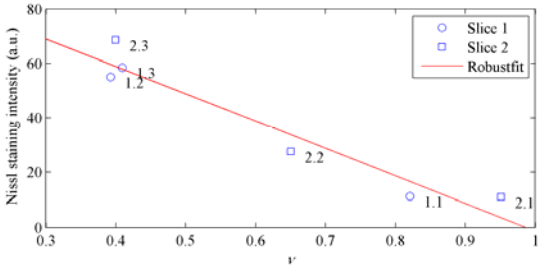


*Fig. 3. Density of cell bodies as estimated from the intensity of the Nissl stain versus the cylinder density.*

**References:**

[1]. S. N. Jespersen et al., appearing in *NeuroImage*. [2] J. D. Tournier et al., *NeuroImage* **23**: 1176-1185 (2004). [3] C. D. Kroenke et al., *MRM* **52**(5):1052-9 (2004). [4] R. H. Hardin and N. J. A. Sloane, *Discrete Comput Geom* **15**: 429-441 (1996). [5] M. Larsen et al., *Histol Histopathol*. **18**(4):1125-30 (2003).