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# Evaluating fibre orientation dispersion in white matter: Comparison of diffusion MRI, histology and polarized light imaging

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ABSTRACT

Diffusion MRI is an exquisitely sensitive probe of tissue microstructure, and is currently the only non-invasive measure of the brain's fibre architecture. As this technique becomes more sophisticated and microstructurally informative, there is increasing value in comparing diffusion MRI with microscopic imaging in the same tissue samples. This study compared estimates of fibre orientation dispersion in white matter derived from diffusion MRI to reference measures of dispersion obtained from polarized light imaging and histology.

Three post-mortem brain specimens were scanned with diffusion MRI and analyzed with a two-compartment dispersion model. The specimens were then sectioned for microscopy, including polarized light imaging estimates of fibre orientation and histological quantitative estimates of myelin and astrocytes. Dispersion estimates were correlated on region – and voxel-wise levels in the corpus callosum, the centrum semiovale and the corticospinal tract.

The region-wise analysis yielded correlation coefficients of r = 0.79 for the diffusion MRI and histology comparison, while r = 0.60 was reported for the comparison with polarized light imaging. In the corpus callosum, we observed a pattern of higher dispersion at the midline compared to its lateral aspects. This pattern was present in all modalities and the dispersion profiles from microscopy and diffusion MRI were highly correlated. The astrocytes appeared to have minor contribution to dispersion observed with diffusion MRI.

These results demonstrate that fibre orientation dispersion estimates from diffusion MRI represents the tissue architecture well. Dispersion models might be improved by more faithfully incorporating an informed mapping based on microscopy data.

## Introduction

By measuring diffusive motion of water molecules in tissue non-invasively, diffusion Magnetic Resonance Imaging (dMRI) aims to unravel tissue features at a much smaller scale than the imaging resolution. Obstruction of diffusion due to the presence of cellular membranes and macromolecules allows us to infer the microstructural tissue architecture that is reflected by the diffusion signal (Beaulieu, 2002). In addition to estimating microstructural tissue properties, a key challenge in dMRI is to recover within-voxel fibre configurations. Methods that have been developed to enable the reconstruction of crossing fibres in the brain are relatively well established (Behrens et al., 2003; Ozarslan et al., 2006; Tournier et al., 2007; Tuch, 2004; Wedeen et al., 2005), especially if the crossings have a high separation angle. More recently, models have been developed for specifically assessing fibre orientation dispersion using dMRI (Sotiropoulos

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et al., 2012; Tariq et al., 2016; Zhang et al., 2012) or dMR spectroscopy (Ronen et al., 2014). Others have focussed on the effect of dispersing geometries on the diffusion signal through Monte Carlo simulations (Kleinnijenhuis et al., 2015; Nilsson et al., 2012). Estimating dispersion has the potential to improve current tractography algorithms for delineating white matter pathways (Behrens and Jbabdi, 2009; Rowe et al., 2013) or serve as a marker of local fibre coherence, which may provide novel markers of neuropathology. In addition, the diffusion MRI signal from a large portion of white matter is better explained by a model that incorporates dispersion (Ghosh et al., 2016) than models of crossing fibres (Jeurissen et al., 2013).

Comparison of estimates against reference measurements is an essential contribution to the development of increasingly advanced models of fibre architecture. One approach is to use simulations (Balls and Frank, 2009; Hall and Alexander, 2009) or physical phantoms (Fieremans et al., 2008) to generate dMRI data that mimic those obtained from real biological tissue. The primary advantage of such an approach is the control over the fibre configuration to be investigated. A different approach is to directly compare dMRI data to a reference measure in the same tissue, for example by acquiring post-mortem MRI data and microscopy in the same tissue sample. Most commonly, the tissue is stained to highlight specific features of interest, from which quantitative measures can be derived relating to the parameters generated by the dMRI model, for example, when tissue is stained for neurites to estimate intra-cellular volume fractions of white matter. Regarding fibre architecture, many studies focus on evaluating primary fibre orientations, for example using Fourier analysis (Budde et al., 2011; Choe et al., 2012) or structure tensor analysis (Budde and Frank, 2012a; Seehaus et al., 2015). The latter was recently applied to 3D confocal microscopy in order to estimate 3D fibre orientation distribution functions (fODF) and compare them to those reconstructed from dMRI data (Schilling et al., 2016). While dispersion has been quantified previously in histological sections, for example in (Budde and Annese, 2013), a direct comparison with dMRI, ideally in the same specimens, is lacking to date.

Scanning post-mortem tissue faces several challenges compared to in-vivo dMRI experiments. For example, the apparent diffusion coefficient (ADC) and the fractional anisotropy (FA) are known to reduce in formalin fixed tissue (D'Arceuil and de Crespigny, 2007). In addition, the $T_2$ relaxation time of fixed tissue is decreased compared to brain tissue of living subjects (Pfefferbaum et al., 2004; Shepherd et al., 2009). However, dMRI data with high SNR can be obtained from post-mortem tissue, because such experiments are less restricted by scan times and can be performed at systems operating at ultra-high field strengths.

In this study, we evaluated estimates of fibre orientation dispersion in white matter from post-mortem human brain specimens using a parametric dMRI dispersion model (Sotiropoulos et al., 2012) and equivalent measures derived from microscopy data. Specifically, we use polarized light imaging (PLI) measures of fibre orientation and immunohistochemical stains for myelin and astrocytes. We demonstrate good agreement between dMRI estimates of fibre orientation and equivalent measures derived from microscopy in the same three tissue samples.

## Methods

### *Tissue specimens*

Three post-mortem human brains were acquired with permission from the Oxford Brain Bank at the John Radcliffe Hospital in Headington, United Kingdom. The brains were immersion-fixed with formalin after extraction from the skull. Details on the history of each specimen can be found in Table 1. At the level of the anterior commissure, coronal slabs of 5 mm thickness were excised that included the medial part of the corpus callosum (CC), the centrum semiovale (CSO), part of the corticospinal tract (CST), the cingulate cortex and the superior frontal cortex. The samples originated from the anterior body of the CC, i.e. regions 3 and 4 according to Witelson's parcellation scheme (Witelson, 1989).

**Table 1**
Post-mortem specimen details. Abbreviations: PMI; post-mortem interval, i.e. time between death and start of fixation, FT; fixation time, i.e. the time between start of fixation and MRI, COD; cause of death.

| # | PMI (hours) | FT (months) | Sex | Age (years) | COD |
|---|---|---|---|---|---|
| 1 | 48 | 22 | M | 65 | Myocardial Infarction |
| 2 | 48 | 30 | M | 51 | Chronic Obstructive Pulmonary Disease |
| 3 | 16 | 7.5 | M | 91 | Heart failure |

Formalin fixation is known to reduce the $T_2$ relaxation time of tissue, which is detrimental to SNR in MRI, but can be reversed by soaking samples in saline (Shepherd et al., 2009). The samples were immersed in phosphate buffered saline to remove excess formalin 72 h prior to imaging. 48 h later the samples were transferred to a syringe filled with Fluorinert (FC-3283, 3 M™, St. Paul, USA), a hydrogen-free liquid that is susceptibility-matched to tissue to maximize field homogeneity, but which contributes no signal. Where necessary, the specimens were immobilized by placing additional gauzes inside the syringe.

### *MRI acquisition*

The imaging pipeline for the specimens is illustrated in Fig. 1. MR imaging was performed on a 9.4 T 160 mm horizontal bore VNMRS preclinical MRI system equipped with a 100 mm bore gradient insert (Varian Inc, CA, USA). The maximum gradient strength was 400 mT/m with a slew rate of 2162 mT/m/ms in all axes. RF transmission and reception was performed using a 26 mm ID quadrature birdcage coil (Rapid Biomedical GmbH, Germany). Diffusion-weighted images were acquired with a spin-echo sequence (TE = 29 ms, TR = 2.4 s) using single line readout and b = 5000 s/mm$^2$ ($\delta$ = 6 ms and $\Delta$ = 16 ms). High b-values were required to obtain sufficient diffusion contrast for estimating dispersion, as demonstrated in (Sotiropoulos et al., 2012) using b-values as high as b = 8000 s/mm2 in post-mortem macaque brain. A total of 120 gradient directions were acquired in addition to four images with negligible diffusion weighting (b $\approx$ 8 s/mm$^2$). The field-of-view covered the samples in the sagittal plane of the scanner (51.2 mm × 38.4 mm) and was sampled with a 128 × 96 matrix. This lead to an in-plane resolution of 0.4 × 0.4 mm, which was matched with a slice thickness of 0.4 mm for isotropic voxels. The average SNR for the b = 5000 s/mm$^2$ data was 15.5 and 18.6 for grey and white matter, respectively. To reduce Gibbs ringing, the complex k-space data of all volumes were filtered with a Tukey window ($\alpha$ = 0.5). Diffusion tensor images (DTI) were obtained using FMRIB's Diffusion Toolbox (FDT) in FSL (Jenkinson et al., 2012) to compute mean diffusivity maps. These maps were solely used image registration with PLI and histology data. However, no diffusivity values were derived from the DTI analysis.

### *dMRI-derived dispersion*

The dMRI dispersion model separates the diffusion signal into isotropic and anisotropic fractions. Dispersion is estimated from the anisotropic fraction, which aims to describe both intra- and extra-cellular compartments. The isotropic fraction likely captures both free water and non-neuronal cells (Azevedo et al., 2009). This model describes the fODF using a Bingham distribution (Fig. 2), which provides a quantitative estimate of fibre dispersion representing the fanning and bending fibre geometries that appear throughout the brain (Sotiropoulos et al., 2012; Tariq et al., 2016). The Bingham distribution