Histological validation of the brain cell body imaging with diffusion MRI at ultrahigh field

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SYNOPSIS

Biophysical modelling of diffusion-weighted MRI (DW-MRI) data can help to gain more insight into brain microstructure. However, models need to be validated.
This work validates a recently-developed technique for non-invasive mapping of brain cell-body (soma) size/density with DW-MRI, by using ultrahigh-field DW-MRI experiments and histology of mouse brain. Predictions from numerical simulations are experimentally confirmed and brain's maps of MR-measured soma size/density are shown to correspond very well with histology. We provide differential contrasts between cell layers that are less expressed in tensor analyses, leading to novel complementary contrasts of the brain tissue. Limitations and future research directions are discussed.

INTRODUCTION

Mapping brain microstructure noninvasively using diffusion-weighted MRI (DW-MRI) remains a formidable challenge due to the complexity of the underlying constituents and the relatively featureless diffusion-driven signal decay. Biophysical modelling can help to gain more insight into the microstructure[1-3]. However, it is very difficult to choose the correct model[4], and validation is necessary.

This work validates a recently-developed technique[5] for non-invasive mapping of brain cell-body (namely soma) size and density with DW-MRI.

Advanced Monte-Carlo simulations, based on a recently-proposed framework[6], showed that soma size/density may have a specific signature on the direction-averaged DW-MRI signal S_{ave} at high b-values[5,7]. Simulations also suggested that at diffusion times t_d<30 ms, the water exchange between neurites and soma may be neglected, supporting the design of a simple three-compartment model to quantify the presence of soma[5]:

$$S_{ave}(b)/S(b=0)=f_{sticks}S_{sticks}(b,D_a)+f_{sphere}S_{sphere}(b,D_{sphere},R_{sphere})+(1-f_{sticks}-f_{sphere})S(b,D_{iso}) \quad [1]$$

where f_{sticks} represents the MR cell-fibres signal fraction, D_a axial diffusivity; f_{sphere} MR soma signal fraction, R_{sphere} soma radius, D_{sphere} and D_{iso} intra-soma and extra-cellular diffusivity.

Here we use ultrahigh field DW-MRI and histology of mouse brain to validate those simulation results[7] and the proposed compartment model[5].

METHODS

Simulations. Detailed three-dimensional geometries were constructed to mimic realistically connected neurites, in different soma size/volume fraction (r_{soma},f_{soma}) conditions, as proposed in[5-7] **(Fig.1a)**. The S_{ave}(b) signals, as measured by a Pulsed-Gradients-Spin-Echo (PGSE) sequence, were computed in CAMINO[8] for b-values=[0:1:40] ms/μm^2, δ/Δ=3/11 ms and direction-average across 40 gradient directions (**Fig.1b**). Only intracellular water diffusion was simulated. However, the effect of extracellular diffusion and membrane permeability were shown to be negligible at very high b-values[9] and diffusion times<20 ms[10].

Specimen preparation. All animal experiments were preapproved by the institutional and national authorities and were carried out according to European Directive 2010/63. Mice (N=2), male, 8 weeks old, were perfused intracardially with 4% paraformaldehyde. The brains were isolated and kept 48h in 4% paraformaldehyde and 5 days in PBS (changed daily), before being transferred to a 10 mm NMR tube filled with Fluorinert (Sigma Aldrich) for susceptibility matching. Upon MRI, tissue was used for microscopy as described in[11] to provide histological banchmark.

MRI experiments. All experiments were performed using a 16.4 T MRI scanner (Bruker BioSpin, Karlsruhe, Germany) operating at 700 MHz for ^{1}H nuclei equipped with a micro5 imaging probe with maximum gradient strength 3000 mT/m isotropically. The brain was kept at constant temperature of 37°C. DW-MRI were acquired using a PGSE sequence with: TE/TR=20/2500 ms; δ/Δ=3/11 ms; b=0,1,2,3,5,8,10,12,16,20,25,40 ms/μm^2; 40 gradient directions, resolution 50x50x250 μm^3, 10 slices, 4 averages.

Data preprocessing and analysis. Data were denoised using MP-PCA[12] and Gibbs ringing corrected[13]. No artifacts from movement or eddy-current were observed. Data at b=3 ms/μm^2 were used to compute diffusion tensor imaging (DTI) maps in mean diffusivity (MD) and fractional anisotropy (FA), using FSL[14]. Relation [1] was voxel-wise fitted to the measured $S_{ave}(b)$ signal to estimate f_{sticks}, D_a, f_{sphere}, R_{sphere}, D_{iso}, fixing D_{sphere}=3 μm^2/ms.

RESULTS

Simulations *vs.* experiments. Simulations predicted that, at Δ=11 ms, $S(b^{-1/2})$ shows specific curvature in the range $0.2<b^{-1/2}<0.5$ induced by specific (r_{soma},f_{soma}) conditions (**Fig.1b**). In agreement, the signal decay extracted from ROIs known to have different (r_{soma},f_{soma}) conditions, like Corpus-Callosum white matter (WM) and cortical gray matter (GM), reveals that these are correct (**Fig.1b**).

MR measured features. Fig.2a shows f_{sphere}, R_{sphere} and f_{sticks} maps, for the two mouse brains. Note the exquisite differential contrasts between layers in the olfactory bulb, cortex, hippocampus and cerebellum. These contrasts are less expressed in DTI maps (**Fig.2b**).

Comparison against histology. A direct comparison of the MR maps with histology of the same brain is shown in **Fig.3b** and **4**. We observe very good correspondence between soma and cell-fibres spatial distributions from histology and f_{sphere}, R_{sphere} and f_{sticks} maps. Quantitative analysis in **Fig.5** shows higher correlation of f_{sphere} with DAPI staining than MD. Comparison against literature-derived histology[15] is also provided in **Fig.3a**. Overall, the predicted brain's composition in terms of MR-measured soma size/density corresponds very well with ground-truth histological measurements.

DISCUSSION and CONCLUSION

Using high resolution DW-MRI data and histology, this work confirms the predictions of numerical simulation[6,7] (**Fig.1**), and validate the source of new contrasts specifically related to soma size/density[5] (**Fig.3-5**). These are dissimilar to the simple tensor analyses (**Fig.2-5**), and therefore represent new complementary information on the brain.

Future work will investigate possible bias on parameters estimates due to cross-correlation and degeneracy[16] as well as multi-compartmental T_1/T_2 effects[17], cell-fibres curvature[18], branching[19] and exchange[20]. However, the present suggests that these novel contrasts in soma size/density may provide a new set of biomarkers of potential great value for biomedical applications and pure neuroscience.

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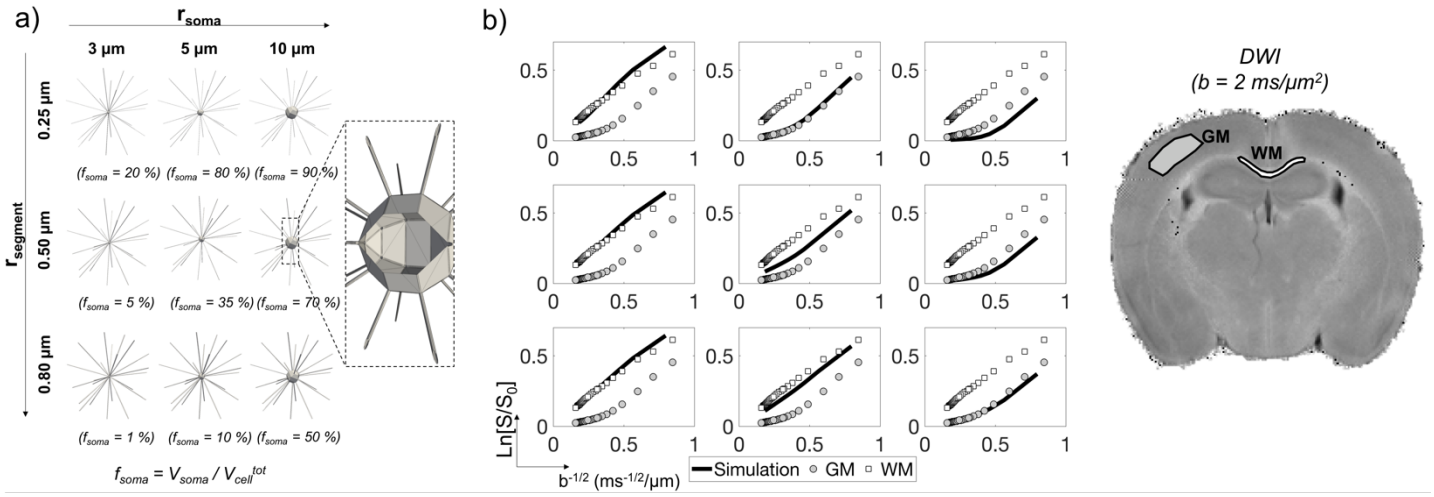

Figure1. Comparison of numerical simulations and experiments. *a)* Computational model: many randomly oriented cylindrical segments of radius $r_{segment}$ and 200 µm long (neurites) were projected from a spherical compartment (soma) of radius r_{soma} (zoomed inset), at different volume fractions of soma f_{soma}. The intracellular diffusion of $5x10^5$ spins was simulated with bulk-diffusivity 2 µm^2/ms. *b)* Normalised direction-averaged DW-MRI signal as a function of $b^{-1/2}$ computed from spins' trajectories (line). Comparison with measured signal from white matter (WM) and gray matter (GM) ROIs shows very good match at $0.2<b^{-1/2}<0.5$ for (r_{soma},f_{soma}) conditions: $(3,0.01\text{-}0.2)_{WM}$ and $(5\text{-}10,0.5\text{-}0.8)_{GM}$, also suggesting negligible extra-cellular contribution for $b^{-1/2}<0.5$.

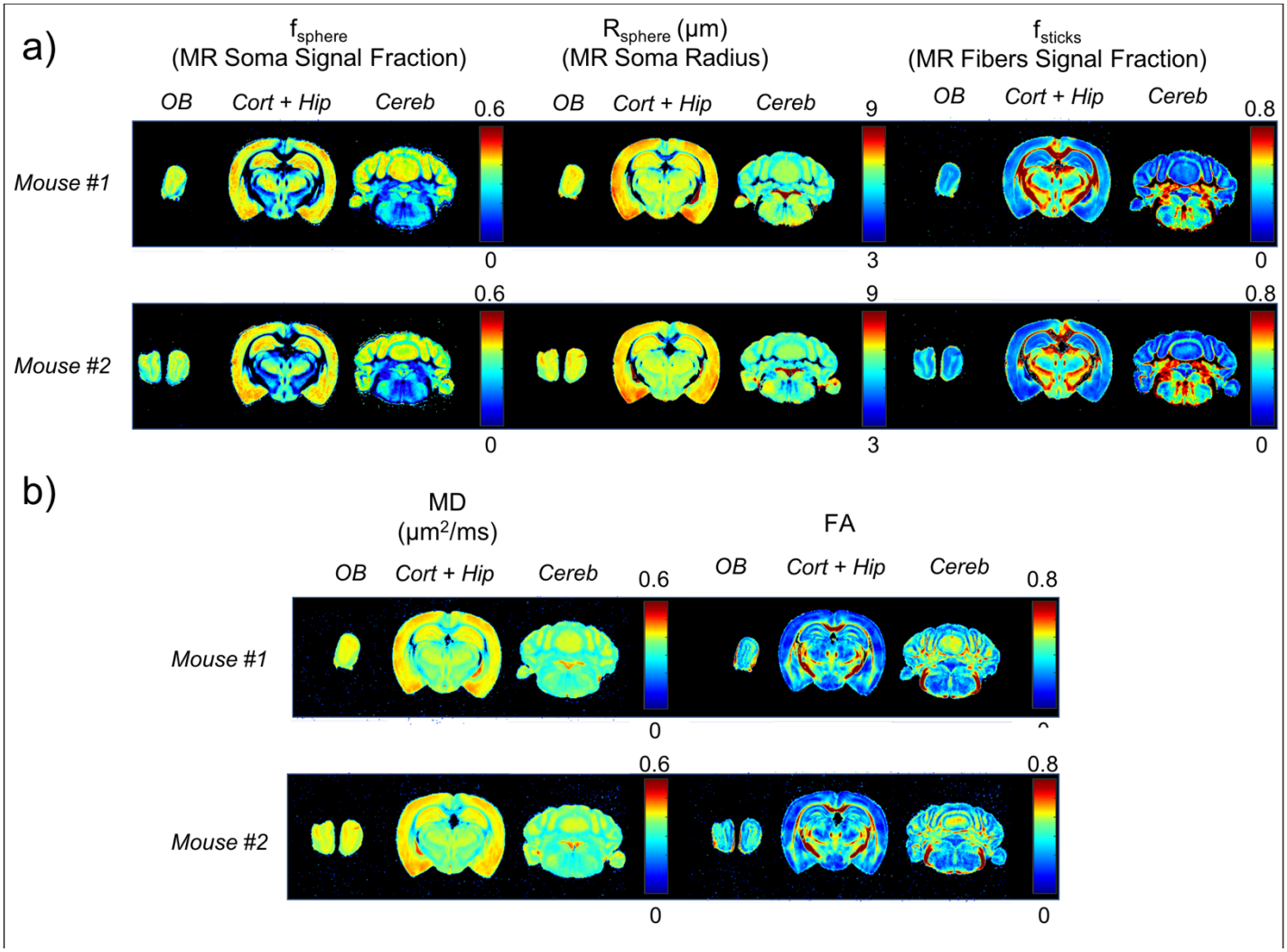

Figure 2. Novel contrasts in soma size and density. *a)* Voxel-wise non-linear fitting of relation [1] provided maps of MR cell body signal fraction f_{sphere} (left), radius R_{sphere} (centre), and MR cell fibers signal fraction f_{sticks} (right). Three slices of interest are displayed, containing olfactory bulb (OB), cortex (Cort), hippocampus (Hip), cerebellum (Cereb). Please note

that given a distribution of real soma size R in each voxel, the MR-measured soma radius R_{sphere} should be proportional to $< R^6 > / < R^3 >$ rather than the mean radius value. *b)* DTI maps of MD and FA computed at b=3 ms/µm^2.

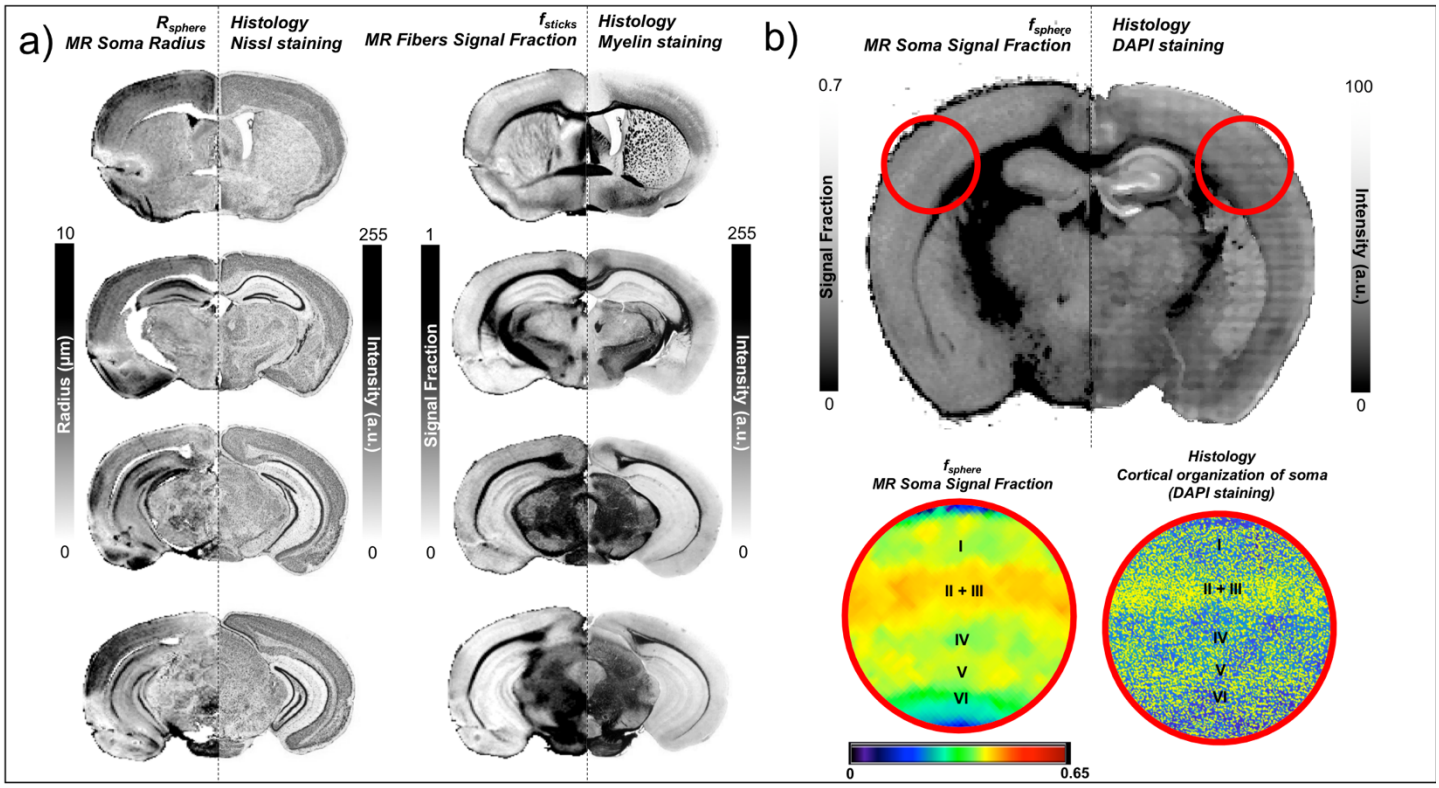

Figure 3. Comparison with histology I. *a)* Direct comparison of MR cell body radius R_{sphere}, and MR cell-fibers signal fraction f_{sticks}, with literature-derived histological images, adapted from[15]. Note the very good match of MR maps with Nissl staining (soma) images and staining of myelinated fibers. *b)* More direct comparison of MR cell body signal fraction f_{sphere} with DAPI staining (soma) of the same mouse brain. Note the very good match of the soma layering in the cortical ROI defined by the red circle, where the six cortical layers: I-VI are clearly distinguishable.

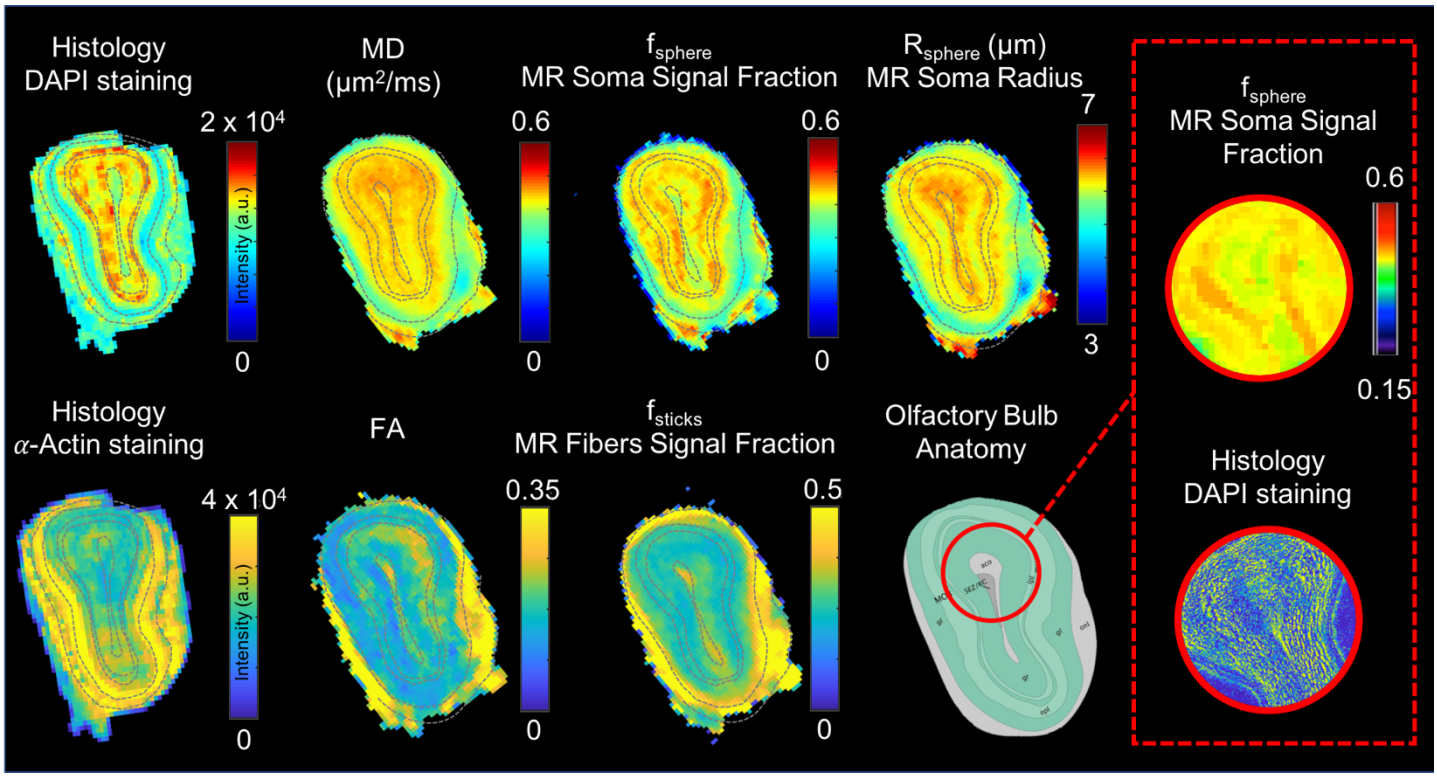

Figure 4. Comparison with histology II. Direct comparison of MR derived maps with histological images (DAPI staining of soma and α-Actin staining of cell membrane) of the same mouse brain olfactory bulb (OB). The OB is characterized by a well-defined layering of soma (see OB anatomy from http://atlas.brain-map.org/) that is perfectly highlighted in maps

of MR soma signal fraction f_{sphere} and radius R_{sphere}, while it is reduced in MD map. Noticeable also the good match between cell membrane arrangement (α-Actin staining) and MR cell-fibres signal fraction f_{sticks} and FA maps.

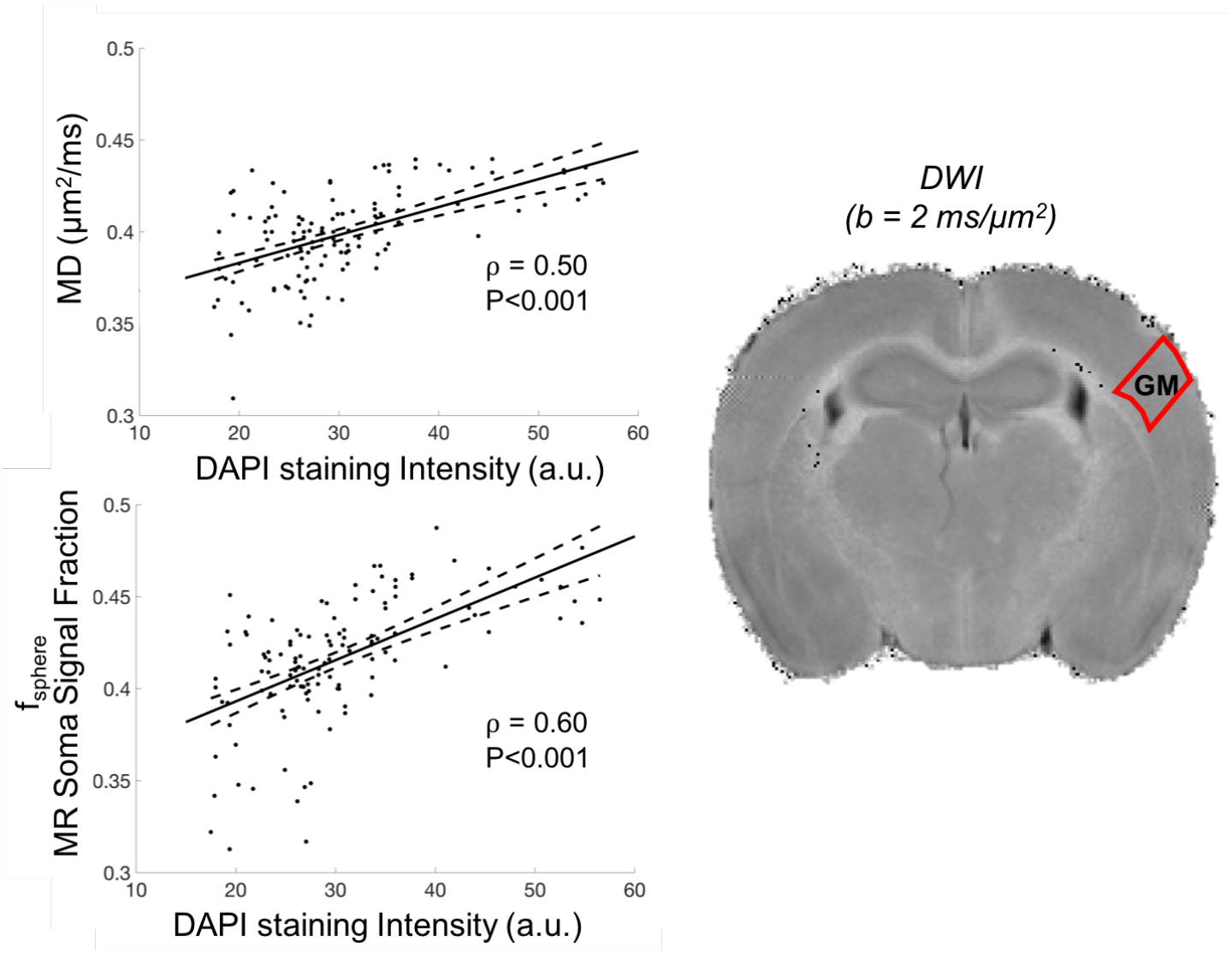

Figure 5. Correlation of MR measured soma signal fraction with histology. Quantitative comparison of MD values and MR soma signal fraction f_{sphere} values with DAPI staining image intensity for a ROI defined in the cortical gray matter (GM) of the same mouse brain, as shown by the reference DWI image. Linear correlation assed by two-tailed t-test shows higher significant correlation between MR soma signal fraction f_{sphere} and DAPI staining image intensity than MD from DTI.