Super-resolution track-density imaging studies of mouse brain: comparison to histology

F. Calamante[1,2], J-D. Tournier[1,2], N. D. Kurniawan[3], Z. Yang[3], E. Gyengesi[4], G. J. Galloway[3], D. C. Reutens[3], and A. Connelly[1,2]
[1]Brain Research Institute, Florey Neuroscience Institutes, Heidelberg West, Victoria, Australia, [2]Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia, [3]Centre for Advanced Imaging, The University of Queensland, Brisbane, Queensland, Australia, [4]Neuroscience Research Australia, Randwick, New South Wales, Australia

Introduction: A new method to achieve super-resolution using diffusion MRI has been recently introduced (1). This technique, known as super-resolution track-density imaging (TDI), is able to increase the spatial resolution of the reconstructed images beyond the acquired MRI resolution by incorporating information contained in whole-brain fibre-track modelling results. The TDI technique not only provides a means to achieve super-resolution, but it also provides very high anatomical contrast with a new MRI contrast mechanism not available from other MRI modalities (1). However, *the anatomical information-content* of this novel contrast mechanism has not been validated yet. It remains to be shown whether the features identified on TDI maps correspond to real brain structures; this would require comparison of the TDI maps with an *anatomical* gold-standard, such as that obtained from histological staining of brain sections. The aim of this work is to perform such a study using diffusion-weighted imaging (DWI) of *ex vivo* mouse brains acquired at 16.4T, and comparing the results of the super-resolution TDI technique to histological staining.

Methods: Two 12-week old adult C57 BL6 mice (*M1* and *M2*) were included in this study. Mice were anaesthetised and perfused with 4% paraformaldehyde containing 0.5% Magnevist. The brains were removed from the skull and placed in Fomblin for MRI.

MRI data acquisition: Data were acquired on a 16.4T scanner (Bruker Biospin) using previously published protocols (2). The acquisition consisted of a 3D DW-SE sequence (TE/TR= 22.8/400ms, 0.1mm isotropic resolution, 30 uniformly distributed DW directions, b=5000s/mm^2, total acquisition time ~32hrs).

Fibre-tracking: whole-brain fibre-tracking was carried out using in-house software based on MRtrix (3). The analysis included constrained spherical deconvolution (CSD) (4) to model multiple fibre orientations (maximum harmonic order, l_{max}=6), and probabilistic fibre-tracking using the 2nd order integration over fibre orientation distributions (iFOD2) algorithm (5), with the following relevant parameters: 0.1mm step-size, maximum angle between steps = 45º, 3 FOD samples/step, any track with length < 0.4 mm was discarded, termination criteria: exit the brain or when the FOD amplitude was < 0.01. Four million tracks were generated for each mouse data-set.

Track-density Imaging: TDI maps were generated in MRtrix by calculating the total number of tracks present in each element of a grid. By selecting a grid-element smaller than the voxel-size of the source data, the spatial resolution of the final map can be increased, thus achieving super-resolution (1). For each mouse data-set, a 20µm isotropic grid was used to generate super-resolution TDI maps (cf. acquired resolution= 100µm isotropic).

Histology was performed with Nissl stain (cell bodies) for the brain of mouse *M1* and myelin stain for mouse *M2*.

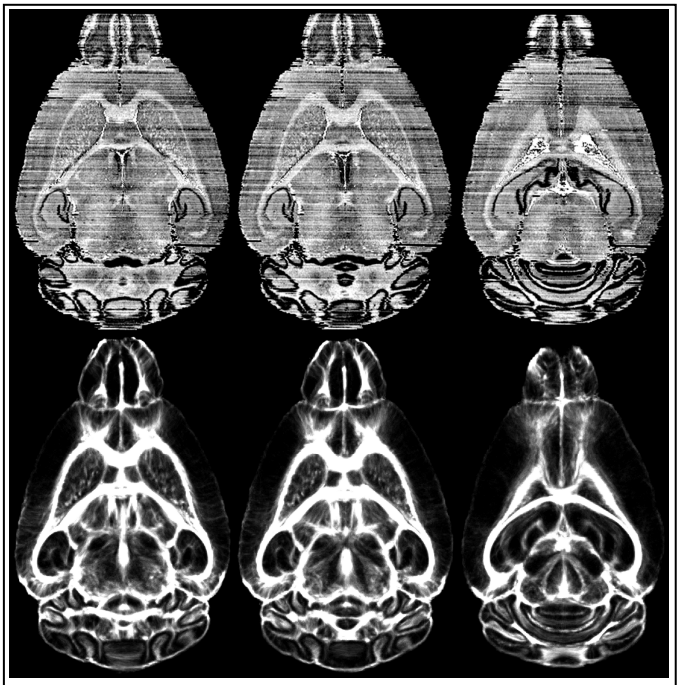

Figure 1: Example horizontal slices from 3D-reconstructed Nissl staining (top row) and TDI maps (bottom row) for the brain of mouse *M1*. Super-resolution TDI voxel size: 20µm isotropic.

Results: A comparison of the structures visualised in the TDI maps and those identified in Nissl staining for the *M1* mouse are shown in Fig. 1. As can be appreciated in these images, there is a striking correspondence between many of the structures detected by both imaging modalities (Note that, due to the different contrast mechanisms, not all structures identified in the TDI maps are expected to have an equivalent in the stained images, and vice-versa). Figure 2 shows an equivalent comparison for the *M2* mouse, with the TDI maps and corresponding myelin staining images. Once again, the similarity between many of the structures visualised by both imaging modalities is readily appreciated.

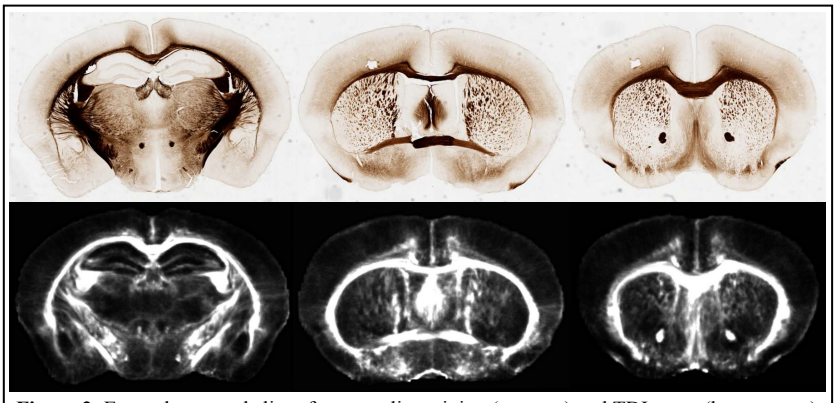

Figure 2: Example coronal slices from myelin staining (top row) and TDI maps (bottom row) for the brain of mouse *M2*. Super-resolution TDI voxel size: 20µm isotropic.

Discussion: The striking agreement between structures visualised in the super-resolution TDI maps and the histological staining corroborates the anatomical information-content of the images generated using the TDI technique. The results therefore show that the TDI methodology does provide meaningful and rich anatomical contrast, in addition to achieving super-resolution The comparison was performed qualitatively; a formal quantitative comparison is not straightforward due to the different image modalities involved (different contrast mechanisms, residual registration errors, etc).

Apart from validating the TDI methodology, this study is the first to show its application to mouse brain imaging. The high-resolution, high-quality images shown in the current study demonstrate the useful complementary information that can be achieved using super-resolution TDI. Due to the increasing interest in mouse brain mapping and imaging phenotypes in mouse models of neurological disorders (7,8), the TDI methodology should provide a very useful imaging modality.

References: (1) Calamante F, et al. *NeuroImage* 2010;53:1233. (2) Moldrich RX, et al. *NeuroImage* 2010;51:1027. (3) *MRtrix*, http://www.brain.org.au/software/ (4) Tournier JD, et al. *NeuroImage* 2007;35:1459. (5) Tournier JD, et al. *Proc. ISMRM*, 2010;18:1670. (6) Basser PJ. *NMR Biomed* 1995;8:333. (7) Wadghiri YZ, et al. *NMR Biomed* 2007;20:151. (8) Waerzeggers Y, et al. *Biochim Biophys Acta* 2010;1802:819.