

Magnetic Resonance Techniques For Imaging White Matter

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Running head: MRI and white matter

Abstract

The white matter is a complex network of brain fibres connecting different information processing regions in the brain. In recent years, the investigation of white matter in humans and in animal models has greatly benefitted from the introduction of *in vivo* non-invasive magnetic resonance imaging (MRI) techniques. MRI allows for multiple *in vivo* time-point whole-brain acquisition in the same subject, thus it can be used longitudinally to monitor white matter brain change, intervention effects, as well as disease progression. However, MRI has low spatial resolution compared to gold-standard cellular techniques and MRI measures are sensitive to a number of tissue properties resulting in a lack of specificity.

The following chapter describes in simple technical terms to non-imaging experts some common MRI techniques that can be used to investigate white matter structure non-invasively, covering some of the advantages and pitfalls of each technique.

Keywords: MRI, white matter, myelin, image analysis.

1. Introduction to MRI

MRI relies on nuclear magnetic resonance (NMR). Nuclei that contain an odd number of protons and/or neutrons spin around their own axis. The most commonly used nucleus in MRI is hydrogen (H), which is present in water molecules and is widely available in body tissue. In a free environment the spins will be randomly oriented, however when placed in a magnetic field, the spins will align with the field and precess around it. In MRI this field is generated by the scanner and is measured in tesla units. In MRI, the alignment to the main magnetic field (named b_0 and conventionally represented as the z axis) is perturbed by a perpendicular magnetic field that emits a brief radio frequency (RF) pulse. This causes the protons to flip their spin and precess in the x,y axis, producing a signal that can be detected as a current by a receiving coil. If the signal is acquired immediately after the RF pulse, most protons will be maximally aligned with the x,y axis and the resulting image will reflect proton density. On the other hand, if the signal acquisition is delayed, proton spins will start to re-align with the main magnetic field, resulting in signal decay in the measurable x,y axis. The recovery time in the longitudinal (z) axis is known as T_1 and varies between different brain tissues which can be a source of tissue contrast. T_2 reflects the decaying time in the transverse plane (x,y) and also varies between tissue types. T_2 decay occurs faster than T_1 due to the small magnetic fields around each proton that affect the spin of the neighbouring nuclei, causing them to dephase and rapidly decrease the signal in the transverse plane. MRI sequences can be tuned in order to maximize or minimize the influence of each type of recovery or decay time in order to obtain images with high contrast between tissues.

To be able to image the location of the protons, it is necessary to use an additional set of small magnetic field gradients distributed across the x,y,z axes. These gradients are rapidly turned on and off, after the RF pulse, to create small linear changes in the main magnetic field along the three orthogonal axes. As a result, protons at different spatial locations will experience slightly different magnetic fields. Since precession frequency depends on the magnetic field experienced by a proton, this means proton precession can be used to estimate the position of the nuclei. Fourier transforms are then used to reconstruct MR images from frequency space, conventionally known as k -space.

2. T1-weighted and T2-weighted imaging

T1-weighted (T1w) and T2-weighted (T2w) imaging are some of the earliest methods used in MR studies to provide image contrast between brain tissue and are sensitive indicators of anatomical structures. The images are generated by exciting and acquiring signal at different time intervals. T1-weighted images allow for a high contrast between white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF), making it ideal for tissue segmentation and tissue volume estimates. The signal intensity from free hydrogen proton filled compartments (such as the CSF) appear attenuated and dark on T1-weighted images, whereas in highly structured materials, such as the lipid rich myelin sheaths (where the macromolecule-bound protons proportion is higher), the T1 signal appears brighter. While T1w and T2w images are sensitive indicators of anatomical structures and the T1w contrast is related to the presence of myelin, these methods are not capable of directly measuring the myelin concentration in the brain due to its qualitative nature (1, 2). Additionally, individual experimental parameters will influence T1w and T2w contrast, precluding the possibility of direct comparisons of T1w and T2w values across patients and healthy controls, or across longitudinal time-points (3, 4).

An alternative approach is to use the ratio of T1w and T2w images to produce maps whose grey-scale intensities have been found to co-localise with myelin-stained histology from the cortical surface (5). These structural images are routinely acquired in MR exams thus eliminate the need of additional complex MR acquisitions and long scan times. Furthermore, they can be acquired at high resolution, which can be beneficial to examine cortical myelination, in a relatively short period of time. However, T1w and T2w are also related to the overall water content in tissues, and thus images individually have low specificity to myelin.

In order to overcome the comparability, reproducibility and specificity issues of qualitative imaging, quantitative mapping that provides absolute values on voxel-by-voxel basis has also been developed (3, 4).

2.1 Quantitative T1 mapping

Quantitative representation of T1 measures offers a more direct assessment of white matter tissue properties. T1 relaxometry refers to quantitative methods that measure and map relaxation times or susceptibility values within tissues. T1 contrast is highly sensitive to the

glycolipids and cholesterol components of myelin sheath (6) and to a lesser degree, iron content, so a decrease in quantitative T1 values relates mainly to an increase in myelin (1).

An alternative approach to T1 relaxometry is to use the ratio of standard structural images to produce maps of myelination. R1 maps ($=1/T1$) positively correlates with the amount of myelination and has been shown to reflect myeloarchitecture in cortical areas (7, 8). Postmortem ultra-high resolution R1 images have shown a good correspondence with histological measures of myelin in cortical areas (1).

Quantitative MRI measurements, including T1 and R1 maps, are theoretically independent of acquisition parameters, therefore the generated information is comparable across healthy participants and pathological conditions, cortical regions, and over different time-points (3, 4). However, long scan times are necessary, which might prevent these quantitative techniques from being routinely included in clinical studies.

3. Magnetisation Transfer Imaging

Magnetisation Transfer (MT) imaging has the ability to indirectly detect macromolecule-bound protons such as membrane lipids and myelin (6). The MT effect relies on exchanges between the macromolecule-bound water and the free water. The protons in the free water have relatively long T2 relaxation times making it easy to detect by conventional MRI systems. By contrast, the magnetisation from the restricted macromolecule-bound protons decays faster. In MT imaging these macromolecule-bound protons are selectively saturated without influencing the free water. This saturated magnetisation is then transferred to the free pool by a continuous exchange of magnetisation, which is referred to cross-relaxation or MT, and this effect is then measured.

MT ratio (MTR) imaging is a semi-quantitative technique with the advantage of short acquisition times at relatively high resolution. To calculate MTR only two types of images are needed: one with MT saturation and one without. The resulting maps are found to correlate with myelin (9). MTR maps have been used to assess changes in myelin content in Multiple Sclerosis (MS) lesions with studies showing higher MTR values in remyelinated WM lesions (9). Despite the strong correlation between MTR and myelin, MTR lacks neuropathological specificity (10). In other words, MTR values are influenced by other factors unrelated to myelin, such as axonal injury, inflammation, edema (11) and experimental parameters, such as direct saturation effects and pulse magnitudes (9, 12).

Furthermore, a lack of standardization across imaging centers makes it hard to compare results (for review see (13)).

Quantitative MT (qMT) is more sensitive and specific to myelin than MTR (10, 14, 15). For instance, qMT is sensitive to regional differences in white matter of healthy participants that are related to tissue myelination (15). Furthermore, qMT is able to detect ageing related changes in myelination in rodent models while MTR is not sufficiently sensitive (10). However, this type of imaging requires more than the two images necessary for the MTR calculation, thus longer scan times are needed for the calculation of qMT. The higher specificity and sensitivity therefore comes at the expense of resolution, coverage and scan time (16-18).

4. Diffusion Imaging

The MRI technique most commonly used to probe white matter structure in humans is Diffusion weighted imaging (DWI). DWI is sensitive to the local diffusion of water molecules. Conventional DWI estimates the overall diffusion at each voxel and can be useful to detect certain types of pathology. However, in many tissue types diffusion will vary along different axes and so an estimate of overall diffusion is less useful.

In the absence of any barriers, water diffusion is roughly uniform in all directions, known as isotropic diffusion. In biological tissue, however, many boundaries alter free water diffusivity, thus it becomes more restricted, or in other words anisotropic. For instance, in the fibrous white matter tracts, the axonal membrane confines the movements of water, diffusion is restricted in the perpendicular direction so water tends to diffuse along the axis of the tract but not across it. In such cases it is useful to estimate diffusion along different directions. The most common approach to do so is diffusion tensor imaging (DTI) (19). In DTI imaging water diffusion is mathematically modelled by an ellipsoid, or tensor. The diffusion tensor is calculated on a voxel-by-voxel basis by applying a mathematical algorithm on multiple diffusion-weighted images, each sensitized to diffusion along a different direction. A minimum of 6 sampled directions are required to estimate the 6 parameters that define any given diffusion tensor: the length of the longest, middle, and shortest axes of the tensor (called eigenvalues) and their orientations in space (called eigenvectors). The larger the number of acquired directions the more accurate is the estimation of the tensor, so most DTI acquisitions sample more than 6 directions at the expense of scan time.

4.1 Imaging white matter microstructure and integrity with DTI

Multiple parameters can be estimated from the diffusion tensor model. Fractional anisotropy (FA) is the most commonly used parameter to measure directional dependence of water diffusion. FA characterizes the shape of the tensor and provides a normalized value to the degree of anisotropy from zero (completely isotropic) to one (completely anisotropic) (20). For instance, at the typical resolution of a DTI acquisition, the diffusion of water molecules shows little directional dependence in the grey matter and cerebrospinal fluid, thus the value of FA in these tissues is close to zero, which is represented as a near-spherical tensor within each voxel. In contrast, in the highly organized white matter fiber bundle the diffusion has a preferred direction, thus the FA value is closer to one and represented by an elongated diffusion ellipsoid.

FA is highly sensitive to various white matter features, such as myelination, fibre caliber and density, fibre organisation and axonal degeneration (21). For instance, FA is higher in white matter tracts that are highly myelinated. Other important measures calculated from the eigenvalues are mean diffusivity (MD), axial diffusivity (AD) also referred to as parallel diffusivity, radial diffusivity (RD), also known as perpendicular diffusivity. MD reflects tissue density, the greater the barriers to diffusion the lower the value; MD is a commonly used measure in both GM and WM. AD is a measure of the diffusivity in the direction of maximum diffusion in a voxel and can be an indirect marker of axonal membrane integrity (22-24). Whereas RD represents the average of the diffusivity values in the two axes perpendicular to the principle direction of diffusion and there is some evidence it might particularly relate to myelination (23, 25). These measures allow for the indirect probing of white matter microstructure and integrity, however, there is not a one-to-one relationship between a particular DTI-derived metric and a specific cellular feature (26). In other words, a decrease in FA, for instance, can be due to a number of causes such as a decrease in myelination, cell swelling, axon degeneration, among others.

Although DTI measures are non-specific, they are very sensitive to events that alter the microstructure of the brain hence this technique has been widely used. For instance it has been used to investigate white matter microstructure in relation to development and ageing (27, 28), brain plasticity during adulthood (29, 30) and brain disorders such as multiple sclerosis (MS) (31) or Alzheimer's disease (32). FA has been found to increase during

development well into adulthood, while MD and RD decrease, partly reflecting increases in myelination (27, 33). FA decreases with ageing while MD and RD have the opposite trajectory, which is associated with decreases in myelination as well as a loss of nerve fibres associated with normal age-related degeneration (27, 28, 34-36). Naturally, diffusion imaging has been used to assess MS lesions during acute phases and it might be useful to monitor disease progression and treatment efficacy. Most studies have shown increased MD and decreased FA in lesions of MS patients when compared with normal appearing white matter or with healthy controls (31, 37, 38). Diffusion imaging has made a large contribution in fostering interest in white matter neuroplasticity during adulthood. Neuroimaging studies have reported that cognitive and motor learning and practice results in changes in white matter structure (29, 30, 39, 40), which are related at least in part to myelination (41, 42).

4.2 Advanced diffusion analysis methods

DTI and its estimated parameters give unprecedented insights into the architectural organization and integrity of the living brain but this simple model has several shortcomings (Assaf et al., 2008; Panagiotaki et al, 2012; De Santis et al., 2014). For instance, DTI does not account for multiple tissue types within a voxel, and a number of different white matter microstructural features (such as the cellular membranes of glial cells or the axon diameter and density) contribute to the obtained DT indices (43, 44). However, the water residing in different tissue compartments has different diffusion patterns. For example, water molecules residing outside the axon (extra-axonal) has different diffusion characteristics than those confined inside an axon (intra-axonal) (43, 45, 46). Hence, by estimating diffusion from the individual compartments, further information could be revealed about white matter microstructure and axonal morphology not provided by DTI. Different multi-compartment models have been proposed to complement DTI measurements and provide more specific estimates of brain microstructural properties.

One of the multi-compartment models is the *Composite hindered and restricted model of diffusion*, or CHARMED (Assaf & Basser, 2005). CHARMED estimates and models the contribution of the extra-axonal and intra-axonal space diffusion compartments. Hindered diffusion characterises the displacement of water in the extra-axonal space that includes extra- and intracellular spaces of glial cells (43). In contrast, restricted diffusion refers to the distribution of water in the intra-axonal space bounded by compartments like axonal

membranes (43). Therefore, CHARMED is able to provide improved local fiber orientations and measures of axonal density.

Based on the two-compartment CHARMED framework, further methods have been developed. The *AxCaliber* model was proposed to estimate the axonal density, the extra-axonal diffusivity and the axon diameter distribution by probing the entire white matter tissue continuously with multiple diffusion times and gradient strengths (47). The diameter distribution of neuronal fibers is an important morphological parameter that may link to various functional properties of white matter fascicles, such as their conduction velocity (48, 49). Additionally, *AxCaliber* is a very promising technique for future insights into the longitudinal changes of axonal diameter distributions *in vivo* during development, ageing and disease.

While most diffusion MRI techniques for the central nervous system have focused on the white matter, recent promising methods for estimating the distribution of orientations and density of neurites (the collective name for axons and dendrites) in either the white matter or grey matter have begun to emerge. Neurite morphology is an important marker of normal brain development, aging, brain connectivity and numerous neurological disorders, such as Alzheimer's disease and Multiple Sclerosis (45, 50). One neuroimaging technique to assess such information is the neurite orientation dispersion and density imaging (NODDI) and its recent extension Bingham-NODDI that aims to estimate the density and orientation dispersion of axons and dendrites in living human brain as little as 10 minutes on standard clinical MRI scanners (45, 50).

4.3 Tracing white matter tracts *in vivo*

MRI can also be used to perform *in vivo* tract tracing. Currently, diffusion imaging is the only method that allows for the tracking of white matter fibers non-invasively. Tractography can reconstruct entire white matter pathways through the human brain by tracing and connecting voxels. The information provided by this method allows the visualization of the three-dimensional architecture of the white matter tracts. It can reveal patterns of anatomical connectivity between different regions, which in turn can provide information about the functional specialization of the brain region.

There are several tracing methods that rely on different assumptions to estimate fibre orientation and in some cases require advanced diffusion acquisition (for more information see (51)). The simplest method of fiber tracing known as deterministic tractography, can use DTI data, and is based on the assumption that the orientation of the largest eigenvector of the fitted tensor corresponds to the direction of the main white matter tracts (19). Probabilistic single-fibre tractography on the other hand, requires sampling of a large number of diffusion directions, and additionally considers the uncertainty on the estimate of principal fiber direction (52). Both these approaches assume a single fibre orientation at each voxel. This is often not the case in white matter voxels where crossing, diverging, or complex pathways mean that a single fibre orientation does not provide a useful representation of the true fibre architecture. Alternative methods, allowing for estimate of multiple fiber populations at each voxel, have therefore been proposed (53). These make it possible to trace crossing fibers and provide a more accurate reconstruction of the underlying anatomy.

Although there has been encouraging evidence on the validation of diffusion tractography relative to gold standard methods (54-57), it is important to highlight that diffusion tractography does not trace axonal fibres directly like injected tracers, and tens of thousands of axons are present in a voxel. This method also does not differentiate between anterograde and retrograde connections thus it does not allow for an identification of the direction of the connection or presence of synapses. Furthermore, crossing-fiber regions, fibre fanning and axon geometry influence tractography performance and accuracy, even when more sophisticated crossing fibre models are used, as such the results should be carefully interpreted in light of these limitations.

5. Conclusions

In the last few decades we have seen a rapid development in MRI techniques that allow for probing of the microstructure of the living brain. This offers huge opportunities for understanding human brain development, ageing, plasticity and pathology. The rapid development of increasingly sophisticated MRI acquisitions and tissue modelling are already providing a better characterization of the microstructure of the brain and more specific markers of the underlying cellular structure. New technical developments will continue to offer more specificity, higher sensitivity, and faster acquisitions, at a higher resolution.

Furthermore, with faster acquisitions, several modalities can be acquired in a single scan session providing complementary information and helping to disentangle and to quantify the different cellular components, offering a more complete picture of the *in vivo* brain.

It is necessary to continue to validate these methods by means of animal models, where both MRI and histological measures can be acquired (41, 42, 54), and human postmortem tissue studies (1, 58). Still, even with further development, MRI will never achieve the same resolution and specificity of gold standard microscopy – but the ability to measure *in vivo* and in humans offers the exciting opportunity to exploit the methods to understand the importance of WM structure to human development, behaviour and disease.

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