Validation approaches for diffusion MRI based techniques

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Tractography is a powerful method enabling non-invasive visualization and segmentation of fibre pathways in the brain and is based on diffusion weighted imaging (DWI). Tractography requires four processing stages: (#1) scanning (#2) post-processing, (#3) fibre reconstruction, and (#4) fibre tracking. Here we will focus on how to validate the reconstruction of crossing fibres and tissue microstructure estimates (stage #3). At the stage of fibre reconstruction, the challenge for the reconstruction algorithm is to generate fibre profiles which serve as optimal models of estimates of the true underlying fibre bundles and their microstructure as reflected by DWI. Based on these fibre profiles, fibre directions can be extracted and used in the ensuing fibre tracking stage to visualise global anatomical connectivity within the brain. Therefore, to achieve reliable tractography it is of great importance that the fibre reconstruction method has high accuracy, especially in regions containing more complex fibre situations such as crossing fibres.

For validation of fibre reconstruction methods a gold standard is needed and here several approaches can be used, such as simulations, phantoms and invasive tracers, each reflecting different degrees of complexity of the real anatomical environment of fibre bundles *in vivo*. To ensure a solid foundation on which to base the selection of a gold standard, it is useful to know about some of the basic anatomical components within white matter (WM) that are thought to be captured by DWI.

The underlying anatomical environment

The anatomical environments probed by water molecules exist on the microscopic level (micro-meter scale) within cellular spaces. Due to its low spatial resolution, the actual DWI captured by MRI represents an integrated version of the micro-environment into a macroscopic one (millimetre-scale), determined by the structural organisation of the fibre bundles.

At the microscopic level the motion of water molecules is generally determined by the fluid and the structural restrictions within the cellular spaces of WM. The fluid is not pure water but generally more a gelatinous, semi-transparent mixture compromised of substances like ions, macromolecules and proteins. Water molecules moving around within such a substance are slowed down due to molecular interactions, such as piece-wise bonding which will lead to decreased diffusivity compared to that of pure water (see LeBihan 2007 for a detailed review).

In terms of molecular restrictions, the axon is akin to a long flexible tube having a membrane that divides the cellular spaces, and a shape determined by the cytoskeleton inside the axon. This cytoskeleton consists of microtubules (in diameter) (~20 nm), neurofilaments (~10 nm) and microfilaments (~5 nm). Although the microtubules are oriented in a highly organised fashion along the axons and are clearly seen on electron microscopy (EM), they are not believed to contribute much to the restricted diffusivity, in comparison to the cell membrane (see Beaulieu, 2003, for review). In WM, axons are often wrapped in several layers of myelin, which in diffusion terms further restricts the motion of water molecules between cellular spaces. The purpose of myelin is to speed up signal

propagation along the axon, and importantly, the speed of propagation is positively correlated with axon diameter. Because of varying communication speed requirements of the component axons, a WM region may contain a broad spectrum of myelination. For example, between contralateral corticocortical regions the corpus callosum includes both myelinated and non-myelinated axons as well as a rather broad distribution of axon diameters (see Lamantia et al. 1990 fig. 6 for an example).

On the macroscopic level we have a highly organised structure of well defined fibre bundles, composed of densely packed axons. The fibre bundles are flexible in shape for optimal utilization of the space within the brain, as for example seen for the corona radiator (see Lawes et al. 2008 for manual dissection of fibres bundles). Therefore, with the voxel resolution of a clinical MR scanner (i.e., 2 mm isotropic resolution), a rather large percentage of the voxels contain crossing fibre bundles (Behrens et al. 2007).

Validation approaches

Simulation: Simulations offer the user full control over all parameters, and which of them to include, when generating a synthetic DWI dataset. They are suitable for validation since a simulated version of the true underlying fibre bundles is known. However, such synthetic datasets are highly dependent upon the user's knowledge and belief on how different anatomical compartments influence the diffusion signal. Synthetic datasets can generally be created in two ways; 1) by test functions or 2) by Monte-Carlo (MC) simulations of spins executing Brownian motion within a user defined environment. Test functions are typically based on a mixture of user-defined Gaussian distributions simulating of the diffusivity of, for example, two crossing fibre bundles (see Alexander 2005 for an example). MC simulations allow highly complex biological environments to be formulated mathematically, as well as the inclusion of scanner parameters, and hence generate synthetic datasets based on a more exact model of the distribution of diffusivity in the true underlying anatomical environment (see Hall et al. 2009, Alexander et al 2010, Fieremans et al. 2008 and Beaulieu et al. 1994 for an example).

Phantom measurements: Phantoms, in contrast to synthetic data based on simulations of diffusivity distributions, offer the generation of suitable DWI validation datasets based upon 'true' Brownian motion of water molecules within a 'real' model of the true underlying fibre bundles (the phantom) and with DWI obtained on a 'real' MR scanner. Examples of micro-structural materials that have been used to mimic the densely packed axons include hollow plastic capillaries (Lin et al. 2003, Tournier et al. 2008) and solid material based on textile rayon fibre (Perrin et al. 2005) and poly-ethylene fibre (Fieremans et al. 2008). Typically, phantoms are filled with pure water. Alternatively, one might construct phantoms on components of postmortem tissue that are believed not to contain crossing fibre bundles, and build a known structure with them, for example the crossing of two spinal cords concreted into agar (Campell et al. 2005).

Invasive tracer studies: Invasive tracers within tissue are the only validation approach that provides the possibility of visualising the actual true underlying anatomical environment from which DWI is obtained (direct validation). Alternatively, a more indirect validation approach can be used where DWI and the gold standard not are obtained within the same brain. Due to the invasive nature of in vivo tracers, animal models such as the pig (Dyrby et al. 2007), rat (Lin et al 2001) and monkey (Schmahmann et al. 2007) are used. The degree of anatomical details visualised by an invasive tracer depends upon the choice of tracer and the visualisation technique used, and can range from a macroscopic level visualisation of fibre bundles, to the more

microscopic visualisation of the distribution of axons (see Dyrby et al. 2007, fig. 1 and 2 for an example where different tracers have been used). Crossing fibres can be validated by means of invasive tracers using a setup where single injections are made in separate animals, and the fibre projections are visualised and then finally projected onto a standard brain (see Schmahmann et al. 2006).

Overall learning objective:

- To understand some of the underlying neurobiology of diffusion processes in white matter tissue as revealed by DWI
- To learn about possible setups for validation of fibre reconstruction methods
- To identify advances and limitations of the different validation setups

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