

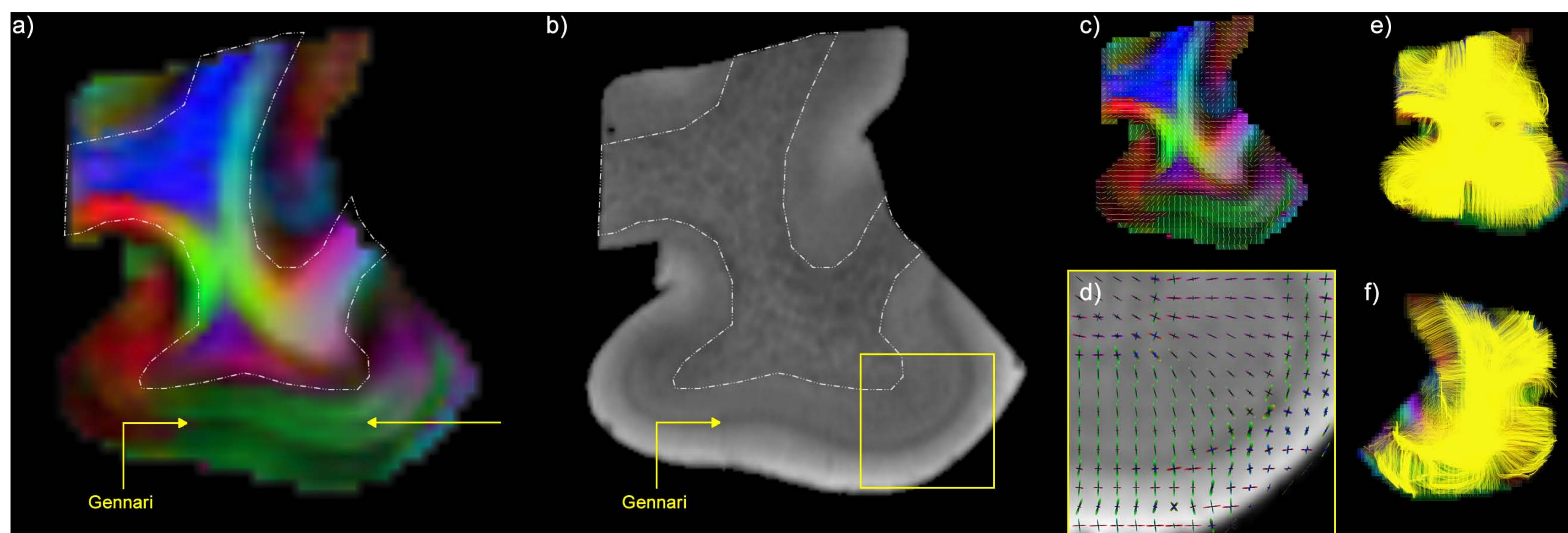
# Validation of Diffusion Weighted Imaging of cortical anisotropy by means of a histological stain for myelin

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## Introduction

To obtain a better understanding of anatomical connectivity *in vivo*, it would be very interesting to track fibers also in and into the cortex. However, the high isotropic component seen in the cortex by current low resolution *in vivo* Diffusion Weighted Imaging (DWI) makes the estimation of cortical connectivity by tractography very challenging. Recently, anisotropy has been shown in cortical grey matter [1,2]. In the cortex, fiber orientation was found to be predominantly radial, but more complex architectures were also observed in the deeper cortical layers of pigs [2]. In this study we aim to examine cortical fibers *in vitro*, as a cross-validation with histological techniques (i.e. myelin staining) as the current gold standard is possible.



**Figure 1: MR results.**  
a) Fractional anisotropy (FA) image showing layer-specific FA (yellow arrows)  
b) MGE image averaged over echoes. The line of Gennari (layer IVb) is visible as a low intensity band.  
c) Principal diffusion direction.  
d) Orientation density functions (PAS ODFs) of the voxels in the yellow square in b). Note crossing fibers at the WM-GM boundary, radial orientation in the deeper layers and multiple directions in the line of Gennari.  
e) DT tractography (seeding in all voxels; FA threshold=0.02; tract length > 5 mm).  
f) Bottom view of e)

## Methods

### Samples:

- Human brain tissue samples of primary visual cortex (V1) including underlying white matter.

### MRI: 11.7T Bruker BioSpec system

- Diffusion Weighted Imaging (DWI) → 0.3 mm isotropic

DW-SE with segmented EPI readout; TR=13.75 s; TE=26.6 ms; 61 directions + 7 non-diffusion-weighted; 14 repetitions; b-value=4000 s/mm<sup>2</sup>; FOV=28.8x28.8 mm; matrix=96x96; 55 slices of 0.3 mm; scan time ~14 h.

- Multi-echo Gradient Echo (MGE) → 0.1 mm isotropic

3D FLASH; TR=40 ms; TE=3.36-38.36 ms; ΔTE=5 ms; flip angle=30°; matrix=256x256x256; FOV=28.8x28.8x28.8 mm; scan time 33 min

### Histology:

- Tissue samples were stained *en bloc* for myelinated nerve fibers with Luxol Fast Blue (LFB).

## Acknowledgements

### VIP Brain Networks



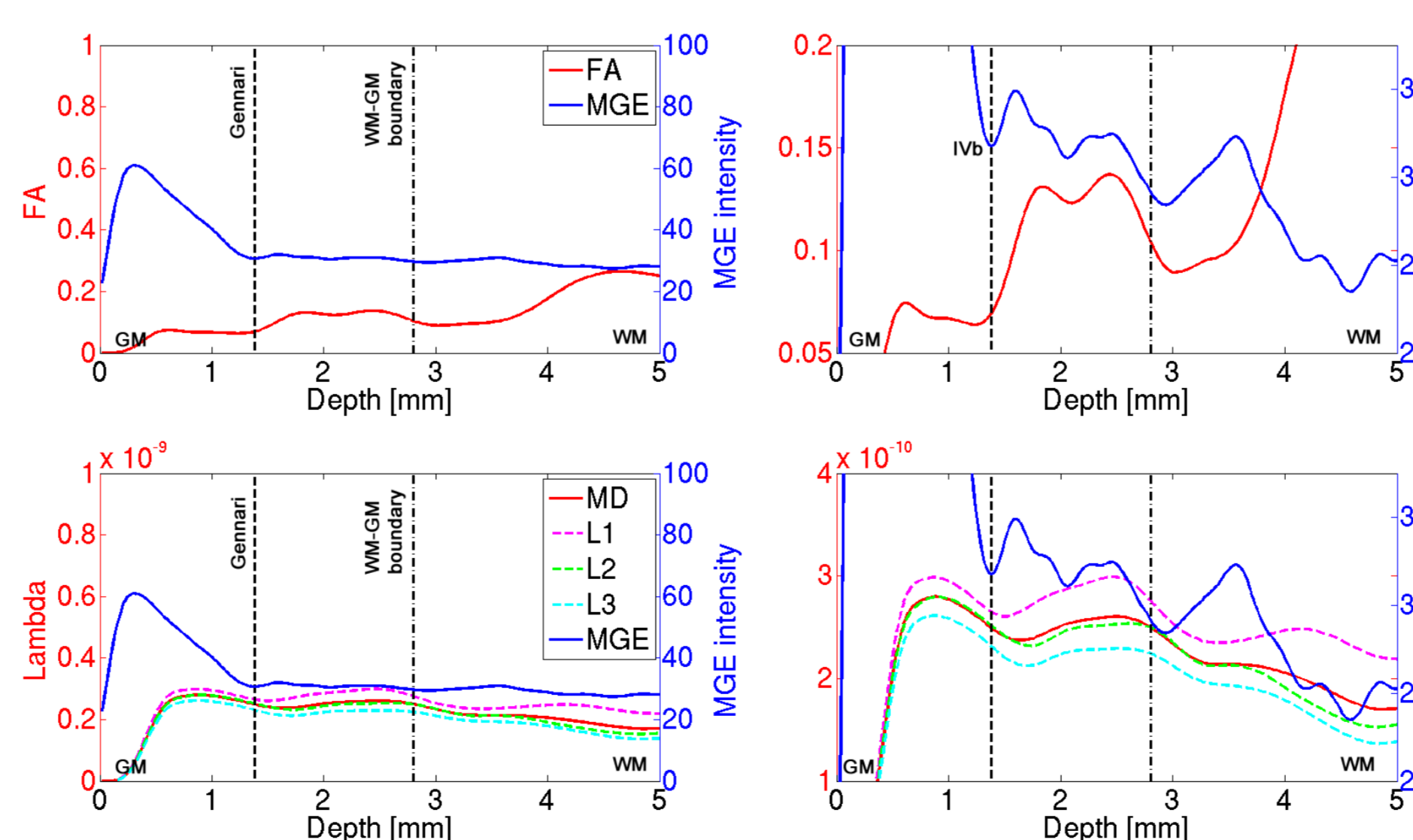
## Results

### MRI:

- Fractional Anisotropy is non-uniform over layers (Fig.1a).
- The line of Gennari shows reduced anisotropy (Fig.2, upper panels) and diffusivity (Fig.2, lower panels).
- In the cortex fiber orientation is predominantly radial (Fig.1c,d), but multifiber reconstructions are seen in e.g. the line of Gennari (Fig.1e) with fibers running horizontally within the layer.

### Histology:

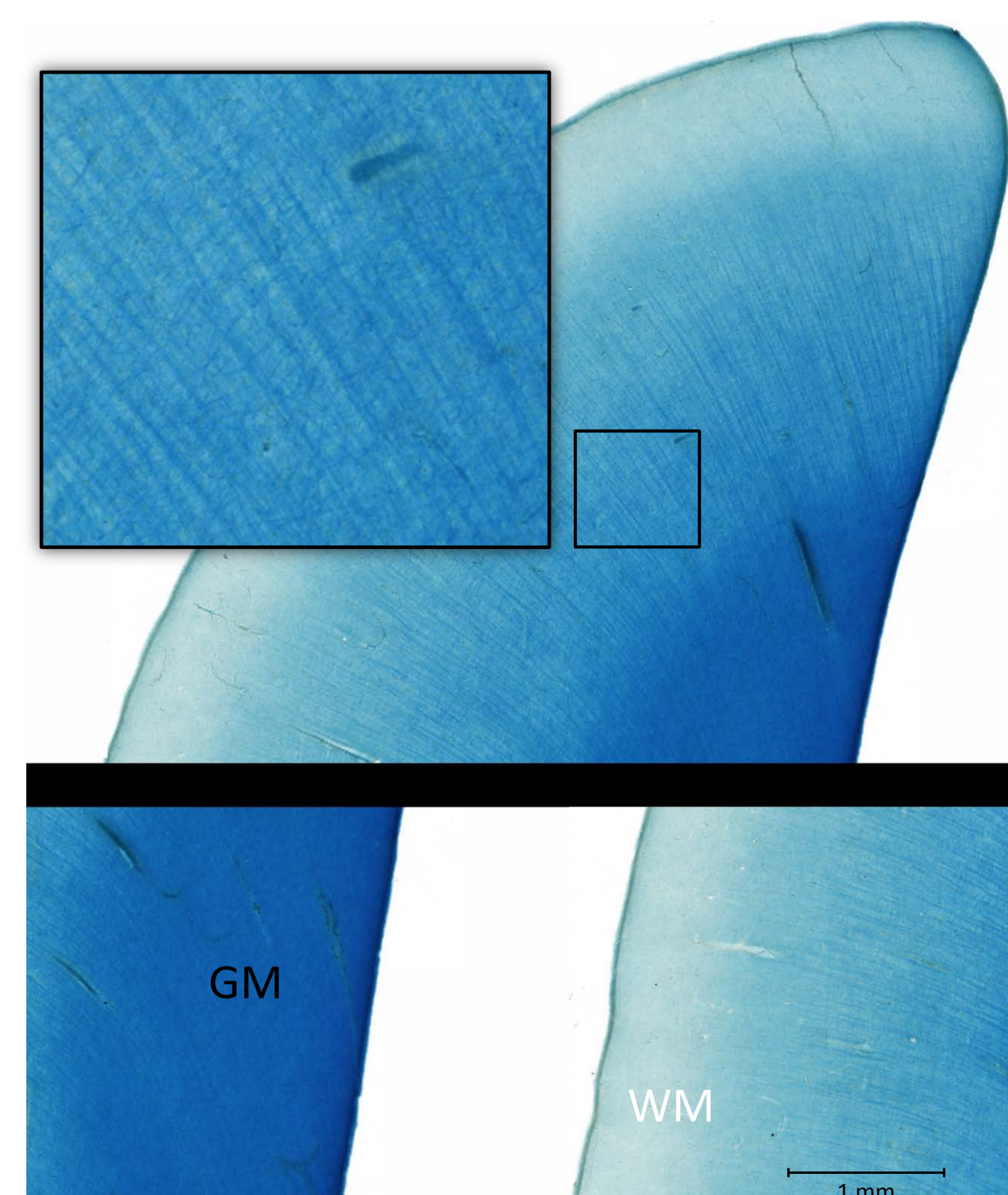
- Myelin-stained sections (Fig.3) clearly show fibers fanning out radially into the cortex and horizontal intracortical fibers.



**Figure 2: Cortical profiles from a small patch of V1 cortex (Ø 2.25 mm).** MGE profiles (blue traces) are included in each panel for anatomical reference. Right panels are blow-outs of left panels. Upper panels: FA profile (red trace). Lower panels: MD and tensor eigenvalue profiles.

## Conclusions

- Layer-specific diffusion parameters have been demonstrated in human primary visual cortex (V1) *in vitro*.
- The usefulness for connectivity research has to be investigated, as tractography within the cortex is challenged by an isotropic component within layers.
- Histological LFB staining successfully showed radial and horizontal cortical fibers and can therefore be used to quantitatively validate DWI results



**Figure 3: A histological virtual slice image at 20x magnification of LFB stained tissue of a 100 µm thick section.** Directionality of myelinated axons is clearly visible in the gray matter. The inset shows radial and horizontal fibers in the gray matter.

[1] Heidemann, R.M. (2010), 'Diffusion Imaging in Humans at 7T Using Readout-Segmented EPI and GRAPPA', MRM, vol. 64, pp. 9-14

[2] Dyrby, T.B. (2010), 'An ex vivo imaging pipeline for producing high-quality and high-resolution diffusion-weighted imaging datasets', HBM, Epub May 13 2010.