From micro- to macro-structures in multiple sclerosis: what is the added value of diffusion imaging

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List of abbreviations

MD	Mean diffusivity
FA	Fractional anisotropy
RD	Radial diffusivity
AD	Axial diffusivity
K	kurtosis
RMSD	root mean square displacement
P_0	probability for zero displacement
dMRI	Diffusion MRI
DTI	Diffusion tensor imaging
PDF	Probability density function
MS	Multiple sclerosis
PPMS	Primary-progressive multiple sclerosis
RRMS	Relapsing-remitting multiple sclerosis
SPMS	Secondary-progressive multiple sclerosis
NAWM	Normal appearing white matter
NAGM	Normal appearing grey matter
CIS	Clinically isolated syndrome
QSI	q-space imaging
DKI	Diffusion kurtosis imaging
EAE	Experimental allergic encephalomyelitis
BBB	Blood brain barrier

Abstract

Diffusion imaging has been instrumental in understanding damage to the central nervous system thanks to its sensitivity to microstructural changes. Clinical applications of diffusion imaging have grown exponentially over the past couple of decades in many neurological and neurodegenerative diseases such as Multiple Sclerosis (MS). For several reasons, MS has been extensively researched using advanced neuroimaging techniques, which makes it an "example disease" to illustrate the potential of diffusion imaging for clinical applications. In addition, MS pathology is characterised by several key processes competing with each other, such as inflammation, demyelination, remyelination, gliosis and axonal loss, enabling the specificity of diffusion to be challenged. In this review we will describe how diffusion imaging can be exploited to investigate micro-, meso- and macro-scale properties of the brain structure and discuss how they are affected by different pathological substrates. Conclusions from literature are that larger studies are needed to confirm exciting results from initial investigations, before current trends in diffusion imaging can be translated to the neurology clinic. Also, for a comprehensive understanding of pathological processes it is essential to take a multiple-level approach where information at micro-, meso- and macroscopic scale are fully integrated.

Introduction:

Diffusion MRI (dMRI) is sensitive to the hindrance of diffusion by tissue microstructure, and thus provides an indirect measure of the size, orientation and shape of cellular structures in vivo[1]. This makes dMRI unique in its ability to provide microstructural information non-invasively. Ever since the introduction of diffusion tensor imaging (DTI)[2] the scalar indices derived from the tensor, such as mean diffusivity (MD), which quantifies the magnitude of diffusion within a voxel, and fractional anisotropy (FA)[3], which measures the directionality of diffusion, have been applied to a wide range of neurological and psychiatric disorders.

The sensitivity of dMRI to the microscopic translational motion of water molecules is also reflected into its sensitivity to the principal direction of diffusion, which can be assumed to coincide with that of the underlying white matter fibers (at least for voxels comprising of a single major fibre bundle). This forms the basis for diffusion 'tractography'[4] a method able to produce 3D 'reconstructions' of probable white matter pathways that offer a reasonable representation of anatomy.

Recently the possibility to reconstruct white matter connections using diffusion tractography has been combined with network analysis and graph theory, to form an integral part of "connectomics", a science branch aiming at mapping all neural connections within the central nervous system (CNS), i..e the brain connectome[5,6].

These features make dMRI a technique sensitive to all scales, ranging from the microscopic properties of tissue to the connections that can be mapped at specific network (mesoscopic) or whole-brain (macroscopic) level[7].

While DTI has proven sensitive to subtle brain abnormalities, its translation into clinical routine (other than for the diagnosis of acute stroke[8,9]) has been limited. To be of real clinical added value, a biomarker would have to provide information more specific than what already available through conventional imaging (i.e. identifying lesional tissue). The lack of specificity, or, in other words the inability to link patterns of diffusion abnormalities with specific pathological substrates, may have contributed to a limited adoption into the clinical setting of DTI. Attempts to address this issue have led to the development of a series of frameworks that are either model-free or employ higher order models of diffusion, with a more direct interpretation of model parameters in terms of their potential biological substrate[10]. The downside of this approach is the increased mathematical complexity of such models, which makes their adoption in clinical settings difficult.

With the aim to review the added value and the pitfalls of dMRI in clinical applications, here we will focus on multiple sclerosis (MS), and on the potentials of dMRI to clarify the effects of this disease on the brain at the micro-, meso- and macro-scopic scales. MS was chosen as "example" disease, as its pathology is characterised by several key processes competing with each other, enabling the specificity of diffusion to be challenged.

Basic diffusion MRI concepts

Details about dMRI methods and techniques are covered by several papers and book chapters, e.g.,[11-13]. Here we will simply introduce the parameters needed for understanding the subsequent discussion.

Diffusion sensitization can be introduced by the use of pulsed magnetic field gradients played out in addition to the standard imaging gradients. The dephasing caused by the gradients results in signal attenuation proportional to the diffusion coefficient[14]. The amount of diffusion sensitization, dependent on the amplitude, separation and duration of the gradients, is summarised by the b factor (or b value)[1]. The time allowed for the diffusion process encoding is referred to as the diffusion time t_{diff} and its definition depends on the pulse sequence used. Typically the acquisition is repeated several times varying the orientation and the amplitude of the gradients. The specific pattern used will depend on the application, the model to be fitted and the techniques used for image processing.

Diffusion Tensor Imaging (DTI)[2] is assuming that water diffusion in tissue can be associated with a diffusion tensor (DT) characterized by three eigenvalues ($\boldsymbol{\varepsilon}_1$, $\boldsymbol{\varepsilon}_2$, $\boldsymbol{\varepsilon}_3$) identifying the principal direction of diffusivity, and the corresponding three eigenvalues (λ_1, λ_2, λ_3). From the eigenvalues it is possible to derive indices reflecting properties of the underlying tissue such as mean diffusivity (MD, average of the three eigenvalues), fractional anisotropy (FA, proportional to

the variance of the eigenvalues)[3], axial diffusivity (AD, equal to λ_1) and radial diffusivity (RD, equal to the average of λ_2 and λ_3).

Q-space imaging (QSI) is a model-free approach that attempts to provide a more accurate description of tissue microstructure and to overcome the limitations of DTI-derived indices such as FA and MD[15,16]. QSI relies on the acquisition of dMRI data at multiple b (or 'q', with q^2 proportional to b/t_{diff}) values to sample the signal decay with q. The Fourier Transform of the signal curve gives the probability density function (PDF) of molecular diffusion. Three parameters, namely the root mean square displacement (RMSD), the probability for zero displacement (P_0), and the kurtosis (K, i.e. a measure of deviation from Gaussian behaviour) can then be derived. If kurtosis is the parameter of interest, this can also be derived using diffusion kurtosis imaging (DKI), a simpler approach to characterize the non-Gaussianity of the probability distribution, less demanding in terms of data acquisition, although still requiring multiple b values[17]. DKI can provide parameters such as the mean kurtosis (DK), independent on the direction of observation, or the $K_{//}$ or $K_{\perp}$, which are the kurtosis along and across the main eigenvector direction (as defined by the diffusion tensor).

The appeal of directly measuring physical properties has prompted the development of models linking the dMRI signal directly to properties of tissue, such as the axon diameter, neurite density (ND) and orientation dispersion (OD)[13,18-20]. Such models are making assumptions to capture the influence of restriction and hindrance on water movement. The non-monoexponential decay

of the diffusion signal at high b values suggests the presence of more than one water compartment with differing diffusion behaviours. In order to capture this compartmentalisation, quite a substantial number of models have been proposed over the years as shown by Ferizi et al.[10], who have compared them head to head thanks to a rich dataset acquired with many diffusion weightings. Three compartment models seem to describe the signal decay at best and fit the data considerably better than the DT model.

Multiple Sclerosis: a quick overview

MS is an inflammatory condition affecting the CNS, and constitutes the greatest cause of non-traumatic disability in young adults in the Western world.

The first MS episode is referred to as clinical isolated syndrome (CIS). Forty to sixty percent of patients presenting with CIS develop a clinically definite MS over the course of the years[21]. The most common MS phenotype is relapsing-remitting (RR) MS, characterized by recurring episodes of inflammation and demyelination (relapses) affecting one or more domains, including for example motor, visual and cognitive systems unpredictably. During a relapse typically the patient experiences acute symptoms, followed by complete or partial recovery. In the majority of patients, the relapses eventually become difficult to distinguish from each other, with a less clear recovery, and the patient enters a phase of progressively accumulating disability, referred to as secondary progressive

multiple sclerosis (SPMS). In 10-15% of patients the clinical course is progressive from the onset and is referred to as primary progressive MS (PPMS).

From a pathological point of view, MS is characterized by the presence of confluent demyelinated lesions in the white and grey matter of the brain, optic nerve and spinal cord. These lesions are caused by immune cell infiltration across the blood–brain barrier (BBB) that promotes inflammation, demyelination, gliosis and neuroaxonal degeneration, leading to disruption of neuronal signalling. Demyelinated areas in the white matter can be partially repaired by remyelination, although histological evidence of this process shows that the myelin sheath does not recover its original structural properties (e.g. it is characterized by thinner layers and a redistribution of the sodium channels along the axonal membrane)[22]. While white matter lesions are visible on conventional T2-weighetd MRI, and BBB leakage can be detected with gadolinium-enhanced T1-weighetd MRI, the correlation between lesion volume and physical disability is moderate in RRMS, and modest at best in the progressive forms due to the complexity of the pathophysiology of MS[23,24]. In addition, subtle damage is known to occur outside of visible lesions, in the so-called normal-appearing white matter (NAWM) and grey matter (NAGM)[25]. This damage is primarily characterised by neuro-axonal loss, which ultimately results in impaired connectivity between different areas of the CNS and can explain patient disability better than macroscopic demyelinated lesions, particularly in SPMS[26].

It has been shown in several studies that inflammation, demyelination and axonal loss, while linked to each other, can also happen independently in MS[27]. Damage can also happen in white matter and grey matter separately, where grey matter atrophy may not be consistent with grey matter demyelination[28]. Given the complexity of MS pathology, we believe that MS offers a good model for investigating whether dMRI can provide added value by offering quantitative indices to disentangle the mechanism of this disease, not captured by conventional imaging. Proposing novel imaging biomarkers at different scales, dMRI could potentially be more specific to the pathophysiological substrate of MS, while also being more sensitive to disease progression.

Microscopic scale: insights about localised pathology

DMRI offers a variety of tissue models that can be used to assess tissue microstructure, from the simplistic DTI model, to more complex mathematical representations that aim at providing biophysically meaningful indices, to model-free approaches requiring specialised acquisition schemes. A fundamental step required for any of the indices derived from these models to be adopted in clinical studies is that of validation, which can be achieved through simulations, animal models, post-mortem tissue and histological comparison. Here we attempt an overview of this enormous ever-growing field in order to provide the added value and limitations of such methods in MS.

MS was one of the first clinical applications of dMRI, and more than 300 MS papers based on DTI have been published to date. The main outcomes can be summarised as follow: DTI parameters are abnormal within MS lesions, with typically increased diffusivity (mean, radial, and axial) and reduced anisotropy

compared to the normal appearing white matter (NAWM)[29,30]. Examples of DTI indices in a patient with MS are shown in figure 1. These results are consistent with increased water content, loss of myelin and axons, and the presence of gliosis. Also, DTI parameters are sensitive to the substrate of lesional damage, as demonstrated by the large variability of DTI indices within lesions[31]. More interestingly, abnormal DTI parameter values are typically found in the NAWM of patients compared to age-matched healthy controls, consistent with subtle but widespread damage known to occur in MS. These initial findings have contributed to establish that white matter damage is widespread in MS even in the early phases, although they did not provide a clear definition of the substrate underpinning these abnormalities. The results obtained in the grey matter (GM) are more intriguing, particularly those obtained in the basal ganglia and the thalamus. Ciccarelli et al.[32] were the first to report a paradoxical increase in FA in the caudate and putamen of patients with MS compared to controls, along with a reduction in the MD. The authors excluded that these findings could be caused by gliosis, which would have resulted in more disorganization (e.g. reduced anisotropy, increased T2) and attributed them to axonal degeneration due to fiber transection in remote focal MS lesions. Later, other authors reported increase of tissue anisotropy of the basal ganglia and the thalamus in RRMS and SPMS patients[33,34] and its correlation with patients disability scores[33-36] . The inability of MD and FA to distinguish and quantify co-existing inflammation, axon injury and myelin damage, however, limits its usefulness in understanding the mechanisms of MS. In particular, it was shown very early on that very different patterns of microstructural changes can result in the same amount of decreased anisotropy[37]. In order to address this limitation, some authors have looked at the

additional information provided by the single eigenvalues, and in particular of "axial" and "radial" diffusivities[38,39]. While results in animal work supports the interpretation of AD as a marker for axonal injury and dysfunction, and of RD as proxy for myelin injury, generalising this interpretation can be dangerous and lead to the wrong conclusions[40,41].

(figure 1)

As already mentioned, the DTI-derived indices are relatively "crude" and non-specific; they are calculated on the assumption of a Gaussian probability density function for the diffusion of water molecules[14]. Several processes occurring at microstructural level, however, may affect the diffusion signal in unpredictable fashion, and their effect might not be accurately captured by Gaussian models. In this situation, model-free approaches (e.g. QSI and DKI introduced above) are preferable and can give parameters that are associated to biophysical properties of the underlying substrate. For example from QSI it is possible to estimate the full width half maximum (FWHM) of the probability distribution function, which is known to be strictly linked to axonal properties such as diameter[42]. QSI requires a large number of data points, and q values beyond those typically achievable in a clinical protocol. For all these more complex approaches, acquisition time and diffusion scheme requirements are demanding, and not necessarily available on all clinical scanners. For these reasons, only a handful of clinical applications are available, and usually limited to pilot studies, demonstrating the sensitivity of these techniques to pathology. As for all novel imaging biomarkers, these methods require validation against histology. Q-space and DKI were applied in animal models and in post-mortem tissue to support studies in vivo in brain and spinal cord of healthy subjects and patients with MS.

One of the most popular models of multiple sclerosis is experimental allergic encephalomyelitis (EAE), which induces inflammation and demyelination and is mainly induced at spinal cord level. EAE can be caused in different animal species[43], and has been extensively used to study MS.

A q-space analysis of EAE diseased swine spinal cords revealed abnormal displacement, probability and anisotropy values within macroscopic plaques (visible on T2-weighted scans), but also in the NAWM[44]. Direct comparison with histology confirmed that areas of abnormal q-space parameters overlapped with areas of pathology as assessed by hematoxylin and eosin, Luxol fast blue, and neurofilaments stains. However, this study did not provide a clear interpretation of these parameters in terms of myelin, axons or glial cells density.

The first example of in-vivo QSI was applied in the brain of MS patients by Assaf et al. who measured q-space parameters in 13 MS patients and 6 healthy controls, using a region of interest approach. Comparing QSI with DTI, they showed that the former provides the highest sensitivity to subtle pathological changes in the NAWM[45]. Representative parametric maps reproduced from this paper are shown in Figure 2. Results of the study indicated that q-space FWHM was more sensitive to damage in the NAWM than DTI-derived indices (e.g. FA), in mild as well as severe cases of MS. The authors explained these findings as a consequence of the higher sensitivity of high b-value dMRI to the slow diffusing water molecules component of the signal, reflecting myelin integrity in white matter. In the attempt to provide further insight into the substrate of these changes, the same Authors have further investigated NAWM with QSI, confirming

their original findings and showing an association with MRS-derived markers of neuronal integrity[46].

(figure 2 approximately here)

Spinal cord damage often correlates better with disability than brain damage in MS[47], therefore efforts are dedicated to implementing novel imaging biomarkers in this structure too. Despite the difficulty of dMRI of the spinal cord, in vivo QSI investigations have been reported[48-50]. The reason for the success of QSI in the spinal cord is that it is possible to take advantage of the highly symmetric microstructure of spinal cord tissue compared to brain; in fact, in the spinal cord fibers are running parallel to its longitudinal axis, hence the acquisition protocol can be simplified by probing diffusion along just two orthogonal directions (along and across the spinal cord). These studies showed consistent results with brain findings, also indicating that the source of specific clinical symptoms might be explained by different biophysical substrates (i.e. RMSD and P_0)[49].

Among q-space parameters, the diffusion kurtosis, K has received more attention than others in MS, especially because it has been associated with myelination[51], as confirmed by studies in the spinal cord of marmosets with chemically induced demyelination, and in shiverer mice[52,53].

Consistently, recent reports based on DKI have demonstrated abnormal K values in both NAWM and NAGM of patients with MS[54-56]. Metrics along specific white

matter tracts were shown to correlate with neuropsychological measures and evoked potentials[57].

While q-space and DKI offer the added value of increased sensitivity to pathological changes, correlating with some histological features, still they do not provide specific information about their nature. Ideally, more direct measurements of microstructure biomarkers, such as axonal density or radius are desirable. In principle diffusion MRI can be exploited to infer this information, but typically these techniques require prohibitively long acquisition times and specialised equipment. Mathematical models of the behaviour of water diffusion in tissue propose an alternative to DTI and QSI.

The specificity of parameters obtained from such models makes them ideal candidates for investigating the pathophysiology of MS. Nevertheless, to date there are no published papers reporting their application to MS populations, and therefore we can only speculate on their usefulness.

One report using diffusion basis spectrum imaging (DBSI[58]), an approach that models axons as anisotropic diffusion tensors, and cells and extracellular space as isotropic diffusion tensors, demonstrated that each of the parameters derived from DBSI correlated with a distinct histological stain[59] in post-mortem MS brain tissue. Surprisingly, to date, other multi-compartments models developed with clinical scanners in mind, such as Neurite Orientation and Density Imaging (NODDI)[13], have not been reported in vivo in MS yet.

To date, the main contribution of dMRI to the characterisation of MS at the microscopic scale has been to provide biomarkers sensitive to subtle changes,

invisible on conventional imaging. The ability to detect damage to the NAWM of both brain and spinal cord, as well as the ability to differentiate lesions that appear similar on T2-weighted MRI, but that are characterised by different substrates, are a clear advantage of dMRI over conventional imaging methods. Despite their potential added value in terms of microstructural specificity, due to lengthy acquisition times and the need for specialised acquisition and analysis, to date advanced methods have been explored only in small samples.

Mesoscopic scale: insights into specific functional systems

Understanding local properties of tissue microstructure that characterise local tissue alterations due to a disease sometimes may not be enough for explaining functional impairment. Therefore, it is important to place the local disruption within the context of the functional networks it affects as current correlation of clinical scores and conventional MRI, such as lesion load, is unsatisfactory and unspecific[60].

DMRI tractography enables the reconstruction of specific white matter bundles, by estimating the principal direction(s) of diffusion within a voxel. Once a tract has been segmented out with tractography, tract-specific measures such as volume, MD, FA, or other MRI parameters, can be estimated within the tract. This offers the unique opportunity to target pathways associated with specific functional impairments, and focus on clinically eloquent areas. Unsurprisingly, several groups investigating MS have focused on the sensory-motor system. Most studies included a quantification of tissue damage along the cortico-spinal tract

(CST) as well as of the primary motor cortex (the latter usually obtained through estimation of atrophy or cortical thickness)[61-63]. MD, AD and RD of the CST were consistently found to be increased in MS patients compared to healthy controls, and associated to measures of functional impairment, such as the timed-walk test or the pyramidal functional system of the expanded disability status scale (EDSS) [62,63]. FA was less significantly associated with disability[62]. One study that recruited patients with lesions within the CST showed that the surface area of the paracentral cortex was inversely correlated to the CST connectivity (estimated by probabilistic tractography) on the affected side, while the opposite was true for the unaffected side[64]. These results suggest the intriguing hypothesis that a lesion in the CST may cause plastic changes to the morphology of the primary motor cortex.

A more sophisticated approach for studying functional systems relies on the use of graph theory[6]. According to this framework, each relevant grey matter area acts as a "node", with various nodes connected by edges. The edges are the white matter connections and can be characterised by dMRI tractography. Topological scores can be used to evaluate measures of integration/segregation and efficiency of the network. Pardini et al.[65] used graph theory to quantify a motor network efficiency score based on FA, tissue volume, and magnetization transfer ratio (MTR, proportional to myelin content[66,67]), which was shown to explain 58% of the variation in EDSS in a group of 71 patients with MS.
Other pathways have been studied with similar methodology, including the cingulum, with both its sections being associated with symptoms such as fatigue[68], and episodic memory and speed of processing[69].

This network approach is particularly interesting as it may help to clarify the frequent paradoxical observation of severe clinical symptoms against radiological evidence of relatively spared brain tissue[60] A speculative explanation could be that when each single pathway within a functional system is only subtly affected by MS pathology (which could range from demyelination, to axonal loss or inflammation), the functional system impairment emerges only when the pathways alterations are combined at network level, ultimately resulting in dysfunction. In other words, the degree of damage of each edge is too modest to result in a significant difference with respect to healthy controls (Figure 3); however, if each single "edge" of the network is affected, graph metrics may be able to capture the abnormalities. This approach comes at the price of losing information about precise localisation of specific tissue damage.

(Figure 3 approximately here)

The added value of studying meso-scale properties of specific functional systems to capture impairment must be weighted against the well-known pitfalls of tractography[70], which make an unsupervised use of such technique in the clinical setting challenging.

Macroscopic scale: the brain as an integrated system

The concept of white matter as the wiring that connects distinct functional areas can be generalised to describe the whole brain as a complex network. DMRI is currently the only technique able to map anatomical connectivity non-invasively,

with the aim of defining the so-called "structural connectome"[71]. Examining the brain as an integrated network can provide new insights about large-scale neuronal communication and provides a platform to understand how brain connectivity relates to human behaviour, and how it may be altered in disease. This approach to investigating the brain is at the opposite end of the microscopic localised scale, far from closing the gap between imaging metrics and microscopy, but it is essential to understanding functional impairment and functional reserve.[72,73]

A whole-brain network can be defined similarly to the system networks introduced above at the mescoscopic scale. It can be seen as an ensemble of neuronal elements, the "nodes", between which some pairwise relationships ("links" or "edges") can be defined. In the case of the structural connectome, typically the nodes are defined through the parcellation of the whole grey matter[74] based on anatomical[75], functional, or cyto-architectonic criteria[76]. Random parcels have also been used[77], as well as data-driven approaches[78]. The links between these nodes are defined by the white matter connections, which are reconstructed by diffusion tractography. The links can be weighted using the number of reconstructed streamlines[79], tract-specific dMRI indices[80], or microstructural properties derived from other quantitative MRI techniques[81]. As for specific networks, graph theory provides a theoretical framework in which the topology of the network can be examined[6]. The power of this approach is in its ability to reduce a very complex structure into a handful of easily treatable summary measures. This is an emerging field that promise to complement more traditional approaches of investigating microscopic local properties.

Two independent studies[82,83] demonstrated that in the early stages of MS the structural connectome is altered while the functional connectome is preserved, providing a new insight in the mechanisms of structural and functional derangement (Figure 4). Interestingly, this sequence of events seems to occur in the opposite direction to neurodegenerative disorders such as Alzheimer disease, where functional changes have been reported to precede structural ones[84,85]. A topological disruption of several sub-networks was also reported in relapsing remitting MS patients[86], with reduced whole-brain network efficiency correlating with disability. These topological features ultimately define the vulnerability or resilience of the network to injuries. Given the impact of white matter damage on the whole brain network efficiency, as demonstrated also in simulation frameworks[87], graph theory provides a powerful tool for exploring the relationship between connectome damage and clinical status. As for the mesoscale approach, this comes at the price of losing the specificity of localisation and biological substrate of the damage. From a methodological point of view, the connectomic approach is still undergoing intense development and lacks of agreement on several technical aspects, including how to optimally parcellate the cortex, how to run the tractography, how to weight the edges of the graph[88] The connectomic approach, though, can capture subtle diffuse changes that spread across several functional and structural systems, independently of the source of damage of affected edges or nodes.

(figure 4 approximately here)

Conclusions:

Current literature is providing a whole new set of techniques that have the potential of revealing the substrates of pathological changes in MS, and more generally in neurological diseases. These tend to be too demanding in terms of image acquisition and analysis to be exploited in large clinical studies, and often are not supported by sufficiently strong evidence of validity. Recent software and hardware developments, such as the development of fast acquisition schemes[89] and the availability of increasingly strong magnetic field gradients[90], are likely to facilitate the clinical adoption of dMRI.

From a microscopic point of view, while models are providing indices potentially specific to a microstructural characteristic, such as axonal density, in reality there is a coexistence of substrates influencing each other.

Despite its current limitations, dMRI provides the added value of some level of multi-modal investigation of pathology (by providing a range of biologically meaningful indices). At the same time dMRI also offers the unique opportunity of interrogating damage from a multi-scale approach by combining microscopic, mesoscopic and macroscopic information.

Clinically, if all pitfalls were to be overcome, dMRI could have the potential of providing complementary information to conventional imaging and clinical data for increasing patient specificity and therefore influencing clinical care.

References

1. LeBihan D. IVIM method measures diffusion and perfusion. Diagnostic imaging 1990;12:133, 6.
2. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. Journal of magnetic resonance Series B 1996;111:209-19.
3. Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G. Diffusion tensor MR imaging of the human brain. Radiology 1996;201:637-48.
4. Conturo TE, Lori NF, Cull TS, et al. Tracking neuronal fiber pathways in the living human brain. Proceedings of the National Academy of Sciences of the United States of America 1999;96:10422-7.
5. Clayden JD. Imaging connectivity: MRI and the structural networks of the brain. Functional neurology 2013;28:197-203.
6. Sporns O. Structure and function of complex brain networks. Dialogues in clinical neuroscience 2013;15:247-62.
7. Ramnani N, Behrens TE, Penny W, Matthews PM. New approaches for exploring anatomical and functional connectivity in the human brain. Biological psychiatry 2004;56:613-9.
8. Lutsep HL, Albers GW, DeCrespigny A, Kamat GN, Marks MP, Moseley ME. Clinical utility of diffusion-weighted magnetic resonance imaging in the assessment of ischemic stroke. Annals of neurology 1997;41:574-80.
9. Moseley ME, Butts K, Yenari MA, Marks M, de Crespigny A. Clinical aspects of DWI. NMR in biomedicine 1995;8:387-96.
10. Ferizi U, Schneider T, Panagiotaki E, et al. A Ranking of Diffusion MRI Compartment Models with In Vivo Human Brain Data. Magn Reson Med 2014;72:1785-92.
11. Cohen Y, Assaf Y. Extracting Geometric Properties of White Matter with q-space Diffusion MRI (QSI). In: Jones DK, ed. Diffusion MRI: Theory, Methods and Applications. Oxford, Englan: Oxford University Press; 2011.
12. Wheeler-Kingshott CA, Barker GJ, Steens CA, van Buchem MA. D: the diffusion of Water. In: Tofts P, ed. Quantiative MRI of the Brain. Chichester, Englansd: Wiley; 2003.
13. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. NeuroImage 2012;61:1000-16.
14. Stejskal EO, Tanner JE. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient. J Chem Phys 1965;42:288-+.
15. Assaf Y, Cohen Y. Structural information in neuronal tissue as revealed by q-space diffusion NMR spectroscopy of metabolites in bovine optic nerve. NMR in biomedicine 1999;12:335-44.
16. Callaghan PT, Macgowan D, Packer KJ, Zelaya FO. High-Resolution Q-Space Imaging in Porous Structures. J Magn Reson 1990;90:177-82.
17. Jensen JH, Helpern JA, Ramani A, Lu H, Kaczynski K. Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. Magnetic resonance in medicine 2005;53:1432-40.

18. Jespersen SN, Bjarkam CR, Nyengaard JR, et al. Neurite density from magnetic resonance diffusion measurements at ultrahigh field: comparison with light microscopy and electron microscopy. NeuroImage 2010;49:205-16.
19. Alexander DC, Hubbard PL, Hall MG, et al. Orientationally invariant indices of axon diameter and density from diffusion MRI. NeuroImage 2010;52:1374-89.
20. Assaf Y, Blumenfeld-Katzir T, Yovel Y, Basser PJ. AxCaliber: a method for measuring axon diameter distribution from diffusion MRI. Magnetic resonance in medicine 2008;59:1347-54.
21. Spelman T, Meyniel C, Rojas JI, et al. Quantifying risk of early relapse in patients with first demyelinating events: Prediction in clinical practice. Multiple sclerosis 2016.
22. Stadelmann C, Bruck W. Interplay between mechanisms of damage and repair in multiple sclerosis. Journal of neurology 2008;255 Suppl 1:12-8.
23. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. The Lancet Neurology 2015;14:183-93.
24. Dutta R, Trapp BD. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Progress in neurobiology 2011;93:1-12.
25. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain : a journal of neurology 2005;128:2705-12.
26. Vrenken H, Seewann A, Knol DL, Polman CH, Barkhof F, Geurts JJ. Diffusely abnormal white matter in progressive multiple sclerosis: in vivo quantitative MR imaging characterization and comparison between disease types. AJNR American journal of neuroradiology 2010;31:541-8.
27. Bodini B, Khaleeli Z, Cercignani M, Miller DH, Thompson AJ, Ciccarelli O. Exploring the relationship between white matter and gray matter damage in early primary progressive multiple sclerosis: an in vivo study with TBSS and VBM. Human brain mapping 2009;30:2852-61.
28. Mallik S, Muhlert N, Samson RS, et al. Regional patterns of grey matter atrophy and magnetisation transfer ratio abnormalities in multiple sclerosis clinical subgroups: a voxel-based analysis study. Multiple sclerosis 2015;21:423-32.
29. Filippi M, Iannucci G, Cercignani M, Rocca MA, Pratesi A, Comi G. A quantitative study of water diffusion in multiple sclerosis lesions and normal-appearing white matter using echo-planar imaging. Arch Neurol-Chicago 2000;57:1017-21.
30. Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis. Neurology 1999;52:1626-32.
31. Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G. Diffusion tensor magnetic resonance imaging in multiple sclerosis. Neurology 2001;56:304-11.
32. Ciccarelli O, Werring DJ, Wheeler-Kingshott CAM, et al. Investigation of MS normal-appearing brain using diffusion tenser MRI with clinical correlations. Neurology 2001;56:926-33.
33. Cavallari M, Ceccarelli A, Wang GY, et al. Microstructural Changes in the Striatum and Their Impact on Motor and Neuropsychological Performance in Patients with Multiple Sclerosis. Plos One 2014;9.

34. Hannoun S, Durand-Dubief F, Confavreux C, et al. Diffusion Tensor-MRI Evidence for Extra-Axonal Neuronal Degeneration in Caudate and Thalamic Nuclei of Patients with Multiple Sclerosis. Am J Neuroradiol 2012;33:1363-8.
35. Calabrese M, Rinaldi F, Seppi D, et al. Cortical Diffusion-Tensor Imaging Abnormalities in Multiple Sclerosis: A 3-year Longitudinal Study. Radiology 2011;261:891-8.
36. Haider L, Simeonidou C, Steinberger G, et al. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. J Neurol Neurosur Ps 2014;85:1386-95.
37. Beaulieu C. The Biological Basis of Diffusion Anisotropy. Diffusion Mri: From Quantitative Measurement to in Vivo Neuroanatomy 2009:105-26.
38. Klawiter EC, Schmidt RE, Trinkaus K, et al. Radial diffusivity predicts demyelination in ex vivo multiple sclerosis spinal cords. NeuroImage 2011;55:1454-60.
39. Song SK, Yoshino J, Le TQ, et al. Demyelination increases radial diffusivity in corpus callosum of mouse brain. NeuroImage 2005;26:132-40.
40. Wheeler-Kingshott CA, Cercignani M. About "axial" and "radial" diffusivities. Magn Reson Med 2009;61:1255-60.
41. Wheeler-Kingshott CA, Ciccarelli O, Schneider T, Alexander DC, Cercignani M. A new approach to structural integrity assessment based on axial and radial diffusivities. Functional neurology 2012;27:85-90.
42. Ong HH, Wright AC, Wehrli SL, et al. Indirect measurement of regional axon diameter in excised mouse spinal cord with q-space imaging: simulation and experimental studies. NeuroImage 2008;40:1619-32.
43. Steinman L. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. Neuron 1999;24:511-4.
44. Biton IE, Mayk A, Kidron D, Assaf Y, Cohen Y. Improved detectability of experimental allergic encephalomyelitis in excised swine spinal cords by high b-value q-space DWI. Experimental neurology 2005;195:437-46.
45. Assaf Y, Ben-Bashat D, Chapman J, et al. High b-value q-space analyzed diffusion-weighted MRI: Application to multiple sclerosis. Magn Reson Med 2002;47:115-26.
46. Assaf Y, Chapman J, Ben-Bashat D, et al. White matter changes in multiple sclerosis: correlation of q-space diffusion MRI and 1H MRS. Magnetic resonance imaging 2005;23:703-10.
47. Lin X, Tench CR, Evangelou N, Jaspan T, Constantinescu CS. Measurement of spinal cord atrophy in multiple sclerosis. Journal of neuroimaging : official journal of the American Society of Neuroimaging 2004;14:20S-6S.
48. Farrell JA, Smith SA, Gordon-Lipkin EM, Reich DS, Calabresi PA, van Zijl PC. High b-value q-space diffusion-weighted MRI of the human cervical spinal cord in vivo: feasibility and application to multiple sclerosis. Magn Reson Med 2008;59:1079-89.
49. Abdel-Aziz K, Schneider T, Solanky BS, et al. Evidence for early neurodegeneration in the cervical cord of patients with primary progressive multiple sclerosis. Brain : a journal of neurology 2015;138:1568-82.
50. Assaf Y, Mayk A, Cohen Y. Displacement imaging of spinal cord using q-space diffusion-weighted MRI. Magn Reson Med 2000;44:713-22.

51. Nossin-Manor R, Duvdevani R, Cohen Y. q-Space high b value diffusion MRI of hemi-crush in rat spinal cord: evidence for spontaneous regeneration. Magnetic resonance imaging 2002;20:231-41.
52. Fujiyoshi K, Hikishima K, Nakahara J, et al. Application of q-Space Diffusion MRI for the Visualization of White Matter. The Journal of neuroscience : the official journal of the Society for Neuroscience 2016;36:2796-808.
53. Tanikawa M, Nakahara J, Hata J, et al. q-Space Myelin Map imaging for longitudinal analysis of demyelination and remyelination in multiple sclerosis patients treated with fingolimod: A preliminary study. Journal of the neurological sciences 2017;373:352-7.
54. de Kouchkovsky I, Fieremans E, Fleysher L, Herbert J, Grossman RI, Inglese M. Quantification of normal-appearing white matter tract integrity in multiple sclerosis: a diffusion kurtosis imaging study. Journal of neurology 2016;263:1146-55.
55. Yoshida M, Hori M, Yokoyama K, et al. Diffusional kurtosis imaging of normal-appearing white matter in multiple sclerosis: preliminary clinical experience. Japanese journal of radiology 2013;31:50-5.
56. Qian W, Chan KH, Hui ES, Lee CY, Hu Y, Mak HK. Application of diffusional kurtosis imaging to detect occult brain damage in multiple sclerosis and neuromyelitis optica. NMR in biomedicine 2016;29:1536-45.
57. Takemura MY, Hori M, Yokoyama K, et al. Alterations of the optic pathway between unilateral and bilateral optic nerve damage in multiple sclerosis as revealed by the combined use of advanced diffusion kurtosis imaging and visual evoked potentials. Magnetic resonance imaging 2016;39:24-30.
58. Wang Y, Wang Q, Haldar JP, et al. Quantification of increased cellularity during inflammatory demyelination. Brain : a journal of neurology 2011;134:3590-601.
59. Wang Y, Sun P, Wang Q, et al. Differentiation and quantification of inflammation, demyelination and axon injury or loss in multiple sclerosis. Brain : a journal of neurology 2015;138:1223-38.
60. Barkhof F. The clinico-radiological paradox in multiple sclerosis revisited. Current opinion in neurology 2002;15:239-45.
61. Bergsland N, Lagana MM, Tavazzi E, et al. Corticospinal tract integrity is related to primary motor cortex thinning in relapsing-remitting multiple sclerosis. Multiple sclerosis 2015;21:1771-80.
62. Hubbard EA, Wetter NC, Sutton BP, Pilutti LA, Motl RW. Diffusion tensor imaging of the corticospinal tract and walking performance in multiple sclerosis. Journal of the neurological sciences 2016;363:225-31.
63. Lin X, Tench CR, Morgan PS, Niepel G, Constantinescu CS. 'Importance sampling' in MS: Use of diffusion tensor tractography to quantify pathology related to specific impairment. Journal of the neurological sciences 2005;237:13-9.
64. Gorgoraptis N, Wheeler-Kingshott CA, Jenkins TM, et al. Combining tractography and cortical measures to test system-specific hypotheses in multiple sclerosis. Multiple sclerosis 2010;16:555-65.
65. Pardini M, Yaldizli O, Sethi V, et al. Motor network efficiency and disability in multiple sclerosis. Neurology 2015;85:1115-22.
66. Dousset V. Magnetization transfer imaging in vivo study of normal brain tissues and characterization of multiple sclerosis and experimental allergic

encephalomyelitis lesions. Journal of neuroradiology Journal de neuroradiologie 1993;20:297.
67. Dousset V, Grossman RI, Ramer KN, et al. Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. Radiology 1992;182:483-91.
68. Pardini M, Bonzano L, Bergamino M, et al. Cingulum bundle alterations underlie subjective fatigue in multiple sclerosis. Multiple sclerosis 2015;21:442-7.
69. Koenig KA, Sakaie KE, Lowe MJ, et al. The relationship between cognitive function and high-resolution diffusion tensor MRI of the cingulum bundle in multiple sclerosis. Multiple sclerosis 2015;21:1794-801.
70. Johansen-Berg H, Behrens TE. Just pretty pictures? What diffusion tractography can add in clinical neuroscience. Current opinion in neurology 2006;19:379-85.
71. Sporns O, Tononi G, Kotter R. The human connectome: A structural description of the human brain. PLoS computational biology 2005;1:e42.
72. Campbell J, Langdon D, Cercignani M, Rashid W. A Randomised Controlled Trial of Efficacy of Cognitive Rehabilitation in Multiple Sclerosis: A Cognitive, Behavioural, and MRI Study. Neural plasticity 2016;2016:4292585.
73. Bonavita S, Sacco R, Della Corte M, et al. Computer-aided cognitive rehabilitation improves cognitive performances and induces brain functional connectivity changes in relapsing remitting multiple sclerosis patients: an exploratory study. Journal of neurology 2015;262:91-100.
74. de Reus MA, Van den Heuvel MP. The parcellation-based connectome: Limitations and extensions. NeuroImage 2013;80:397-404.
75. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 2002;15:273-89.
76. Scholtens LH, de Reus MA, de Lange SC, Schmidt R, van den Heuvel MP. An MRI Von Economo - Koskinas atlas. NeuroImage 2016.
77. Hagmann P, Cammoun L, Gigandet X, et al. Mapping the structural core of human cerebral cortex. PLoS biology 2008;6:e159.
78. Craddock RC, James GA, Holtzheimer PE, 3rd, Hu XP, Mayberg HS. A whole brain fMRI atlas generated via spatially constrained spectral clustering. Human brain mapping 2012;33:1914-28.
79. van den Heuvel MP, Sporns O, Collin G, et al. Abnormal rich club organization and functional brain dynamics in schizophrenia. JAMA psychiatry 2013;70:783-92.
80. van den Heuvel MP, Mandl RC, Stam CJ, Kahn RS, Hulshoff Pol HE. Aberrant frontal and temporal complex network structure in schizophrenia: a graph theoretical analysis. The Journal of neuroscience : the official journal of the Society for Neuroscience 2010;30:15915-26.
81. Lemkaddem A, Daducci A, Kunz N, et al. Connectivity and tissue microstructural alterations in right and left temporal lobe epilepsy revealed by diffusion spectrum imaging. NeuroImage Clinical 2014;5:349-58.
82. Shu N, Duan Y, Xia M, et al. Disrupted topological organization of structural and functional brain connectomes in clinically isolated syndrome and multiple sclerosis. Scientific reports 2016;6:29383.

83. Romascano D, Meskaldji DE, Bonnier G, et al. Multicontrast connectometry: a new tool to assess cerebellum alterations in early relapsing-remitting multiple sclerosis. Human brain mapping 2015;36:1609-19.
84. Gili T, Cercignani M, Serra L, et al. Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. J Neurol Neurosur Ps 2011;82:58-66.
85. Palesi F, Castellazzi G, Casiraghi L, et al. Exploring Patterns of Alteration in Alzheimer's Disease Brain Networks: A Combined Structural and Functional Connectomics Analysis. Frontiers in neuroscience 2016;10:380.
86. Shu N, Liu Y, Li K, et al. Diffusion tensor tractography reveals disrupted topological efficiency in white matter structural networks in multiple sclerosis. Cerebral cortex 2011;21:2565-77.
87. Aerts H, Fias W, Caeyenberghs K, Marinazzo D. Brain networks under attack: robustness properties and the impact of lesions. Brain : a journal of neurology 2016;139:3063-83.
88. Fornito A. Graph Theoretic Analysis of Human Brain Networks. Neuromethods 2016;119:283-314.
89. Moeller S, Yacoub E, Olman CA, et al. Multiband multislice GE-EPI at 7 tesla, with 16-fold acceleration using partial parallel imaging with application to high spatial and temporal whole-brain fMRI. Magnetic resonance in medicine 2010;63:1144-53.
90. Fan Q, Nummenmaa A, Witzel T, et al. Investigating the capability to resolve complex white matter structures with high b-value diffusion magnetic resonance imaging on the MGH-USC Connectom scanner. Brain connectivity 2014;4:718-26.

Figure Legends

Figure 1. DTI maps in a patient with MS. (A) FLAIR scan showing the distribution of macroscopic lesions: (B) Mean diffusivity; (C) Radial diffusivity; (D) Fractional anisotropy. Note the diversity in the lesional tissue appearance in different maps. The red arrows point at the same lesion on the 3 maps.

Figure 2. In-vivo q-space imaging in a patient with MS. (a) q-space probability (zero-filled), (b) q-space displacement (zero-filled), (c) Fractional anisotropy , (d) FLAIR, and (e) T1-weighted images. Reproduced from Assaf et al., 2002[45] with permission from John Wiley &Sons, Inc.

Figure 3. Sketch of potential multiple damage of different sources affecting network edges in MS. (a) Fully connected healthy network. A, B, C, D are the nodes and ab, ac, ad, bc, cd and bd are edges that can be characterised by different weights and properties. (b) Fully connected network attacked by several disease processes. Edges are either healthy or damaged to different extents. Changes at edge and node level will have a cumulative effect on the overall properties of the network. Graph metrics may be able to capture differences between the healthy and damaged networks, at the price of loosing the local specificity of tissue alterations.

Figure 4. Whole-brain network comparison between patients with clinically isolated syndrome (CIS), patients with MS, and healthy controls (NC). The node sizes indicate the significance of between-group differences in

the regional efficiency. (**A**) For the structural network (SC), nodes in blue showed reduced efficiency in CIS and MS patients compared with controls, and decreased efficiency in MS compared with CIS. (**B**) For the functional network (FC), nodes in red showed increased efficiency in CIS compared with controls and nodes in blue showed decreased efficiency in MS compared with CIS or controls. Reproduced from Shu et al., 2016[82] with permission from Nature Publishing Group.