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Investigating white matter fibre density and morphology using fixel-based analysis

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ABSTRACT

Voxel-based analysis of diffusion MRI data is increasingly popular. However, most white matter voxels contain contributions from multiple fibre populations (often referred to as crossing fibres), and therefore voxel-averaged quantitative measures (e.g. fractional anisotropy) are not fibre-specific and have poor interpretability. Using higher-order diffusion models, parameters related to fibre density can be extracted for individual fibre populations within each voxel ('*fixels*'), and recent advances in statistics enable the multi-subject analysis of such data. However, investigating within-voxel *microscopic* fibre density alone does not account for *macroscopic* differences in the white matter morphology (e.g. the calibre of a fibre bundle). In this work, we introduce a novel method to investigate the latter, which we call fixel-based morphometry (FBM). To obtain a more complete measure related to the total number of white matter axons, information from both within-voxel microscopic fibre density and macroscopic morphology must be combined. We therefore present the FBM method as an integral piece within a comprehensive fixel-based analysis framework to investigate measures of fibre density, fibre-bundle morphology (cross-section), and a combined measure of fibre density and cross-section. We performed simulations to demonstrate the proposed measures using various transformations of a numerical fibre bundle phantom. Finally, we provide an example of such an analysis by comparing a clinical patient group to a healthy control group, which demonstrates that all three measures provide distinct and complementary information. By capturing information from both sources, the combined fibre density and cross-section measure is likely to be more sensitive to certain pathologies and more directly interpretable.

1. Introduction

The importance of white matter axons facilitating microsecond communication between different brain regions is evident from the severe brain dysfunction that arises in disconnection syndromes (Catani and Ffytche, 2005). Furthermore, many neurological disorders (including Motor Neurone Disease (Kassubek et al., 2012), Multiple Sclerosis (Haines et al., 2011), Epilepsy (Otte et al., 2012), and Alzheimer's disease (Radanovic et al., 2013)) involve reduction or disruption of brain 'connectivity' due to pathological changes to the number and density of white matter axons. In vivo methods to quantify white matter changes that alter connectivity are also of interest in relation to psychiatric disorders (Kubicki

Abbreviations: AFD, apparent fibre density; CHARMED, composite hindered and restricted model of diffusion; CUSP-MFM, cube and sphere multi-fascicle model; DWI, diffusion-weighted imaging; FA, fractional anisotropy; FC, fibre-bundle cross-section; FD, fibre density; FDC, fibre density & cross-section; Fixel, A specific fibre population within a voxel; FBA, fixel-based analysis.; FBM, fixel-based morphometry; FOD, fibre orientation distribution; FWE, family-wise error; FWHM, full width at half maximum; MRI, magnetic resonance imaging; SNR, signal-to-noise ratio; SPM, statistical parametric mapping; TBM, tensor based morphometry; VBA, voxel-based analysis.

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et al., 2007), development (Mills and Tamnes, 2014), aging (Lebel et al., 2012), individual differences and brain-behaviour correlations (Johansen-Berg, 2010), genetics (Meyer-Lindenberg, 2009), structural plasticity (Scholz et al., 2009), treatment response (Szeszko et al., 2014) and neuroscientific efforts to relate structural and functional connectivity (Calamante et al., 2013; Stephan et al., 2009; Van Den Heuvel et al., 2009).

Voxel-based analysis (VBA) of diffusion MRI is a common method for studying white matter, providing evidence of altered brain connectivity by detecting differences at a *local level* (Buchsbaum et al., 1998). By far the most popular approach to VBA of diffusion MRI is the analysis of diffusion tensor-derived fractional anisotropy (FA) (Basser and Pierpaoli, 1996), with voxel- or cluster-level statistical inference using packages such as SPM (http://www.fil.ion.ucl.ac.uk/spm/) or FSL (www.fmrib.ox.ac.uk/fsl). However, most white matter voxels are known to contain crossing fibres (Jeurissen et al., 2012), and voxel-averaged measures such as FA are not fibre-specific (or even erroneous) in such regions, which confounds interpretation of apparent differences (Douaud et al., 2011; Pierpaoli et al., 2001; Wheeler-Kingshott and Cercignani, 2009).

In recent years, a number of more advanced diffusion MRI models have been proposed that can resolve multiple fibre populations in a single voxel (Tournier et al., 2011). A major benefit of these so-called mixture models (Tournier et al., 2011) is that quantitative measures can be associated with a single fibre population within a voxel (Assaf and Basser, 2005; De Santis et al., 2016; Dell'Acqua et al., 2013; Raffelt et al., 2012b, 2014; Scherrer et al., 2016; Scherrer and Warfield, 2012). We refer to such a single *fi*bre population within a vo*xel* as a *fixel*,[1] as introduced in Raffelt et al. (2015). Unlike VBA, fixel-based analysis (FBA) can identify effects in specific fibre pathways even within regions containing crossing fibres (Raffelt et al., 2015),

In this work, we first discuss from a theoretical viewpoint why intra-axonal volume (which is a common quantitative measure derived from diffusion mixture models) is of biological interest in FBA of white matter. We then discuss possible mechanisms by which differences in the intra-axonal volume may manifest. This provides the basis for our assertion that when investigating intra-axonal volume, *macroscopic* white matter tract morphology should also be investigated. We therefore introduce a novel method to achieve the latter, which we call fixel-based morphometry (FBM).

The proposed FBM method provides information derived exclusively from morphology differences in fibre bundle cross-section. However, as demonstrated in our previous work (Raffelt et al., 2012b), fibre density and cross-section information can be combined to enable a more complete investigation of white matter. We therefore present the FBM method as an integral piece within a comprehensive fixel-based analysis framework to investigate measures of fibre density, fibre-bundle cross-section, and a combined measure of fibre density and bundle cross-section.

To demonstrate that FBM is appropriate for assessing fibre bundle cross-section, we performed quantitative simulations by applying a number of linear and non-linear transformations to a numerical phantom. Finally, to show how all three measures provide different yet complementary information, we include an example of a fixel-based analysis of temporal lobe epilepsy patients compared to a group of healthy control subjects.

[1] Previous publications have used the word 'fibre' (Assaf and Basser, 2005), 'fascicle' (Rokem et al., 2015; Scherrer and Warfield, 2012) or 'fibre population' (Behrens et al., 2007; Raffelt et al., 2012b) to refer to a specific population of fibres within a single voxel. However, these terms can be ambiguous in certain contexts. For example, when performing statistical analysis of 'fibres' or 'fascicles', this may be misinterpreted as belonging analysis of an entire fibre pathway (e.g. a tractography-based analysis). Here, we use the word 'fixel' to eliminate this ambiguity when discussing fixel-specific measures and fixel-based analysis (FBA).

2. Background

For a fixel-based analysis to be sensitive to white matter changes that affect brain 'connectivity', quantitative measures should ideally reflect the local white matter's 'ability to relay information'. Many DWI models assume that diffusion within axons is restricted in the radial orientation (Alexander, 2008; Assaf and Basser, 2005; Barazany et al., 2009; Jespersen et al., 2007; Lu et al., 2006; Raffelt et al., 2012b; Stanisz et al., 1997; Zhang et al., 2012), and that the exchange of water between the intra-axonal and extra-axonal space is negligible on the timescale of a diffusion MRI experiment (Quirk et al., 2003). DWI models that estimate parameters related to the volume of intra-axonal restricted water are consequently of biological interest since this volume is influenced by the number of axons. It is therefore reasonable to consider that the intra-axonal volume (of axons within a given fixel) is a quantity related to the white matter's local 'ability to relay information'.

In addition to the number of axons, changes in axon diameter may also influence the intra-axonal volume assigned to a given voxel or fixel. Axon diameter plays a role in the 'ability to relay information' via modulating transmission speed, timing and firing rate (Perge et al., 2012; Waxman, 1980). Accounting for axon diameter distributions when investigating intra-axonal volume would provide additional information and potentially more biologically meaningful metrics, however current approaches to estimate axon diameters using DWI are not able to assign estimates to individual fixels in crossing fibre regions (Alexander et al., 2010; Assaf et al., 2008). Furthermore the vast majority of axons in the human brain are smaller than 2 μm (Liewald et al., 2014), and are therefore too small to discriminate between using clinical MRI systems (Drobnjak et al., 2015).

The degree of myelination also influences white matter's capacity to transfer information. Recent work estimates fixel-specific myelin content via T1 relaxometry (De Santis et al., 2016), which would provide useful additional information when investigating fibre density. However, the current acquisition time for the required inversion recovery diffusion weighted imaging sequence is ~1 h (for whole-brain coverage), which is not suitable for most clinical populations.

2.1. Fibre density (FD)

In the last decade there have been numerous DWI models proposed that estimate parameters related to the "intra-axonal restricted compartment", and the terminology employed to describe this compartment varies considerably in the literature (e.g. population fraction of the restricted compartment (Assaf and Basser, 2005), restricted fraction (De Santis et al., 2014a, 2014b), axonal density (Assaf et al., 2008; De Santis et al., 2014a, 2014b; Dyrby et al., 2013), partial volume fraction (Jbabdi et al., 2010), fibre density (Alexander et al., 2010; Assaf et al., 2013; Reisert et al., 2013, 2014), apparent fibre density (Dell'acqua et al., 2010; Raffelt et al., 2012b), neurite density (Jespersen et al., 2010; Zhang et al., 2012), intra-axonal volume fraction (Panagiotaki et al., 2012) fibre volume fraction (Cabeen et al., 2015), fascicle fraction of occupancy (Scherrer et al., 2016)). While there are advantages and disadvantages to the different terminologies, in this work we refer to it as "fibre density" (FD) (see Section 5 for further comment on nomenclature).

Fig. 1 shows different ways that the intra-axonal volume of a fibre bundle may vary. Fig. 1a illustrates a reduced *volume of restricted water* within any given voxel (for example due to disease-induced axonal loss). This scenario manifests entirely as a *within-voxel* change that would be detected as a change in the diffusion-weighted signal and therefore DWI model-derived estimates of FD. While the simple schematic in Fig. 1 only depicts a single fibre bundle, we emphasise that the goal of a fixel-based analysis is to detect fibre density changes belonging to specific