ABSTRACT

Multiple sclerosis (MS), a demyelinating disease, occurs principally in the white matter (WM) of the central nervous system. Conventional magnetic resonance imaging (MRI) is sensitive to some, but not all, brain changes associated with MS. Diffusion-weighted imaging (DWI) provides information about water diffusion in tissue and diffusion tensor MRI (DT-MRI) about fiber direction, allowing for the identification of WM abnormalities that are not apparent on conventional MRI images. These techniques can quantitatively characterize the local microstructure of tissues. MS-associated disease processes lead to regions characterized by an increased amount of water diffusion and a decrease in the anisotropy of diffusion direction. These changes have been found to produce different patterns in MS patients presenting different courses of the disease. Changes in water diffusion may allow examination of the type, appearance, enhancement, and location of lesions not readily visible by other means. Ongoing studies of MS are integrating conventional MRI and DT-MRI measures with connectivity-based regional assessment, aiming to provide a better understanding of the nature and the location of WM lesions. This integration and the development of novel image-processing and visualization techniques may improve the understanding of WM architecture and its disruption in MS. This article presents a brief history of DWI, its basic principles and applications in the study of MS, a review of the properties and applications of DT-MRI, and their use in the study of MS. In addition, this article illustrates the methodology for the analysis of DT-MRI in ongoing studies of MS.

Key words: Diffusion-weighted imaging, diffusion tensor, DT-MRI, multiple sclerosis, magnetic resonance imaging, diffusivity, lesion, tractography.

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Multiple sclerosis (MS) is a demyelinating disease occurring in the white matter (WM) and gray matter (GM) of the central nervous system. In conventional magnetic resonance imaging (MRI), MS lesions located in the WM produce a hyperintense signal in both proton density and T2-weighted images, while the hypointense T1-weighted lesions are considered to be chronic.1

In MS patients, WM may be disrupted in areas not apparent on conventional T2-weighted MRI. Abnormalities of normal-appearing WM (NAWM) on T2-weighted MRI have been detected using magnetization transfer imaging (MTI),2 4 diffusion-weighted imaging (DWI),5 9 diffusion tensor MRI (DT-MRI),6 10 13 and magnetic resonance spectroscopy (MRS).14 16 (For separate, detailed discussions of MTI and MRS, please see the accompanying articles in this supplement.4 16)

In general, MS patients present an increased amount of water diffusion and a decreased anisotropy of diffusion direction in the region of the lesions, in the surrounding lesion tissue, and in the remote NAWM. These changes are believed to be the result of either damage and removal of highly aligned cellular structures or replacement of axonal fibers with amorphous cells5 9 17 and are apparently dependent on the clinical course of the patient.

The correlation between WM lesion burden in MRI and clinical outcome measures is significant but not strong.19 WM lesion burden is typically measured over the entire brain, which may underestimate the significance of the underlying connectivity of WM and the...
cumulative effects on WM damage in functionally eloquent WM tracts. 

Recent advances in DT-MRI and image-processing techniques are providing the image acquisition and visualization technology to enable in vivo assessment of the WM architecture of the human brain. These technologies will enable future studies of the relationship between WM disruption, WM connectivity, and clinical measures and will ultimately lead to improved monitoring of patients, better prediction of the course of the disease, and more rapid assessment of new treatments or therapies. (For separate, detailed discussions of image-processing techniques, please see the accompanying article in this supplement 21)

This article presents a brief history of DWI and its basic principles and applications in the study of MS, followed by a review of the properties and applications of DT-MRI and its use in the study of MS. In addition, it presents a proposed methodology for the analysis of DT-MRI in ongoing studies of MS.

**Diffusion-Weighted Imaging**

**Brief History**

DWI allows quantitative measurement of the molecular motion of water. DWI is based on the continuous agitation of minute suspended particles, which is a phenomenon known as Brownian movement, named after Robert Brown, who observed the constant movement of pollen grains in 1827. Brown suspended some of the pollen grains in water and examined them closely, only to see them “filled with particles” that were very evidently in motion.” He was soon satisfied that the movement “arose neither from currents in the fluid, nor from its gradual evaporation, but belonged to the particle itself.” The kinetic force in Brownian movement is directly related to particle size, and the vector of the force that gives rise to the movement is not consistent, nor does it result in motion in a specific direction. Through his investigation of Brownian movement (frequently referred to as Brownian motion), Albert Einstein showed that according to the molecular-kinetic theory of heat, bodies of microscopically visible size suspended in a liquid perform movements of such magnitude that they can be easily observed using a microscope, on account of the molecular motions of heat. This theoretical formulation was also independently derived by Sutherland, and it has been referred to as the Sutherland-Einstein equation. This analysis of Brownian motion led to the formulation of the Boltzmann constant.

Early nuclear magnetic resonance (NMR) efforts to observe diffusion were performed by Stejskal and Tanner. They derived the effect of a time-dependent magnetic gradient on the spin-echo experiment, particularly in the presence of spin diffusion. In 1986, Le Bihan et al developed an MRI technique to observe intravoxel incoherent motions resulting from the distribution of phases in a single voxel when appropriate magnetic field gradient pulses are applied. They studied the diffusion coefficients measured on images of water and acetone phantoms. They also analyzed neurological images from healthy controls and patients by estimating the amount of water diffusion in tissue by means of the apparent diffusion coefficient (ADC) and found differences between various normal and pathologic tissues. ADC is a scalar measure that reflects the amount of apparent diffusivity in a particular direction. One of the first and most widely used clinical applications of DWI is the evaluation of brain stroke (ischemia). DWI is the most sensitive method for detecting acute ischemia in vivo, allowing for the distinction between old and new strokes and helping to differentiate early stroke from other focal brain processes, which is not possible with conventional MRI.

**Basic Principles**

The measurement and characterization of water diffusion in tissues is based on the quantification of the random motion of water molecules in tissues. This random motion provides microscopic in vivo information about tissue architecture that is not provided by conventional MRI. In pure water, individual molecules present a constant random motion in all directions, but in the environment of brain tissue, this random motion is restricted in different ways. The main factors affecting water diffusion are the structural components and temperature of the tissues. Isotropic diffusion occurs largely in tissues with incoherent structures such as those in the brain ventricles and in adult cortical GM, where the apparent diffusion restriction is equal in all directions. Anisotropic diffusion is present in tissues such as those in regions of WM fiber tracts, where water diffusion has a preferred orientation.

In DWI, a single field gradient pulse is applied during image acquisition, providing a quantitative measurement of water diffusion. This quantitative measurement of the diffusion in the gradient direction is determined by the amount of attenuation in the signal resulting from the randomization of the NMR spin phase caused by the diffusion of water molecules. Thus, only diffusion in the direction of the applied gradient can be detected. As the diffusion of water is 3-dimensional (3D), 3 orthogonal measures of direction are required to calculate the mean
diffusivity $\langle \mathbf{D} \rangle$ for each voxel. The bulk $\mathbf{D}$ is the 3D analog of the ADC and is an intrinsic property of the tissues and has no directional dependence. It has also been called trace ADC or mean trace. 28

**DWI in MS**

In the brain’s WM, the mobility of the water is restricted by structures such as myelinated and unmyelinated axons that are oriented along the fiber tracts, and the direction of highest diffusivity coincides with the tissue’s fiber tract axis. 32,33 The pathological elements of MS have the potential to alter the permeability or geometry of structural barriers to molecular diffusion of water in the brain. 7

The first study to use DWI in MS was performed by Larsson et al. 34 They studied acute and chronic MS lesions and found diffusion to be higher in acute plaques compared with chronic plaques, suggesting a probable relation between the degree of demyelination and the increase of extracellular water space. Another study performed by Droogan et al 35 found a higher $\mathbf{D}$ and reduced anisotropy in MS lesions as compared with NAWM, with the highest $\mathbf{D}$ values measured in T1-weighted hypointense and enhanced lesions. A slightly higher $\mathbf{D}$ and reduced anisotropy were also found in the WM of the MS patients as compared with healthy controls. However, no differences in $\mathbf{D}$ between patients with different disease phenotypes were observed, and no correlations with disability were seen. In a study of lesions presenting different patterns of enhancement (eg, nonenhancement, homogeneously enhanced, and ring enhanced), Roychowdhury et al 36 aimed to determine whether the $\mathbf{D}$ pattern corresponded to the MRI findings and whether it accounted for histopathologic characteristics of different lesion types. This correspondence was observed, as all 3 types of lesions had a higher $\mathbf{D}$ than the NAWM. There was also a significant difference in the mean $\mathbf{D}$ between homogeneously enhanced and ring-enhanced as well as between homogeneously enhanced and nonenhanced lesions.

Longitudinal studies have analyzed the changes in diffusion properties in NAWM regions and subsequently presented new enhancing lesions, finding an increasing $\mathbf{D}$ in the prelesion NAWM 37,38 and at the time of lesion enhancement. 32 These observations suggested that alterations in the tissue integrity, such as edema and demyelination, occur before the formation of new MS lesions. Werring et al 37 also observed an increase in $\mathbf{D}$ in matched contralateral NAWM regions at the time of the first noted lesion enhancement, suggesting that structural damage is also caused in connected areas of NAWM. In an additional longitudinal study performed by Caramia et al, 39 $\mathbf{D}$ was monitored to identify changes occurring in the NAWM, the linkage to T2-weighted lesion load, and the correlation with clinical parameters in early MS patients with clinically isolated symptoms. At baseline, they did not find any difference in the $\mathbf{D}$ between patients and healthy controls; after 12 months, the $\mathbf{D}$ in patients was significantly higher and correlated with T2-weighted load. For those patients presenting an increase in $\mathbf{D}$ above a confidence interval, disability status also deteriorated. Similarly, Schmierer et al 40 observed the changes in $\mathbf{D}$ in NAWM in patients with primary-progressive MS (PPMS) over a 1-year period. Serial DWI showed progressive changes in the NAWM in patients with PPMS, with an increment of the $\mathbf{D}$ associated with an increase of the T1-weighted and T2-weighted lesion load. They also found the $\mathbf{D}$ in frontal NAWM to be associated with disability.

In MS, the changes in diffusion are not exclusively located in the WM; they can also be observed in the normal-appearing GM (NAGM). Cercignani et al 41 measured $\mathbf{D}$ in both the NAWM and NAGM in MS patients and found that $\mathbf{D}$ in healthy controls was higher in both NAWM and NAGM than in MS patients. Fabiano et al 42 analyzed the changes in $\mathbf{D}$ in the thalamus of MS patients and observed increased water diffusion, which was partly associated with clinical course, lesion load, and brain atrophy. These results suggest that in MS, subtle changes also occur in the NAGM.

**Diffusion Tensor MRI**

**Estimation of DT From DT-MRI**

DT-MRI, initially proposed by Basser et al 32 differs from DWI in that in DT-MRI, a tensor describing local water diffusion is calculated for each voxel from measurements of diffusion of at least 6 noncollinear, noncoplanar gradients. The tensors $\mathbf{D}$ are then estimated by solving a system of equations with the form 43

$$\ln(S_i) = \ln(S_0) - k \mathbf{g}_k^T \mathbf{D} \mathbf{g}_k,$$

where $k = 1 \ldots n$, in which $n$ is the number of gradients. $S_i$ represents the signal intensities in the presence of diffusion-sensitizing gradients, and $g_k - S_0$ is the baseline signal intensity obtained from the absence of a diffusion-sensitizing field gradient.

Diffusion tensors are often visualized as ellipsoids with the size and shape reflecting the degree of diffusion along each principal axis and may be represented by symmetric $3 \times 3$ matrices. The principal axes correspond to the eigenvectors of the tensor ($e_1$, $e_2$, and $e_3$), and the relative size of each axis is determined by the eigenvalues of the tensor ($\lambda_1$, $\lambda_2$, and $\lambda_3$). 32 An elliptical representation of a tensor is depicted in Figure 1.
Tensor Representation

In DT-MRI, a tensor describes the local water diffusion per voxel. In isotropic diffusion, characteristic of the adult human brain GM and cerebrospinal fluid, the magnitude of the diffusion is equal in all directions, and the corresponding tensor shape is spherical. Anisotropic diffusion, found in the adult human brain WM, is represented by ellipsoids with variable magnitude of their axes. The shape characteristics of this ellipsoid may be summarized with basic geometric measures: (1) linear, when the diffusion is mainly in the direction of the largest eigenvalue ($\lambda_1 > \lambda_2 \approx \lambda_3$); (2) planar, when the diffusion is restricted to a plane spanned by the 2 eigenvectors of the 2 largest eigenvalues ($\lambda_1 = \lambda_2 > \lambda_3$); and (3) spherical, when the diffusion is equal in all directions ($\lambda_1 = \lambda_2 = \lambda_3$). By using the largest eigenvalues of the tensor, the linear, planar, and spherical measures can be obtained by, respectively,

$$c_l = \frac{\lambda_1 - \lambda_2}{\lambda_1} \quad (2)$$

$$c_p = \frac{\lambda_2 - \lambda_3}{\lambda_1} \quad (3)$$

$$c_s = \frac{\lambda_3}{\lambda_1} \quad (4)$$

where $c_l$, $c_p$, and $c_s$ lie in the range $[0, 1]$ and their sum is equal to 1.

Scalar Measurements

The 2 primary measurements derived from DT-MRI and its tensor representation, based on the normalized variance of the eigenvalues, include (1) the bulk mean diffusivity, a measure of the amount of water diffusion in tissue, which is equal to one third of the trace of the diffusion tensor:

$$D = \frac{\text{Trace}(D)}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (5)$$

and (2) the fractional anisotropy (FA), a measure of the anisotropy of diffusion direction,

$$FA = \frac{1}{\sqrt{2}} \left(\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_1 - \lambda_3)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}\right) \quad (6)$$

Tractography

Tensor visualization allows for representation of the information contained in DT-MRI data. This is the first step for the construction of fiber tracts. The eigenvector corresponding to the largest eigenvalue indicates the primary orientation of the local brain structure; therefore, it is possible to assess a bulk average of the in vivo axonal connectivity using DT-MRI. The in-plane component of the measured fiber direction is usually represented by headless arrows, with the length of the arrows being proportional to the relative anisotropy and orientation indicated by color coding. DT-MRI may be used to delineate WM fiber tracts and thus construct 3D tracts, traces of the pathways representing WM fiber tracts by means of connected diffusion tensors. Mori et al constructed such representations by starting from a seed pixel and stepping along a line in both the retrograde and orthograde directions according to the largest eigenvector at each pixel. Different criteria were defined to determine when and where to terminate the tracking procedure and hence to identify particular WM pathways of interest. The tracking was performed for every pixel inside the brain, and only those fibers that penetrated previously defined regions of interest were retained. Wakana et al applied the same technique to reconstruct the 3D trajectories of 17 prominent WM tracts. These tracts were superimposed on coregistered anatomic MR images, and parcellation maps of the WM were created, which were later coregistered to DT-imaging color maps to assign visible structures.

Recent work has been carried out in the construction of intersubject atlases from DT-MRI data. Jones et al described spatial averaging of scans from 10 healthy adults and demonstrated success in bringing fiber tracts into correspondence. Mori et al successfully extracted certain corresponding fiber tracts and then detected a different pattern of anisotropy along a certain tract in an adrenoleukodystrophy patient as compared to the...
healthy individuals. The feasibility of selection and extraction of specific tracts from scans has also been recently demonstrated,\textsuperscript{55} as shown also in a study of pyramidal tracts in MS patients.\textsuperscript{18} Average DT-MRI atlases have been constructed by aligning the DT-MRI data using a nonlinear registration approach, which uses all the components of the tensor.\textsuperscript{56} This enables the topology and morphology representative of a group of patients to be modeled by the combination of the intensity average and the shape average derived from the mean of deformation fields.\textsuperscript{57}

**DT-MRI in MS**

The geometry of diffusion tensors can quantitatively characterize the local structure in tissues. The density of the fibers, the degree of myelination, the average fiber tract diameter, and the directional similarity of the fibers in the voxel all affect the shape of the diffusion ellipsoid.\textsuperscript{43} Investigation of these properties through several ongoing research studies may provide a better understanding of the pathologic processes involved in MS.

DT-MRI–derived metrics have been found to show tissue damage not only in the area of the T1- and T2-weighted lesion but also in the lesion’s surrounding area and in remote NAWM and NAGM as a result of Wallerian degeneration. Kealy et al\textsuperscript{46,59} compared the T2-weighted size of MS lesions with lesion size as defined on FA maps. They found a significantly reduced anisotropy both inside the T2-weighted lesions and in the immediately adjacent NAWM regions, indicating that the real size of the lesions is often substantially greater in DT-MRI than what is seen in conventional MRI. Similarly, Guo et al\textsuperscript{60} found that the anisotropy and the \( \bar{D} \) values were more abnormal in the lesions and their periphery than in more distant regions. A generally increased \( \bar{D} \) and a reduced FA, especially in brain regions normally presenting a high anisotropy, such as the corpus callosum (CC) and the internal capsule,\textsuperscript{61,62} are common changes in the DT-MRI parameters in the NAWM of MS patients. In a more detailed and recent study of the NAWM of the CC of MS patients,\textsuperscript{63} a significantly reduced FA in the anterior and posterior midbody subdivisions of the CC was observed. Compared to healthy controls, almost no changes in the FA of the genu of the CC were found, while the splenium showed an insignificant trend to reduced FA values.

Some studies have investigated the pathologic severity of tissue damage in MS based on the DT-MRI metrics observed in different types of lesions. Werring et al\textsuperscript{44} observed the highest diffusion in destructive T1-weighted hypointense lesions, whereas the greatest change in anisotropy was found in inflammatory contrast-enhanced lesions. Two different studies\textsuperscript{6,45} found that in nonacute enhanced T1-weighted hypointense lesions, the FA was lower than that of T1-weighted isointense lesions. However, significantly higher \( \bar{D} \) and similar \( D \) values\textsuperscript{6} were also observed when comparing enhancing and nonenhancing MS lesions.

DT-MRI also has been used with the aim of studying and characterizing the damage caused by MS in its different stages and phenotypes. A study comparing early-onset MS (mean disease duration <1.5 years) in young patients (mean age 14.1 years) with healthy controls with DT-MRI\textsuperscript{64} found only a slight increase in \( \bar{D} \) of the NAWM. Griffin et al\textsuperscript{65} analyzed the changes in macroscopically normal-appearing brain tissue (NABT) in early relapsing-remitting MS (RRMS) patients (mean disease duration 1.7 years), finding significant differences in FA, \( \bar{D} \), and volume ratio between lesions and NAWM. However, they did not find a significant difference in the NAWM and NAGM between patients and healthy controls, nor did they find any correlation with clinical outcome measures. To determine whether DT-MRI observable disease effects could be detected in early MS (mean disease duration 1.6 years), Rashid et al\textsuperscript{66} analyzed the changes in FA, \( \bar{D} \), and volume ratio in both the NABT and the whole brain tissue, finding only an increased FA. Studying the normal-appearing CC in RRMS patients having a relatively short disease duration of 2.7 years\textsuperscript{67}, revealed that FA and \( \bar{D} \) are more severe in normal-appearing CC regions than in other NAWM regions. These studies suggest that in early-stage disease, the pathological changes are minor or localized in specific brain structures such as the CC.

Studies comparing DT-MRI–derived measures concur that the damage caused by MS is higher in more progressive forms of the disease. Cercignani et al\textsuperscript{68} quantified tissue damage on T2-weighted lesions and NAWM by means of \( \bar{D} \), FA, and intervoxel coherence (\( C \), which represents the degree of similarity of orientation of adjacent voxels) from PPMS, RRMS, and secondary-progressive MS (SPMS) patients. They found that the CC of SPMS patients had a higher \( \bar{D} \) and a lower FA and C than did patients with either RRMS or PPMS. SPMS patients also had a higher average lesion \( D \) than both RRMS and PPMS did. Similar results were obtained by Rovaris et al\textsuperscript{69} for both NAWM and NAGM, with a significantly different \( \bar{D} \) and not significantly different FA when comparing SPMS to PPMS patients. In another study quantifying the extent of GM damage as seen on DT-MRI maps of RRMS, PPMS, and SPMS patients,\textsuperscript{70} significant differences were found between controls and patients with only the progressive forms of the disease. The authors also found some differences between RRMS and SPMS patients as well as some differences between PPMS and
SPMS patients, confirming GM damage in MS, which is also related to disease progression.

Longitudinal studies have also been carried out using DT-MRI–derived parameters. Cassol et al71 monitored the evolution of trace and of FA in the NAWM of MS patients and found that both trace and FA indicated a recovery after the acute phase in RRMS patients and a progressive shift toward abnormal values in SPMS patients. In another study, over a 1-year period,72 tissue changes beyond the resolution of conventional MRI were detected in the NAGM of patients with progressive MS. These observations indicated that the damage in the GM does not depend only on the T2-weighted lesion load and the reduction of brain volume and may be an additional result of accumulated disability in progressive MS.

The microscopic and more localized analysis of tissue damage that is possible with DT-MRI, as well as its capability to detect remote damage in normal-appearing tissue, is allowing some improvement in the correlation of MRI studies with clinical disability. Ciccarelli et al11 observed that the FA and $\bar{D}$ in the cerebral peduncles were inversely correlated with the Expanded Disability Status Scale (EDSS) and pyramidal functional scores. RRMS patients had a strong correlation between the FA and the EDSS in both supra- and infratentorial NAWM, while in PPMS and SPMS patients, disease duration correlated strongly with $\bar{D}$ in infratentorial NAWM and with FA in the cerebral peduncles. In a study of the correlation between cognitive impairment in RRMS patients and DT-MRI,17 no correlation between any of the neuropsychological test scores and brain volume, average lesion FA, and whole brain tissue FA was found. However, moderate correlations were found between neuropsychological scores exploring language, attention, and memory and DT-MRI quantitative metrics such as FA and $\bar{D}$ histograms of whole brain tissue, NABT, NAWM, and NAGM. This seems to reflect the severity of language, attention, and memory deficits in RRMS patients. Using an algorithm to identify specific tracts and measure disease burden within them, Wilson et al18 assessed pyramidal tract damage caused by MS with a measure derived from the relative anisotropy along the tracts. This measurement in the pyramidal tract correlated significantly with both the EDSS and, to a greater extent, with the pyramidal functional system score (FSS). In a similar way, Lin et al20 measured the average $\bar{D}$ ( $\bar{D}_{av}$ ) along the pyramidal tract and CC. They found a correlation between the pyramidal tract $\bar{D}_{av}$ and pyramidal FSS and between CC $\bar{D}_{av}$ and the Paced Auditory Serial Addition Test, a neuropsychological test commonly used on MS patients. They also found that the global, but not the localized, T2-weighted lesion correlated with $\bar{D}_{av}$ of both the pyramidal tract and CC. The results obtained by Wilson et al18 and Lin et al20 show that an increased specificity in monitoring the progression of motor and cognitive impairment in MS may be obtained by matching specific WM tracts with specific clinical scoring systems.

Proposed Methodology for the Analysis of DT-MRI in Ongoing Studies of MS

Image Acquisition

High-resolution MRI was acquired from MS patients and healthy controls using a 3T Signa System (GE Medical Systems, Milwaukee, WI). The acquired sets of images included the following: (1) line scan diffusion images (TR/TE = 93/55 milliseconds, field of view [FOV] = 270 cm, matrix size = 256 $\times$ 256) using a b = 1000 s/mm$^2$, 1 baseline and 6 noncollinear and noncoplanar directions, 60 contiguous 2-mm-thick axial sections for each direction; (2) MPRAGE3D T1-weighted (TR/TE = 8/3.2 milliseconds, inversion preparation time = 725 milliseconds, postdelay time = 1400 milliseconds, FOV = 240 cm, matrix size = 256 $\times$ 256), 124 contiguous 1.3-mm-thick sagittal sections; (3) 3DFSE T2-weighted (TR/TE = 4300/8 milliseconds, FOV = 240 cm, matrix size = 256 $\times$ 256), 160 contiguous 1-mm-thick sagittal sections; and (4) fluid-attenuated inversion recovery (FLAIR; TR/TE = 8000/8 milliseconds, inversion time = 2450 milliseconds, FOV = 240 cm, matrix size = 256 $\times$ 256), 80 contiguous 1.5-mm-thick sagittal sections.

Alignment of Conventional and DT-MRI

Although the T1-weighted, T2-weighted, and FLAIR images were acquired during the same session, slight head movements were expected to occur. Therefore, T2-weighted and FLAIR images were aligned with the T1-weighted images using an ITK implementation (www.itk.org) of a rigid registration algorithm based on the maximization of mutual information.73 Following the rigid registration of conventional MRI, an outline of the intracranial cavity (ICC) masks containing brain parenchyma and cerebrospinal fluid were manually obtained from both the DT-MRI baseline and the T1-weighted MRI. These ICC masks were later applied to the original baseline and T1-weighted sets of images, respectively, to get the segmentation of the ICC. The T1-weighted ICC mask was also used to segment the ICC from the T2-weighted and FLAIR images.

The T1-weighted, T2-weighted, and FLAIR ICCs were then registered onto the baseline ICC using an affine registration algorithm.74 This algorithm computes an optimal alignment using a robust least-squares method to esti-
mate the rigid body or affine transform that best fits a set of local block match calculations.

**Interactive Identification of Particular Fiber Tracts**

Raw data were converted to derive DT information using custom software and were then loaded into 3D slicer (www.slicer.org). The regions of interest (ROIs) were outlined by an expert based on information from the FA map and an additional map, which encodes the direction of the largest eigenvector. This map is known as a color by orientation (CBO) map. The FA map was thresholded to identify regions of anisotropic WM. Based on the CBO map, which allows ready visualization of changes in fiber orientation, the binary label map was segmented into different anatomically defined ROIs. Ambiguities in the CBO map were resolved by seeding single tracts and observing the trajectory.

**Assessment of Disease Burden From DT- and Conventional MRI**

The correlation between conventional MRI-derived measures of MS lesion burden and clinical measures of disease status has not been strong and may be at least in part because most MRI-derived measures ignore the different functional significance of different regions of the WM. (For an in-depth discussion of the clinical–MRI paradox, please see the accompanying article in this supplement.) This could be addressed by defining a specific set of regions of WM fiber tracts as observed with DT-MRI and measuring lesion burden from DT- and conventional MRI that reflect alterations in the WM in those regions.

Several measures are possible, including the minimum (min), maximum (max), and mean ± standard deviation of the FA and ADC, as well as for the linear, planar, and circular measurements from (1) inside the lesion, (2) a peripheral ROI surrounding the lesion, and (3) a “healthy” symmetric area corresponding to the mirror-image reflection across the midline. MS lesions can be identified and segmented by analyzing the previously aligned sets of images (e.g., conventional MRIs registered to the DT-MRI baseline) using established methods.

Beyond single summary measures, distributions of parameters such as the FA along the fiber tracts contained in anatomically defined ROIs may be determined and may reflect the resulting disruption caused by MS. This analysis includes the estimation of tracts aligned with conventional MRI from which the MS lesions are segmented.

**Quantitative Assessment**

Compared to the information obtained from the FA map, the information about changes in fiber direction contained in the CBO map is more useful for the interactive identification of fiber tracts. In Figure 2A, for example, the internal capsule appears as a bright structure in the FA map, and separating the anterior and posterior crus of the internal capsule is not possible. In contrast, in the CBO scalar map (Fig 2B), it is possible to separately identify the anterior crus (blue contour on the right and yellow contour on the left) and posterior crus (orange contour on the right and green contour on the left). Differentiation between different ROIs lying close to each other and appearing bright in the FA map (Fig 2A) is also possible due to the different orientation visible in the CBO map (Fig 2B). In this way, the limbic system (sky blue contour on the right and pink contour on the left) was differentiated from the CC (Fig 2B in green). The identification of the major fiber tracts allows the construction of DT-MRI-based digital atlases such as the one depicted in Figure 2C along with representations of fiber tracts generated anatomically.

The min, max, and mean ± standard deviation of the FA, ADC, linear, planar, and spherical measurements were obtained for an MS lesion visible on conventional MRI. Table 1 summarizes the measurements from inside the lesion, from the lesion’s periphery, and from the lesion’s mirror-image area. As can be observed, there is a decrease in the mean FA and an increase in the mean ADC as the measurement is done closer to the area where the lesion is located. Similar results were obtained for the planar and spherical measurements.

Figure 3A shows the analyzed lesion (arrow) as seen in the baseline image slice of an MS patient’s DT-MRI scan, while Figure 3B presents the same slice depicting diffusion tensors represented as glyphs and color coded by the spherical measure of the local diffusion tensor.

To illustrate the analysis of changes in FA along the fiber tracts, an MS lesion on the posterior part of the CC was identified. Identification of the lesion was performed using the sets of conventional MRI (Fig 4A). In addition, an apparent area of NAWM located relatively close to the MS lesion was identified from the FA (Fig 4B) and CBO (Fig 4C) maps. From the obtained changes in FA measured along the fibers of the constructed fiber tracts (Figs 5A, B), a significant drop in the FA value corresponding to the lesion (yellow in Figs 5A, B) may be observed, while the slight drop corresponds to the apparent area of NAWM (blue in Figs 5A, B).

**Summary**

DWI and DT-MRI have been widely used in studies conducted with the purpose of better understanding the pathogenesis of MS, its natural course, and the nature and location of WM abnormalities, as well as the correlation
between MS lesions and the clinical outcome measures. It is now known that the FA decreases while the ADC increases in the areas affected by MS abnormalities, the NAWM and the NAGM. These changes have been found to be particularly high in patients presenting a more severe course of the disease, such as SPMS, than in less severe courses, such as early-onset MS and RRMS patients. Changing patterns of DT-MRI measurements depending

**Fig 2.** Axial slice from the diffusion tensor magnetic resonance imaging of a healthy volunteer, shown as a fractional anisotropy map (A) and as a color by orientation (CBO) map (B). In both images, a manually drawn labeled atlas of the major fiber tracts is superimposed. The fiber tracts were identified based on the information provided by the CBO map. In this way, the tracts were drawn on every single slice in which they appeared. (C) This figure illustrates white matter fiber tracts in 2 ways. Some white matter fiber tracts generated by streamline tractography are visualized as thin lines (blue for fibers associated with the corpus callosum and peach for fibers generated in the pons and continuing into the medial pedunculi of the cerebellum). In joint analysis of DT-MRI and conventional MRI, it is of interest to associate particular voxels of the conventional MRI with streamline tractography from particular regions and, in doing so, to define volumetric regions of interest. In this figure, several such white matter regions are visualized as color-coded solid 3-dimensional models.
on the type, appearance, and enhancement of MS lesions and/or their location in different brain structures have also been found.

The integration of both DT- and conventional MRI measures together with connectivity-based regional assessment and the development of novel image analysis and visualization techniques could provide better means to understand the nature and the location of WM abnormalities. The relationship between WM disruption, WM connectivity, and clinical measures will potentially allow clinicians to better correlate fiber tract disruption and MS symptoms such as cognitive impairment. Furthermore, it would ultimately lead to improved monitoring of patients, better prediction of the course of the disease, and more rapid assessment of new treatments or therapies.

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Table 1. Minimum, Maximum, and $\bar{x} \pm \text{SD}$ for the FA, ADC, Linear, Planar, and Spherical Measurements for an Identified MS Lesion

<table>
<thead>
<tr>
<th></th>
<th>Inside the Lesion</th>
<th>Lesion’s Periphery</th>
<th>Lesion’s Mirror Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.102</td>
<td>0.133</td>
<td>0.120</td>
</tr>
<tr>
<td>Max</td>
<td>0.385</td>
<td>0.600</td>
<td>0.590</td>
</tr>
<tr>
<td>$\bar{x} \pm \text{SD}$</td>
<td>0.344 ± 0.092</td>
<td>0.395 ± 0.084</td>
<td>0.385 ± 0.092</td>
</tr>
<tr>
<td>ADC ($\times 10^{-3}\text{mm}^2/\text{s}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.840</td>
<td>0.745</td>
<td>0.798</td>
</tr>
<tr>
<td>Max</td>
<td>1.511</td>
<td>1.280</td>
<td>1.196</td>
</tr>
<tr>
<td>$\bar{x} \pm \text{SD}$</td>
<td>1.230 ± 0.150</td>
<td>1.023 ± 0.101</td>
<td>1.000 ± 0.070</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.015</td>
<td>0.024</td>
<td>0.001</td>
</tr>
<tr>
<td>Max</td>
<td>0.278</td>
<td>0.374</td>
<td>0.293</td>
</tr>
<tr>
<td>$\bar{x} \pm \text{SD}$</td>
<td>0.137 ± 0.057</td>
<td>0.156 ± 0.069</td>
<td>0.132 ± 0.064</td>
</tr>
<tr>
<td>Planar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.008</td>
<td>0.011</td>
<td>0.021</td>
</tr>
<tr>
<td>Max</td>
<td>0.515</td>
<td>0.661</td>
<td>0.664</td>
</tr>
<tr>
<td>$\bar{x} \pm \text{SD}$</td>
<td>0.190 ± 0.115</td>
<td>0.243 ± 0.145</td>
<td>0.285 ± 0.132</td>
</tr>
<tr>
<td>Spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>0.300</td>
<td>0.223</td>
<td>0.229</td>
</tr>
<tr>
<td>Max</td>
<td>0.874</td>
<td>0.853</td>
<td>0.847</td>
</tr>
<tr>
<td>$\bar{x} \pm \text{SD}$</td>
<td>0.644 ± 0.116</td>
<td>0.566 ± 0.120</td>
<td>0.550 ± 0.125</td>
</tr>
</tbody>
</table>

FA = fractional anisotropy, ADC = apparent diffusion coefficient, MS = multiple sclerosis. Measurements were obtained from inside the lesion, the lesion’s periphery, and the lesion’s mirror-image area.

Fig 3. (A) The baseline source of the diffusion tensor magnetic resonance imaging scan of a patient with multiple sclerosis presenting with white matter (WM) lesions. (B) Diffusion tensors represented as glyphs and color coded by the degree of isotropy of the local diffusion tensor: red where the diffusion is most isotropic and blue where the diffusion is most anisotropic. Note that the WM lesions present an increased isotropic diffusion. Results in Table 1 correspond to the WM lesion on the left hemisphere (arrow).
A multiple sclerosis (MS) lesion (yellow) and an apparent area of normal-appearing white matter (NAWM; blue) detected in the posterior part of the corpus callosum. Different from the MS lesion, which can be observed in conventional magnetic resonance images (MRIs) (A) and diffusion tensor (DT)–MRI–derived maps such as fractional anisotropy (B), the NAWM can be observed only from DT-MRI–derived color by orientation maps (C).
References


Fig 5. (A) Tractography of the posterior part of the corpus callosum, which is disrupted by a multiple sclerosis lesion (yellow) and an apparent area of normal-appearing white matter (NAWM; blue, see also Fig 3). (B) A magnified view of the fibers clearly passing through the lesion and the NAWM. (C) Changes in fractional anisotropy (FA) measured along the fibers tracts depicted in (A). These changes are represented as the mean (solid line) ± standard deviation (dashed lines). Position from the brain midline is indicated on the abscissa. The significant drop in the FA value corresponds to the lesion (yellow in [A] and [B]), while the slight drop corresponds to the apparent area of NAWM (blue in [A] and [B]).


