Biol Res 40: 385-400, 2007

Diffusion Signal in Magnetic Resonance Imaging: Origin and Interpretation in Neurosciences

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ABSTRACT

Diffusion Magnetic Resonance Imaging provides images of unquestionable diagnostic value. It is commonly used in the assessment of stroke and in white matter fiber tracking, among other applications. The diffusion coefficient has been shown to depend on cell concentration, membrane permeability, and cell orientation in the case of white matter or muscle fiber tracking; yet a clear relation between diffusion measurements and known physiological parameters is not established.
The aim of this paper is to review hypotheses and actual knowledge on diffusion signal origin to provide assistance in the interpretation of diffusion MR images. Focus will be set on brain images, as most common applications of diffusion MRI are found in neuroradiology.
Diffusion signal does not come from two intra- or extracellular compartments, as was first assumed. Restriction of water displacement due to membranes, hindrance in the extracellular space, and tissue heterogeneity are important factors. Unanswered questions remain on how to deal with tissue heterogeneity, and how to retrieve parameters less troublesome to work with from biological and clinical points of view. Diffusion quantification should be done with care, as many variables can lead to variation in measurements.

Key terms: Diffusion, Magnetic Resonance Imaging, Interpretation, non Gaussian.

INTRODUCTION

Nowadays Magnetic Resonance (MR) provides images of unquestionable diagnostic value. It offers millimeter-scale images with high soft tissue contrast, particularly useful in brain studies. Yet, one limitation of MRI is that images offer macroscopic information, i.e. "gross" anatomic information: voxels of the order of a millimeter contain thousands and thousands of cells. Dysfunction will only be visible after important damage gets to anatomical scale. This is one of the reasons why the recently developed Diffusion Magnetic Resonance Imaging (dMRI) has been attracting so much interest.

Diffusion MR imaging consists in weighting the acquired signal by the amount of translational random motion of water molecules, known as Brownian motion (Fick 1855). In free media, motion is indeed random, and the displacement probability is normally distributed. This is not true in biological tissue as different barriers and biophysical phenomena are in the way of water molecules, modifying their behavior (Le Bihan 2003). Diffusing molecules explore their microscopic environment, changes in the image contrast obtained from this process give information that reflects tissue microstructure. Water displacement is understood to be modified by biophysical events, membrane permeability, relative importance of intra- and extra-cellular space, etc. dMRI is more than a new source of image contrast; it allows the quantification of water translational motion.

dMRI has proved useful in assessing many white matter and psychiatric pathologies. It is of particular interest to

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Received:April 19, 2007. Accepted: March 3, 2008

determine ischemic brain injury as diffusion images are the only early sensitive method to identify stroke (Sotak 2002, Rivers et al., 2006, Moseley et al., 1990). Diffusion Tensor Imaging (DTI) has shown potential in characterizing normal brain development and aging (Huppi and Dubois, 2006; Mukherjee and McKinstry, 2006; Salat et al 2005; Miller et al.,2003); in psychiatric disorder (Mori and Zhang 2006, Horsfield and Jones 2002, Lim and Helpern 2002), in schizophrenia (Kanaan et al., 2005), in cognitive disorders (Catani 2006); in characterizing head injury, where reductions in white matter integrity serve as a biomarker for degree of diffuse axonal injury (Budde et al.,2007; Huisman et al.,2004) and in demyelinating and neurodegenerative diseases such as multiple sclerosis (Schmierer et al., 2007; Patel et al.,2005; Bammer and Fazekas, 2002, Rovaris et al.,2005).

Despite the recent wide use of dMRI, various hypotheses and many works published, a clear relation between diffusion and known physiological parameters is not yet established. It is still necessary to obtain a relation between this quantification and some known physiological parameters in order to define a useful correlation between quantitative imaging and image interpretation. As it is not fully understood, quantification should be done with care, due to its dependence on many parameters. The aim of this paper is to review different hypotheses and actual knowledge on diffusion signal origin in order to provide a better insight on how to use the information retrieved from diffusion MR images.

DIFFUSION PHENOMENA

Diffusion is the process according to which molecules move from one place to another in a non-homogeneous medium, due to random molecular motion. The first mathematical bases of diffusion were established by Fick (1855). Considering an isotropic medium, the diffusion coefficient definition is related to variation of concentration, C, according to equation 1.

$$\frac{\partial C}{\partial t} = D\nabla^2 C \qquad . [1]$$

Diffusion is a process linked to the kinetic energy of molecules, a process that depends on the temperature, T. The Stokes-Einstein relation relates the diffusion coefficient to the temperature, T, to the solvent viscosity, η, to the hydrodynamic radius of the molecule, R_D, as follows:

$$D = \frac{k_B T}{6\pi\eta R_D} \qquad [2]$$

where k_B is the Boltzmann constant. As D is linearly dependent on temperature, *in vivo* diffusion measurements give information on temperature variation. Sensitivity of temperature measurements through diffusion imaging has been evaluated *in vitro* to 2.4% /°(LeBihan et al., 1989; Tofts et al., 2000).

This description is valid in an isotropic medium, such as free liquids like water, oil, etc. In case of anisotropic diffusion, D in equation 1 must be replaced by a second-order tensor, symmetric and positive definite:

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix} \qquad . [3]$$

Even if interpretation of the diffusion coefficient is clear in an isotropic system, the physical meaning of the diffusion tensor is not trivial. The different cross-terms $D_{\alpha\beta}$ ($\alpha \neq \beta$) do not represent diffusion in direction $\alpha + \beta$ but rather the influence of the concentration gradient in direction α over displacements in direction β. For instance, if we consider a system in which diffusion is more important along the x-axis, in which the concentration gradient would also have a component along the y-axis, then the displacement along the x direction would also depend on the concentration gradient on the other axis. This effect is taken into account in the $D_{\alpha\beta}$ entries of the tensor.

In inhomogeneous biological tissues, diffusion is not a purely random process. The presence of membranes, that can be

more or less permeable to water, restricts diffusion and modifies the exchanges between compartments. Water molecules are hindered in their displacement by membranes, organelles. In biological tissues, the measured diffusion coefficient is referred to as Apparent Diffusion Coefficient *ADC*.

DIFFUSION MEASUREMENT PRINCIPLES

Before discussing diffusion signal interpretation, it is necessary to briefly review the acquisition principles. In presence of a strong magnetic field B_0, spins precess at Larmor frequency f_0, where γ stands for gyromagnetic ratio depending on the nucleus (Haacke et al., 1999):

$$f_0 = \gamma B_0 / (2\pi) \quad [4]$$

By adding a magnetic field gradient G_z along the z axis for instance, precessing frequency at location z_1 is modified according to:

$$f_1 = (B_0 + G_z z_1)(\gamma / 2\pi) \quad [5]$$

If a first gradient, called diffusion gradient $diff_1$, of duration δ, is applied, then all spins precess with different frequencies according to their respective position. Different frequencies imply having different phases at the same time instant. Two spins i, j at different positions z_i, z_j will have phases ϕ_i and ϕ_j given by

$$\begin{aligned} \phi_{i,diff1} &= 2\pi f_i \delta = \gamma (B_0 + G_{diff1} z_i)\delta \\ \phi_{j,diff1} &= 2\pi f_j \delta = \gamma (B_0 + G_{diff1} z_j)\delta \end{aligned} \quad [6]$$

Considering only variation due to gradient with respect to the main magnetic field and the main frequency constant, the phase expressions in [6] reduce to:

$$\begin{aligned} \phi_{i,diff1} &= \gamma G_{diff1} z_i \delta \\ \phi_{j,diff1} &= \gamma G_{diff1} z_j \delta \end{aligned} \quad [7]$$

If both spins are static, a second gradient $diff_2$ with the same duration δ and magnitude, $G_{diff2}=G_{diff1}$, but with inverse polarity, induces the following phases:

$$\begin{aligned} \phi_{i,diff2} &= -\gamma G_{diff2} z_i \delta \\ \phi_{j,diff2} &= -\gamma G_{diff2} z_j \delta \end{aligned} \quad [8]$$

At echo time, the phase difference between both spins cancels out. However if one spin moves due to diffusion phenomena, the "rephasing" effect of the second gradient is not perfectly equal to "dephasing" of the first gradient. Imperfect rephasing over the entire voxel will create smaller signal magnitude, as depicted in figure 1. This signal loss depends on the amount of motion that occurred between applications of the two diffusion gradients. This is called signal diffusion-weighting preparation; "dephasing" and "rephasing" diffusion gradients are applied before the imaging gradients of the MR pulse sequence. The most commonly used sequence is called Pulsed Gradient Spin Echo PGSE (Stejskal and Tanner 1965). Further details on acquisition techniques are found in (Basser and Jones 2002, Mori and van Zijl 2002, Le Bihan et al. 2006).

More or less diffusion weight can be given to the acquired signal by varying diffusion gradient magnitude G, application time d, or delay between the two gradients D. The degree of diffusion sensitization is quantified through the so-called "b-factor" (Le Bihan et al., 1986, Le Bihan 1995, Basser and Jones 2002) given by

$$b = (\gamma G \delta)^2 (\Delta - \delta/3) \quad [9]$$

The measured signal magnitude S_1 is given by the simple expression

$$S_1 = S_0 e^{-bD} \quad [10]$$

where S_0 stands for signal magnitude with no diffusion-weighting. The b-factor is a user-defined parameter. To compute D, two images have to be acquired, one, S_1, with diffusion gradient on ($b \neq 0$) and the other, S_0, with $b=0$.

Whereas in the isotropic case one single measure in one direction is enough to calculate D because it is the same along all directions, in the anisotropic case measurements in more directions are needed. Specifically, to fully calculate

diffusion tensor **D** as defined in equation 2, six non-collinear diffusion-weighted acquisitions are needed (Basser et al., 1994, Pierpaoli and Basser 1996). After calculation of the tensor in each voxel, rotationally invariant indices and maps can be derived. The most common of them are Mean Diffusivity *MD,* an index of average diffusivity in all directions, and Fractional Anisotropy, *FA*, which indicates how strongly oriented is diffusion in only one direction in space (Basser et al., 1994).

DIFFUSION APPLICATIONS

Since the beginning of the '90s clinical applications of dMRI and DTI have been exploited. The most successful of them is the early diagnosis of brain ischemia. In this pathology, mean diffusivity decreases in the infarcted regions few minutes after the stroke (Moseley et al., 1990) while all the other imaging techniques require much more time after the accident to have a visible effect. Although the underlying mechanism is still not clear, in this pathology dMRI sensitivity has a very crucial role as it enables the activation of suitable treatments which can change the destiny of brain tissues (Sotak 2002, Rivers et al., 2006). Another important application of dMRI is related to the detection of cancer or metastases, where there is a significant decrease in MD in the case of malignant tumors, maybe because of cell proliferation (Takahara et al., 2004, Goldman et al., 2006, Rubesova et al., 2006, Mulkern et al., 2006).

Much interest has been put in studies of brain development and maturation of newborns. Structural and functional modifications at microscopic level can be assessed by the specific sensitivity of dMRI. In fact, during the first phases of life, brain begins to myelinate and connections between regions start to be created so that tensor indexes changes are evident and quantifiable. In newborn babies mean diffusivity is higher than in mature brain, and diminishes with aging, while diffusion anisotropy is lower at birth and increases fast (Neil et al., 1998, Neil et al., 2002).

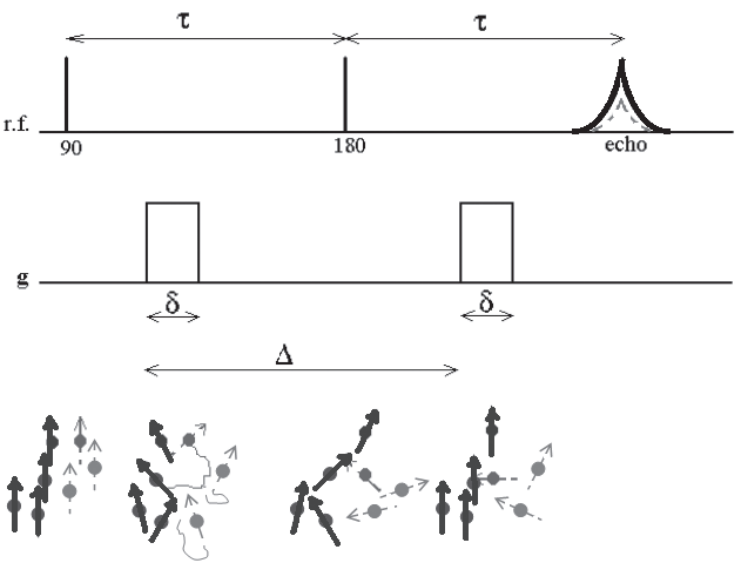

Figure 1: Principle of diffusion-weighting preparation: static spins (thick line) are dephased by the first diffusion gradient according to their respective position. 180° pulse and a second diffusion gradient perfectly rephase those spins according to equations 7 and 8, creating echo thick line represented. Yet moving spins (interrupted lines) do not have the same position when the second diffusion gradient is applied, therefore at echo time all spins are not perfectly in phase, resulting in a echo of smaller magnitude (interrupted line).

In healthy brains, higher anisotropy indices are found in white matter, in particular in corpus callosum, while cerebral cortex is often considered to have homogeneous diffusion along all directions, see table 1 (Pierpaoli et al., 1996, Shimony et al., 1999) and figure 2. Anisotropy of white matter, is mainly due to organization in bundles and fibers overlapped with sheaths of lipidic membrane, myelin. Water molecules preferentially diffuse along the length of axons, making the diffusion coefficient larger in the direction parallel to the axons than perpendicular to them. An important consequence of this observation is that it is possible to estimate the orientation of the fibers by means of the direction of maximum water diffusion. Maps of directionalities and fiber tracking algorithms exploit this principle to characterize and rebuild in three dimensions the path of the main white matter fibers and bundles (Mori and van Zijl, 2002, Mangin et al., 2002). Applications of fiber tracking are in course of validation (Lin et al., 2003). Reconstruction of fiber tracks in patients with brain tumors is a undoubted aid in the pre-surgical planning (Schonberg et al., 2006, Arfanakis et al.,2006) as the regions and connections may be delocalized by the presence of the mass of tumor itself. All these applications are based on the Diffusion Tensor model which, on the other hand, has some intrinsic limitations. Specifically, DTI is not always able to correctly model the behavior of water molecules as, for example, in voxels where fibers cross. To overcome this, High angular Resolution Diffusion Imaging (HARDI) methods have been proposed (Weeden et al. 1999; Assaf and Cohen, 1999 Jansons and Alexander, 2003; Ozarslan et al., 2006, Tuch, 2004, Descoteaux et al., 2006, Tournier et al., 2004, Dell'Acqua et al., 2007) and nowadays are widely applied to better define water motion direction.

DIFFUSION ACQUISITIONS

As diffusion measurement through MRI is obtained from signal loss quantification, the main acquisition difficulty is linked to Signal-to-Noise Ratio (SNR) that is usually quite low, and depends inversely on the value of b: the higher b – i.e. the diffusion weight – the lower the SNR. Thus, as often needed in MRI in order to maintain clinically compatible acquisition time, a compromise must be made between increasing repetition number (to improve SNR), increasing spatial resolution or increasing angular resolution through a precise spatial characterization of the diffusion tensor **D** along various directions. Usually, 5 to 15 minutes are needed to obtain data with voxels of the order of a few mm resolution and acceptable image quality.

Another important problem related to diffusion acquisition is motion artifact. As diffusion consists in measuring microscopic motion, acquisitions are sensitive to water motion and more generally to patient motion. To reduce patient motion artifacts, images must be acquired fast. The fastest sequence is single-shot Echo-Planar-Imaging (Mansfield 1977). Although much faster than all the other pulse sequences, EPI does not offer a perfect solution as images show strong geometric distortions in the presence

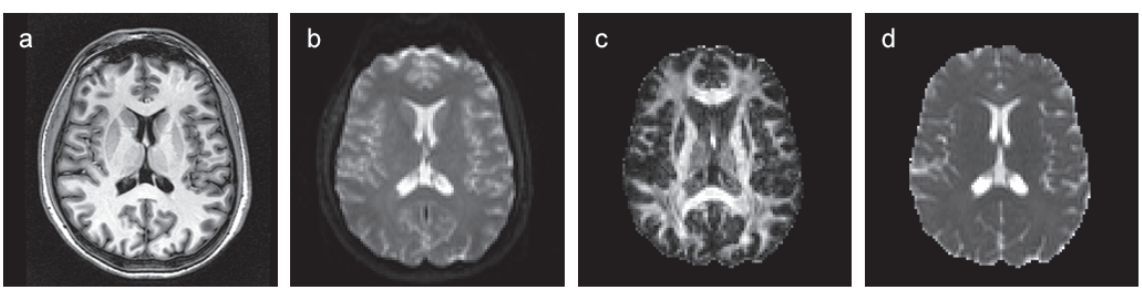

Figure 2: (a) T1-weighted axial slice of a healthy brain. (b) T2-weighted image. (c) Fractional Anisotropy map; gray matter presents almost no anisotropy, contrary to white matter. (d) Mean Diffusivity map; almost no contrast is visible between white and gray matter.

of inhomogeneous magnetic field. (Le Bihan et al., 2006, Jezzard et al., 1998). Different strategies to reduce acquisition time and to counteract motion artifacts have been proposed, such as cardiac gating to reduce influence of brain pulsation (Brockstedt et al. 1999, Robson and Porter 2005), or additional echo, "navigator echo", used to monitor and correct spurious phase due to motion (Anderson and Gore1994, Butts et al. 1996, Robson et al., 1997, Liu et al. 2004). Most recent and successful has been the implementation of parallel imaging (Pruessman et al. 1999, Griswold et al., 1999), taking advantage of acquiring signal with various coils, which permits to reduce acquisition time by the same coil number. Acquisition time reduction yields smaller artifacts and better image quality. Parallel imaging improved spinal cord dMRI (Cercignani et al., 2003), and cardiac diffusion imaging (Hsu and Henriquez, 2001) among other applications (Bammer et al., 2001, Golay et al., 2002).

DIFFUSION SIGNAL DECAY IS NOT BICOMPARTIMENTAL

Apparent Diffusion Coefficient measurements previously described are useful in both clinical and research contexts. The question is what is measured exactly, and what modification of tissue microstructure relates to ADC variations. Diffusion signal has been measured not to decay in a mono-exponential way *in vivo*, contrary to signal decay in free media described in equation 10. Numerous observations showed that signal decay was better fitted by various exponentials, as illustrated in figure 3 (Niendorf et al., 1996, Assaf et al., 2002, Mulkern et al., 1999, Pfeuffer et al., 1999, Clark and Le Bihan, 2000). Questions arise on the interpretation of multiexponential decay: which relation exists between diffusion quantification and some known physiological parameters, so that fine image interpretation can be done.

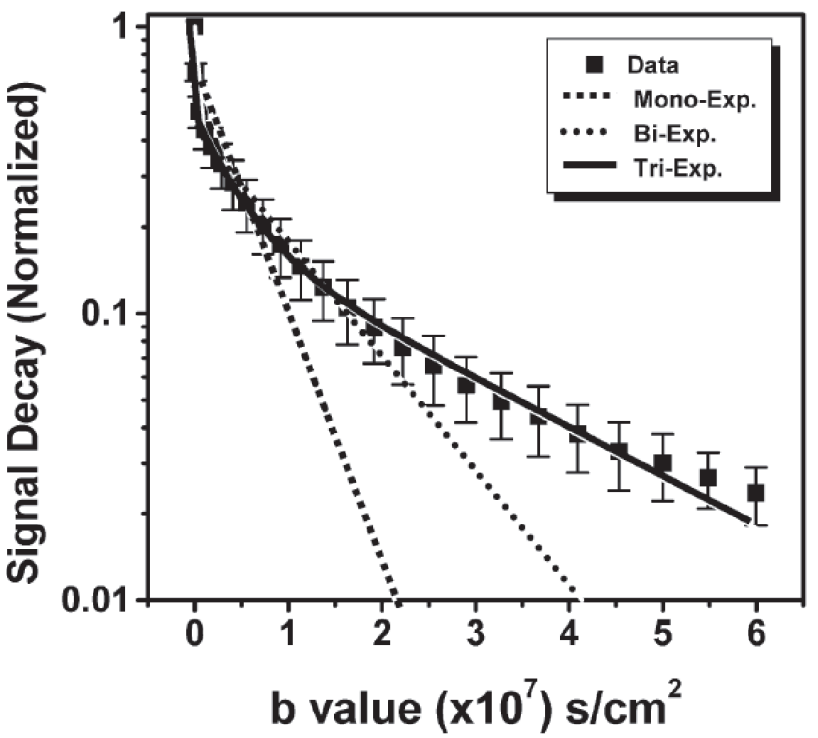

Figure 3: Water signal decay in excised rat sciatic nerves in diffusion experiments with respect to the *b*-values, showing the attempt to fit the experimental data with a mono-, a bi-, and a tri-exponential function (Assaf et al., 2002)

Bicompartmental hypothesis

Cerebral tissue is divided into two main spaces: intra- and extra-cellular spaces, separated by semi-permeable membranes, assumed to have different diffusive properties. When diffusion time t_d is much shorter than average time molecules spend in each compartment, *i.e.* when there is no exchange between compartments, echo attenuation is given by superposition of the two decaying signals (Karger et al., 1988). The following equation is adjusted:

$$\frac{S(b)}{S(0)} = fe^{-bADC_{fast}} + (1-f)e^{-bADC_{slow}} \quad [11]$$

where $ADC_{fast(slow)}$ represent now the fast and slow apparent diffusion coefficients, whose biophysical origin is unknown; f stands for the fast diffusing compartment volume fraction. Fast and slow diffusion tensors can be defined the same way as for the mono-exponential analysis, leading to fast and slow mean diffusivity and fractional anisotropy.

It was first assumed that intracellular diffusion would be slower as protein concentration is higher, cytoplasm is more viscous (Niendorf et al., 1996). Yet, measurements show that fast diffusing compartment volume fractions were equal to about 70%, as shown in table 2. Diffusion measurements do not correspond at all with anatomy, as about 20% of total volume is extra-cellular. Numerical simulations based on known anatomy showed that assignment of fractions obtained from biexponential fits of fast and slow diffusion attenuation to extra- and intra-cellular space volume ratios is not correct (Chin et al., 2002).

Various theoretical models put forward the importance of intra- and extra-cellular volumes on diffusion signal (Szafer et al. 1995, Latour et al., 1994, Stanisz et al.,1997). Yet, these models are highly idealized, considering in general cells as regularly organized geometrical structures, using a high number of parameters; they do not constitute sufficient validation supporting the bicompartmental hypothesis. Experimental observations also set the emphasis on the influence of intra- and extra-cellular compartments: *in vitro*, using iontophoresis and provoking cellular volume changes by osmotic shock Sykova (1997) showed that a decrease in extra-cellular volume implies increase in extra-cellular tortuosity, and decrease in diffusion coefficient. Similar results were obtained using NMR, *in vitro*, showing besides that *ADC* decreased when cellular density increased (Anderson et al., 2000). With a similar experimental design, bi-exponential measures were done showing that cellular volume variation did not make fast and slow diffusion coefficients vary but their fractions do (Sheperd et al., 2003), sustaining the hypothesis that diffusion signal comes from two compartments.

Compartment definition

The question remained on the definition of such two compartments. Diffusion coefficients were measured similar in both intra- and extra-cellular compartments for NAA and 2FDG-6P (Duong et al. 1998). This is true for macromolecules bigger than water, but remains to be demonstrated for water signal. The hypothesis according to which intracellular diffusion is slower than extracellular is still to be verified. Measuring only intracellular water diffusion decay in the isolated *Aplysia* giant neuron Grant et al. (2001) found various *ADC*, which would imply the presence of several intracellular compartments. By destroying cellular membranes, and by eliminating extra-cellular space, Schwarcz et al. (2004) still observed diffusion signal biexponential decay. There is no need to have intra- and extra-cellular compartments to observe diffusion biexponential signal decay; the intracellular compartment itself can present as various "compartments", that could consist of the nucleus and various organelles (endoplasmic reticulum, Golgi apparatus, etc.).

Exchange between compartments

Influence of exchange between compartments has been demonstrated experimentally by Thelwall et al. (2002), as

diffusion measures were varied by blocking exchange canals of intra- and extra-cellular water using pCMBS. Yet, equation 11 is based on the hypothesis that no exchange occurs between compartments during observation, i.e. diffusion time. Another variable is then added, as different groups disagree on time spent by water in each intra- and extra-cellular compartment. Based on diffusion MR measures, Pfeuffer et al. (1998) evaluated intracellular time to 15 – 20 ms. As these values are based on diffusion measures, they are subject to controversy. Various other groups evaluate intracellular time to be 550 ms, and extra-cellular time as 120 ms, basing measures on longitudinal relaxation time (Duong et al. 1998, Quirk et al. 2003, Meier et al. 2003). Moreover, Meier et al. (2003) affirm that intracellular time is variable according to cell size. As most diffusion times are about a few tens of a millisecond, the assumption of observation time much shorter than average time spent in each compartment would be valid and equation 11 holds. The diffusion time defined explicitly, or implicitly through *b*-value choice, is an important parameter.

Transverse relaxation time

It has been argued that the difference between known cellular volumetric fractions and diffusion volume fractions could come from transverse relaxation time (T2) differences between compartments. Indeed, different T2 have been measured in the various nervous tissue components, myelin, intra-axonal and extra-cellular water (Stanisz and Henkelman,1998, Peled et al., 1999, Inglis et al., 2001), which weight each compartment in a different way. Yet simulations showed that the sensitivity to *T*2 is small and thus *T*2 differences are unlikely to explain this mismatch (Chin et al., 2002).

Multiexponential does not imply multicompartment

There is no evidence that there are two compartments, there may be more compartments, and there may be no diffusive compartments. According to Assaf et al. (2002) data are best fitted with 3 exponentials rather than with 2, as illustrated in figure 3. Pfeuffer et al. (1999) agree as they find 3 peaks analyzing data through Laplace transform.

It is highly probable that the multi-exponential signal decay does not represent intra- and extra-cellular compartments. Cell membranes and extra-cellular space play important roles, but compartmentation is not sufficient to explain signal variations.

Numerical simulations set forward that there was no need of the presence of multiple compartments to observe multiexponential decay (Kiselev and Il'yasov, 2007, Sustanskii and Yablonskiy, 2002). Modulation of the signal decay curve can be obtained from a heterogeneous distribution of cell size (Peled et al., 1999). Observation of multiexponential decay does not imply the presence of multicompartments.

RESTRICTION AND HINDRANCE PHENOMENA ARE IMPORTANT

Another hypothesis is based on the importance of restriction of water molecule mobility inside the cell, and/or motion hindrance in the extracellular space.

Restriction hypothesis

Simulations show that restriction and hindrance can modulate signal decay to the multiexponential curve observed. Membranes have been considered as primary determinants of water restriction (Beaulieu and Allen, 1994). A model of packed myelinated axons treating white matter fascicles as an array of identical thick-walled cylindrical tubes arranged periodically in a regular lattice and immersed in an outer medium suggested that intracellular properties (diffusivity and dimensions) are not primary causes for ADC variations and anisotropy but rather outer axon diameter, myelin sheaths and particularly extracellular space (Sen and Basser, 2005). Cell package and organization affect diffusion measures in white matter, but do not explain gray matter biexponential observations.

Experimental evidence

Restriction of diffusion motion inside cellular space implies obtaining signal variation with diffusion time, as below certain time threshold no restriction should occur. To obtain experimental measures to provide realistic data into theoretical models is difficult due to hardware limitation of magnetic field gradient capacities, and in particular difficulties to diminish diffusion time. According to methodology used, all groups do not agree on measures such as diffusion coefficients inside myelin itself, as myelin T2 is very short (Andrews et al., 2006, Peled et al., 1999, Stanisz and Henkelman,1998).

Variation of slow diffusion coefficient fraction while cellular volume changed made Le Bihan et al. (2006) advance the hypothesis about the importance of layers of water molecules that are most hindered in their motion, such as a membrane-bound water pool. This hypothesis is closer to biological tissue complexity, but is challenged by the difficulty to obtain experimental evidence.

Restriction observations could not be made *in vivo*, using diffusion time down to 8 ms (Clark 2001). Yet, a diffusion time of 4 ms was estimated necessary to visualize these restriction effects (Stanisz et al., 1997, Beaulieu and Allen, 1996), which is difficult to achieve *in vivo*. Experimental observations seem to reinforce the importance of restriction and hindrance phenomena: as myelin thickness increases, the mean *ADC* progressively drops; experimental measures made by Neil et al. (1998) correspond to the model developed by Sen and Basser (2005). Displacements measured perpendicular to white matter fibers in excised rat spinal cord are constant, measured from 50 to 250 ms (Nossin-Manor et al., 2005). Similarly, metabolites in bovine optic nerve show broad displacement distribution of fast diffusing component, appearing not to be restricted, while slow diffusing component is highly restricted to milieu in the order of 1 - 2 μm, as shown in figure 4 (Assaf and Cohen, 1999).

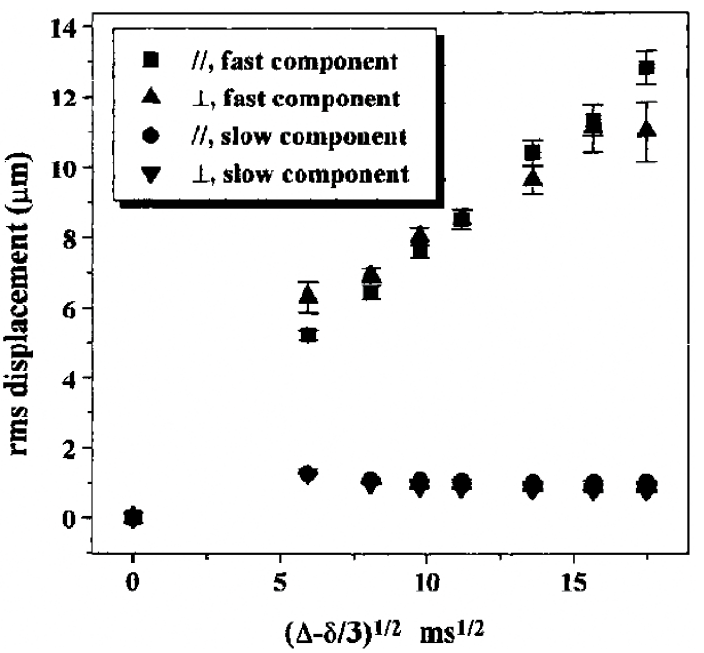

Figure 4: Root mean square (rms) choline displacements observed in excised bovine optic nerve as function of the square root of diffusion time for the slow and fast diffusing components in both orientations (Assaf and Cohen, 1999). Slow diffusion distance is in good agreement with the known inner-axonal diameter of bovine optic nerve of around 1-2 mm.

Conciliation model: inclusion of two types of behavior.

Restriction and hindrance phenomena seem more plausible to explain water diffusion signal decay, yet experimental evidence is hard to obtain. Following Assaf and Cohen (1999) observations, a model proposed recently by the same authors (Assaf et al., 2004, Assaf and Basser, 2005), Composite Hindered And Restricted Model of Diffusion CHARMED, is considering water to have two different types of behavior: restricted in the intra-axonal space, and hindered in the extra-axonal space, characterized by normal distribution with varied mean and standard deviation. No number of compartments, restricted or hindered, is imposed. Assaf et al. (2006) were able to obtain on spinal cord *in vitro* tissue segmentation that corresponds to histology, and inferred axonal diameter distribution. This model still lacks *in vivo* validation, but shows promise in the study of white matter structures.

ANISOTROPY

Diffusion anisotropy origin is prone to be connected to the same restriction and hindrance effects that affect diffusion multiexponential decay (Moseley et al., 1991, Le Bihan et al., 1993). Anisotropy is different according to white matter bundle (table 1). It is particularly high in the corpus callosum. No simple relationship has been established with known microstructural elements (Pierpaoli and Basser, 1996). Recently Golabchi et al. (2006) showed existence of correlation between FA and axon density *in vitro* in human axial spinal cord.

Some anisotropy has also been measured for different metabolites (N-acetyl aspartate, phosphocreatine, and choline) in both white and gray matter (Ellegood et al., 2006). Yet function and size of such molecules are different from water, hindering straightforward conclusion extrapolation. Up to the present there is no direct explanation for diffusion anisotropy. The main hypothesis is that membrane restriction effects are mostly responsible for this anisotropy. Water diffusivity was measured to be similar inside axons perpendicular and parallel to the axon main axis (Takahashi et al., 2002). Beaulieu and Allen (1994) showed that anisotropy did not come from the presence of microtubules, or of active axonal transport.

Even though not indispensable (Beaulieu 2002), myelin sheaths are important as they modulate anisotropy: FA decreases in absence of myelin (Gulani et al., 2001, Neil et al., 1998, Neil et al., 2002), demyelination processes induced by cuprizone could be observed *in vivo* (Sun et al., 2006). Recent results measured myelin water diffusion to be by itself anisotropic (Andrews et al., 2006), contrary to Stanisz and Henkelman observations (Stanisz and Henkelman, 1998). Discrepancy comes from the use of different myelin signal separation techniques. Three effects can account for anisotropy diminution: increase in transversal diffusivity, decrease in longitudinal diffusivity or both (Song et al. 2002).

Brain cortex is considered as isotropic at regular clinical magnet image scale (about 1.5 x 1.5 x 4 mm^3) (Shimony et al., 1999). At higher magnetic field, signal is high enough to reduce voxel size (0.39 x 0.39 x 2 mm3) and to measure diffusion anisotropy in mature cortex as Ronen et al. (2003) have shown in cat mature cortex. Cortex anisotropy was even stronger considering only slow diffusion processes (Maier et al., 2004). In various brain regions, it seems that anisotropy is more important for slow diffusion than for fast diffusion processes (Maier et al., 2004, Chabert, 2004). It is probable that anisotropy phenomena will be better understood once unidirectional diffusion signal variations are explained.

INTEGRATION OF ALL PARAMETERS

Many parameters influence diffusion signal variation as discussed earlier in this review. Main hypotheses include water signal compartmentalization, restriction and hindrance in intra- and extracellular spaces, and tissue heterogeneity (Peled et al., 1999). There is a need to look at data in another way.

TABLE 1

Mean Diffusivity and Fractional Anisotropy values in different regions of interest of healthy human brain

	MD (x $10^{-3}mm^2/s$)	FA
Ventricules (CSF) (Pierpaoli et al., 1996)	3.19 ± 0.10	0.11 ± 0.05
Grey Matter (frontal lobe) (Shimony et al., 1999)	0.88 ± 0.04	0.15 ± 0.02
Grey Matter (putamen) (Shimony et al., 1999)	0.73 ± 0.03	0.14 ± 0.01
Corpus Callosum (body) (Shimony et al., 1999)	0.72 ± 0.05	0.71 ± 0.02
Optic Radiations (Shimony et al., 1999)	0.74 ± 0.06	0.49 ± 0.02
White Matter (occipital lobe) (Shimony et al., 1999)	0.78 ± 0.06	0.33 ± 0.01
Internal Capsule (Shimony et al., 1999)	0.70 ± 0.05	0.59 ± 0.02
Pyramidal Tract (Pierpaoli et al., 1996)	0.71 ± 0.04	0.87 ± 0.04
U-fibers (Pierpaoli et al., 1996)	0.65 ± 0.05	0.65 ± 0.11

TABLE 2

Fast and Slow Mean Diffusivities(MD_{fast}, MD_{slow}), fast diffusive volume fraction *f* in different regions of interest of healthy human brain.

	MDfast (x $10^{-3}mm^2/s$)	MDslow (x $10^{-3}mm^2/s$)	f
Grey Matter (Clark and LeBihan 2000)	1.01 ± 0.10	0.18 ± 0.10	0.73 ± 0.08
Corpus Callosum (Maier et al., 2004)	1.176 ± 0.050	0.195 ± 0.015	0.699 ± 0.046
Internal Capsule (Maier et al., 2004)	1.201 ± 0.040	0.176 ± 0.007	0.643 ± 0.014
Thalamus (Maier et al., 2004)	1.139 ± 0.055	0.237 ± 0.012	0.689 ± 0.028

Several attempts have been made to get closer to the complex reality of biology. Yablonskiy et al. (2003) developed a statistical model of diffusion signal decay, based on the hypothesis that there is a high number of diffusion pools, and the resulting probability distribution of diffusion coefficients is Gaussian. Standard deviation was found to be almost constant relative to *ADC*, of 36%. Even though average diffusivity is different in each tissue, the broadness of diffusion coefficient distribution is similar everywhere, meaning that the same phenomena could make diffusion coefficients span in the same manner whatever the tissue structure may be. This result is quite complex and difficult to interpret.

Characterization of "tissue heterogeneity", or of multiplicity of pools, was proposed by Bennett et al. (2003), based on stretched exponential model, where DDC stands for Distributed Diffusion Coefficient, and α is used to parameterize heterogeneity:

$$S(b) = S_0 \exp\left[-(b * DDC)^{\alpha}\right] \quad . [12]$$

At variance with Yablonskiy's findings, α is not homogeneous throughout the brain; higher heterogeneity is found in white matter. This finding emphasizes the importance of restriction and barriers of axonal membranes on diffusion signal modification. Bennett's characterization was shown to be sensitive to early pathological change of cancerous tissue (Bennett et al., 2004). Interestingly, α does not seem to vary according to the direction of measurement (Bennett et al., 2006).

If this observation is confirmed, in spite of low SNR and big voxel size (3.4 x 3.4 x 5 mm^3) images, measurements along only one direction would be sufficient to

characterize tissue heterogeneity; this could result in a fast method to evaluate fine changes in tissue microstructure. Both Bennett's and Yablonskiy's observations are counter-intuitive since important differences in tissue structure are well-known.

There is much non-exploited data at high *b*-values (Meca et al., 2004). As all aforementioned parameters seem to matter in unknown proportions, characterization of the entire signal decay is necessary, using other descriptors. Quantification of deviation from monoexponential decay has been derived using a normalized kurtosis index value (Chabert et al., 2004, Lu et al., 2006). No brain region presents monoexponential decay, major differences are found in white matter measured perpendicular to the main axis. This finding reinforces a coexistence of various phenomena, and the significance of the particular organization of fibers and/or myelin. *In vivo* diffusion signal is not monoexponential, yet a monoexponential hypothesis is used when computing *ADC* with equation 10. Use of a monoexponential hypothesis underestimates displacement, leading to differences up to 50% in highly non-monoexponential regions (Chabert 2004, Liu et al., 2005).

CONCLUSION

Diffusion MRI provides *in vivo* images highly sensitive to tissue microstructure. Diffusion measurement is a non-invasive way to obtain *in vivo* information at the microscopic scale, where cell size and organization matter. Diffusion signal is not bicompartmental, and not separated into intra- and extracellular compartments. Restriction, hindrance and tissue heterogeneity are important factors that modify measured diffusion signals. In spite of various attempts, the unanswered question remains on how to deal with tissue heterogeneity, and how to retrieve parameters easy to work with on a biological and clinical point of view. Diffusion quantification should be done with great care, as many variables can lead to different measures.

In spite of difficulties, there are some promising results such as correlation between diffusion anisotropy and the presence and preservation of myelin sheaths (Beaulieu 2002), or *in vitro* correlation between anisotropy and axonal density (Golabchi et al., 2006). Another further step would be to achieve *in vivo* inference of spinal axonal diameter distribution such as done in rat spinal cord *in vitro* (Assaf et al., 2006). Essays have been undertaken using *q*-space diffraction pattern in rat corpus callosum at different ages (Weng et al., 2005), with no coincidence between diffusion measurements and known cell sizes. Diffusion measurements are so sensitive that there are still numerous possibilities of unexplored applications. A recent example is the demonstration of the feasibility of monitoring brain activity through diffusion imaging (Le Bihan et al., 2006).

ACKNOWLEDGEMENT

S.C. acknowledges funding from FONDECYT grant number 11060036.

REFERENCES

ANDERSON AW, GORE JC (1994) Analysis and correction of motion artifacts in diffusion weighted imaging. Magn Reson Med 32: 379-387

ANDERSON A, XIE J, PIZZONA J, BRONEN R, SPENCER D, GORE J (2000) Effects of cell volume fraction changes on apparent diffusion in human cells. Magn Reson Imaging 18: 689-695

ANDREWS TJ, OSBORNE MT, DOES MD (2006) Diffusion of Myelin Water. Magn Reson Med 56: 381-385

ARFANAKIS K, GUI M, LAZAR M (2006) Optimization of white matter tractography for pre-surgical planning and image-guided surgery. Oncol Rep 15: 1061-1064

ASSAF Y, COHEN Y (1998) Non mono-exponential attenuation of water and N-acetyl aspartate signals due to diffusion in brain tissue, J Magn Reson 131: 69-85

ASSAF Y, COHEN Y (1999) Structural information in neuronal tissue as revealed by q-space diffusion NMR spectroscopy of metabolites in bovine optic nerve. NMR Biomed 12: 335-344

ASSAF Y, KAFRI M, SHINAR H, CHAPMAN J, KORCZYN AD, NAVON G, COHEN Y (2002) Changes in axonal morphology in experimental autoimmune neuritis as studied by high b-value q-space ^{1}H and ^{2}H DQF diffusion magnetic resonance spectroscopy. Magn Reson Med 48: 71-81

ASSAF Y, FREIDLIN RZ, ROHDE GK, BASSER PJ (2004) New Modeling and Experimental Framework to

Characterize Hindered and Restricted Water Diffusion in Brain White Matter. Magn Reson Med 52: 965-978

ASSAF Y, BASSER PJ (2005) Composite hindered and restricted model of diffusion (CHARMED) MR imaging of the human brain. NeuroImage 27: 48-58

ASSAF Y, BLUMENFELD T, LEVIN G, YOVEL Y, BASSER PJ (2006) AxCaliber – A Method to Measure the Axon Diameter Distribution and Density in Neuronal Tissues. In Proceedings of the 14th ISMRM Scientific Meeting, Seattle, USA, p.637

BAMMER R, KEELING SL, AUGUSTIN M, PRUESSMANN KP, WOLF R, STOLLBERGER R, HARTUNG HP, FAZEKAS F (2001) Improved diffusion-weighted single-shot echo-planar imaging (EPI) in stroke using Sensitivity Encoding (SENSE) Magn Reson Med 46: 548-554

BAMMER R, FAZEKAS F. Diffusion imaging in multiple sclerosis (2002) Neuroimaging Clin N Am;12: 71-106

BASSER PJ, MATTIELLO J, LE BIHAN D (1994) Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B 103: 247-264

BASSER PJ, JONES DK (2002) Diffusion tensor MRI: theory, experimental design and data analysis – technical review. NMR Biomed 15: 456-467

BEAULIEU C, ALLEN P (1994) Determinants of anisotropic water diffusion in nerves. Magn Reson Med 31: 394-400

BEAULIEU C, ALLEN P (1994) Water diffusion in the giant axon of the squid: implications for diffusion-weighted MRI of the nervous system. Magn Reson Med 32: 579-583

BEAULIEU C, ALLEN P (1996) An in vitro evaluation of the effects of local magnetic-susceptibility-induced gradients on anisotropic water diffusion in nerve. Magn Reson Med 36: 39-44

BEAULIEU C (2002) The basis of anisotropic water diffusion in the nervous system – a technical review. NMR Biomed 15: 435-455

BENNETT K, SCHMAINDA K, BENNET R, ROWE D, LU H, HYDE J (2003) Characterization of continuously distributed cortical water diffusion rates with a stretched-exponential model. Magn Reson Med 50: 727-734

BENNET K, HYDE J, RAND SD, BENNET R, KRAUWER H, REBRO, KJ, SCHMAINDA K (2004) Intra-voxel distribution of DWI decay rates reveals C6 glioma invasion in rat brain. Magn Reson Med 52: 994–1004

BENNET K, HYDE J, SCHMAINDA K, (2006) Water diffusion heterogeneity index in the human brain is insensitive to the orientation of applied magnetic field gradients. Magn Reson Med 56: 235-239

BROCKSTEDT S, BORG M, GEIJER B, WIRESTAM R, THOMSEN C, HOLTAS S, STAHLBERG F (1999) Triggering in quantitative diffusion imaging with single-shot EPI. Acta Radiol 40: 263-269

BUDDE MD, KIM JH, LIANG HG, SCHMIDT RE, RUSSEL JH, CROSS AH, SONG SK (2007) Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. Magn Reson Med 57: 688-695

BUTTS K, DE CRESPIGNY A, PAULY J, MOSELEY ME (1996) Diffusion weighted interleaved echo planar imaging with a pair of orthogonal navigator echoes. Magn Reson Med 35: 763-770

CATANI M (2006) Diffusion tensor magnetic resonance imaging tractography in cognitive disorders. Curr Opin Neurol 19: 599-606

CERCIGNANI M, HORSFIELD MA, AGOSTA F, FILIPPI M (2003) Sensitivity-encoded diffusion tensor MR imaging of the cervical cord. Am J Neuroradiol 24: 1254-1256.

CHABERT S, MECA CC, LE BIHAN D (2004) Relevance of the information about the diffusion distribution in vivo given by kurtosis in q-space imaging. In Proceedings of the 12th ISMRM Scientific Meeting, Kyoto, Japan, p. 1238

CHABERT S (2004) Développements méthodologiques pour l'imagerie cérébrale de diffusion in vivo chez l'homme. Ph.D. Thesis. Université de Technologie de Compiègne, France

CHIN CL, WEHRLI FW, HWANG SN, TAKAHASHI M, HACKNEY DB (2002) Biexponential Diffusion Attenuation in the Rat Spinal Cord: Computer Simulations Based on Anatomic Images of Axonal Architecture. Magn Reson Med 47: 455-460

CLARK C, LEBIHAN D (2000) Water diffusion compartmentation and anisotropy at high b values in the human brain. Magn Reson Med 44: 852-859

CLARK C (2001) Diffusion Time Dependence of the Apparent Diffusion Tensor in Healthy Human Brain and White Matter Disease. Magn Reson Med 45: 1126-1129

DELL'ACQUA F, RIZZO G, SCIFO P, ALONSO CLARKE R, SCOTTI G, FAZIO F. A model-based deconvolution approach to solve fiber crossing indiffusion-weighted MR imaging. (2007), IEEE Trans Biomed Eng 54: 462-72

DESCOTEAUX M, ANGELINO E, FITZGIBBONS S, DERICHE R (2006) Apparent diffusion coefficients from high angular resolution diffusion imaging: estimation and applications. Magn Reson Med 56: 395-410

DUONG T, ACKERMAN J, YING H, NEIL J (1998) Evaluation of extra- and intracellular apparent diffusion in normal and globally ischemic rat brain via ^{19}F NMR. Magn Reson Med 40: 1-13

ELLEGOOD J, HANSTOCK CC, BEAULIEU C (2006) Diffusion Tensor Spectroscopy (DTS) of Human Brain. Magn Reson Med 55: 1-8

FICK A (1855) Ueber diffusion. Poggendorff's Annalen der Physik und Chemie 94: 59-86

GOLABCHI FN, BROOKS DH, HOGE WS, MAMATA H, MAIER SE (2006) Comparison of MR Diffusion Anisotropy with Axon Density. in Proceedings of the 14th ISMRM Conference, Seattle, USA, p. 639

GOLAY X, JIANG H, VAN ZIJL PCM, MORI S (2002) High-resolution isotropic 3D diffusion tensor Imaging of the human brain. Magn Reson Med 47: 837-843

GOLDMAN M, BOXERMAN JL, ROGG JM, NOREN G (2006) Utility of apparent diffusion coefficient in predicting the outcome of Gamma Knife-treated brain metastases prior to changes in tumor volume: a preliminary study. J Neurosurg 105: 175-182

GRANT S, BUCKLEY D, GIBBS S, WEBB A, BLACKBAND S (2001) MR microscopy of multicomponent diffusion in single neurons. Magn Reson Med 46: 1107-1112

GRISWOLD MA, JAKOB PM, CHEN Q, GOLDFARB JW, MANNING WJ, EDELMAN RR, SODICKSON DK (1999) Resolution enhancement in single-shot imaging using simultaneous acquisition of spatial harmonics (SMASH) Magn Reson Med 41: 1236-1245

GULANI V, WEBB A, DUNCAN I, LAUTERBUR P (2001) Apparent diffusion tensor measurements in myelin-deficient rat spinal cord. Magn Reson Med 45: 191-195

HAACKE ME, BROWN R, THOMPSON M, VENKATESAN R (1999) Classical response of a single nucleus to a magnetic field. In: Magnetic

resonance imaging: physical principles and sequence design, New York: Wiley-Liss. pp: 17-32

HORSFIELD MA, JONES DK (2002) Applications of diffusion weighted and diffusion tensor MRI to white matter diseases. NMR Biomed 15: 570-577

HSU EW, HENRIQUEZ CS (2001) Myocardial fiber orientation mapping using reduced encoding diffusion tensor imaging J Cardiovasc Magn Reson 3: 339-347

HUISMAN TAGM, SCHWAMM LH, SCHAEFER PW, KOROSHETZ WJ, SHETTY-ALVA N, OZSUNAR Y, WU O, SORESEN AG (2004) Diffusion Tensor Imaging as Potential Biomarker of White Matter Injury in Diffuse Axonal Injury AJNR Am J Neuroradiol 25: 370 - 376

HUPPI PS, DUBOIS J (2006) Diffusion tensor imaging of brain development. Semin Fetal Neonatal Med 2006 11: 489-97

INGLIS B, BOSSART E, BUCKLEY D, WIRTH E, MARECI T (2001) Visualization of neural tissue water compartments using biexponential diffusion tensor MRI. Magn Reson Med 45: 580-587

JANSONS KM, ALEXANDER DC (2003) Persistent Angular Structure: new insights from diffusion MRI data. Inf Process Med Imaging 18: 672-83

JEZZARD P, BARNETT A, PIERPAOLI C (1998) Characterization of and correction for eddy current artifacts in echo planar diffusion anisotropy studies in NMR microimaging. Magn Reson Med 39: 801-812

KANAAN RAA, KIM JS, KAUFMANN WE, PEARLSON GD, BARKER GJ, MCGUIRE PK (2005) Diffusion tensor imaging in schizophrenia. Biological Psychiatry 58: 921-929

KARGER J. PFEIFER H, HEINK W (1988) Principles and applications of self diffusion measurements by nuclear magnetic resonance. Adv Magn Reson 12: 1-89

KISELEV VG, IL'YASOV KA (2007) Is the "biexponential diffusion" biexponential? Magn Reson Med 57: 464-469

LATOUR L, SVOBODA K, MITRA P, SOTAK C (1994) Time dependent diffusion of water in a biological model system. Proc Natl Acad Sci USA 91: 1229-1233

LE BIHAN D, BRETON E, LALLEMAND D, GRENIER P, CABANIS E, LAVAL-JEANTET M (1986) MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. Radiology 161: 401-407

LEBIHAN D, TURNER R, MACFALL, J (1989) Effects of intravoxel incoherent motions (IVIM) in steady-state free precession (SSFP) imaging: application to molecular diffusion imaging. Magn Reson Med 10: 324-337

LE BIHAN D, TURNER R, DOUEK P (1993) Is water diffusion restricted in human brain white matter? An echo-planar NMR imaging study. Neuroreport 4: 887-890

LE BIHAN D (1995) Diffusion and perfusion magnetic resonance imaging. Applications to functional MRI. Raven Press, New York

LE BIHAN D (2003) Looking into the functional architecture of the brain with diffusion MRI. Nature Rev Neuroscience 4: 469-480

LE BIHAN D, POUPON C, AMADON A, LETHIMONNIER F (2006). Artifacts and pitfalls in diffusion MRI. J Magn Reson Imaging 24(3): 478-88

LE BIHAN D, URAYAMA S, ASO T, HANAKAWA T, FUKYYAMA H (2006) Direct and fast detection of neuronal activation in the human brain with diffusion MRI. Proc Natl Acad Sci USA 103: 8265-8268

LIN CP, WEDEEN VJ, CHEN JH, YAO C, TSENG WY. (2003) Validation of diffusion spectrum magnetic resonance imaging with manganese-enhanced rat optic tracts and ex vivo phantoms. Neuroimage 19: 482-95

LIM KO, HELPERN JA (2002) Neuropsychiatric application of DTI – a review. NMR Biomed 15: 587-593

LIU C, BAMMER R, KIM D, MOSELEY ME (2004) Self-navigated interleaved spiral (SNAILS): application to high-resolution diffusion tensor imaging. Magn Reson Med 52: 1388-1396

LIU C, BAMMER R, MOSELEY ME (2005) Limitations of Apparent Diffusion Coefficient-Based Models in Characterizing Non-Gaussian Diffusion. Magn Reson Med 54: 419-428

LU H, JENSEN JH, RAMANI A, HELPERN JA (2006) Three-dimensional characterization of non-gaussian water diffusion in humans using diffusion kurtosis imaging. NMR Biomed19: 236-47

MAIER S, VAJAPEYAM S, MAMATA H, WESTIN C, JOLESZ FA, MULKERN RV (2004) Biexponential diffusion tensor analysis of human diffusion data. Magn Reson Med 51: 321-330

MANGIN JF, POUPON C, COINTEPAS Y, RIVIERE D, PAPADOPOULOS-ORFANOS D, CLARK CA, REGIS J, LEBIHAN D (2002) A framework based on spin glass models for the inference of anatomical connectivity from diffusion-weighted MR data – a technical review. NMR Biomed 15: 481-492

MANSFIELD P (1977) Multi-planar image formation using NMR spin echoes. J Phys C 10: L55-L58

MECA C, CHABERT S, LE BIHAN D (2004) Diffusion MRI at large b values: what's the limit? In Proceedings of the 12th ISMRM Scientific Meeting, Kyoto, Japan. p 1196

MEIER C, DREHER W, LEIBFRITZ D (2003) Diffusion in compartmental systems. A comparison of an analytical model with simulations. Magn Reson Med 50: 500-509

MILLER JH, MCKINSTRY RC, PHILIP JV, MUKHERJEE P, NEIL JJ (2003). Diffusion-tensor MR imaging of normal brain maturation: a guide to structural development and myelination. AJR Am J Roentgenol 180: 851-859

MORI S, VANZIJL PCM (2002) Fiber tracking: principles and strategies - a technical review. NMR in Biomed 15: 468-480

MORI S, ZHANG J (2006) Principles of Diffusion Tensor Imaging and Its Applications to Basic Neuroscience Research. Neuron 51: 527-539

MOSELEY ME, COHEN Y, MINTOROVITCH J, CHILEUITT L, SHIMIZU H, KUCHARCZYK J, WENDLAND MF, WEINSTEIN PR (1990) Early detection of regional cerebral ischemia in cats: comparison of diffusion- and T2-weighted MRI and spectroscopy. Magn Reson Med 14: 330–346

MOSELEY M, KUCHARCZYK J, ASGARI H, NORMAN D. (1991) Anisotropy in diffusion-weighted MRI. Magn Reson Med 19: 321-326

MUKHERJEE P, MCKINSTRY RC (2006) Diffusion tensor imaging and tractography of human brain development. Neuroimaging Clin N Am 16: 19-43 MULKERN R, GUDBJARTSSON, WESTIN C, ZENGINGONUL H, GARTNERR W, GUTTMAN C, ROBERTSON R, KYRIAKOS W, SCHWARTZ C, HOLTZMANN D, JOLESZ F, MAIER S (1999) Multicomponent apparent diffusion coefficients in human brain. NMR Biomed 12: 51-62

MULKERN R, BARNES AS, HAKER SJ, HUNG YP, RYBICKI FJ, MAIER SE, TEMPANY CMC (2006) Biexponential characterization of prostate tissue water diffusion decay curves over an extended b-factor range. Magn Reson Imaging 24: 563-568

NEIL JJ, SHIRAN SI, MCKINSTRY RC, SCHEFFT GL, SNYDER AZ, ALMLI CR, AKBUDAK E, ARONOVITZ JA, MILLER JP, LEE BC, CONTURO TE (1998) Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging. Radiology 209: 57-66

NEIL JJ, MILLER JP, HUPPI PS (2002) Diffusion tensor imaging of the developing human brain, NMR Biomed 17: 543-550

NIENDORF T , DIJKUIZEN R, NORRIS D, VAN LOOKEREN CAMPAGNE M, NICOLAY K (1996) Biexponential diffusion attenuation in various states of brain tissue: implications for diffusion-weighting imaging. Magn Reson Med 36: 847-857

NOSSINMANOR R, DUVDEVANI R, COHEN Y (2005) Effect of experimental parameters on high *b*-value *q*-space MR images of excised rat spinal cord. Magn Reson Med 54: 96-104

OZARSLAN E, SHEPHERD TM, VEMURI BC, BLACKBAND SJ, MARECI TH (2006): Resolution of complex tissue microarchitecture using the diffusion orientation transform (DOT). Neuroimage 31: 1086-103

PATEL S, HUM B, GONZALEZ C, SCHWARTZMAN R, FARO S, MOHAMED F (2005) Use of a voxelwise approach in the analysis of fractional anisotropy data in multiple sclerosis patients. In Conference Proceedings of the IEEE Eng Med Biol Soc 7: 7032-5

PELED S, CORY D, RAYMOND S, KIRSCHNER D, JOLESZ F (1999) Water diffusion , T2 and compartmentation in frog sciatic nerve. Magn Reson Med 42: 911-918

PFEUFFER J, DREHER W, SYKOVA E, LEIBFRITZ D (1998) Water signal attenuation in diffusion weighted ^{1}H NMR experiments during cerebral ischemia: influence of intracellular restrictions, extracellular tortuosity and exchange. Magn Reson Imaging16: 1023-1032

PFEUFFER J, PROVENCHER S, GRUETTER R (1999) Water diffusion in rat brain in vivo as detected at very large b values is multicompartmental, MAGMA 8: 98-108

PIERPAOLI C, BASSER PJ (1996) Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 36: 893-906

PIERPAOLI C, JEZZARD P, BASSER P, BARNETT A, DICHIRO G (1996) Diffusion tensor MR imaging of the human brain. Radiology 201: 637–648

PRUESSMANN KP, WEIGER M, SCHEIDEGGER MB, BOESIGER P (1999) SENSE: Sensitivity encoding for fast MRI. Magn Reson Med 42: 952-962

QUIRK J, BRETTHORST G, DUONG T, SNYDER A, SPRINGER C, ACKERMAN J, NEIL J (2003) Equilibrium water exchange between the intra- and extracellular spaces of mammalian brain. Magn Reson Med 50: 493-499

RIVERS CS, WARDLAW JM, ARMITAGE PA, BASTIN ME, CARPENTER TK, CVORO V, HAND PJ, DENNIS MS (2006) Do acute diffusion- and perfusion-weighted MRI lesions identify final infarct volume in ischemic stroke? Stroke 37: 98-104

ROBSON M, ANDERSON A, GORE J (1997) Diffusion weighted multiple shot echo planar imaging of humans without navigation. Magn Reson Med 38: 82-88

ROBSON MD, PORTER DA (2005) Reconstruction as a source of artifact in nongated single-shot diffusion-weighted EPI. Magn Reson Imaging 23: 899-905

RONEN I, KIM I, GARWOOD M, UGURBIL K, KIM D (2003) Conventional DTI vs. slow and fast diffusion tensors in cat visual cortex. Magn Reson Med 49: 785-790

ROVARIS M, GASS A, BAMMER R, HICKMAN SJ, CICCARELLI O, MILLER DH, FILIPPI M (2005). Diffusion MRI in multiple sclerosis. Neurology 65: 1526-1532

RUBESOVA E, GRELL AS, DEMAERTELAER V, METENS T, CHAO SL, LEMORT M (2006) Quantitative diffusion imaging in breast cancer: A clinical prospective study. J Magn Reson Imaging 24: 319-324

SALAT DH, TUCH DS, HEVELONE ND, FISCHL B, CORKIN S, ROSAS HD, DALE AM (2005) Age-related changes in prefrontal white matter measured by diffusion tensor imaging. Ann N Y Acad Sci 1064: 37-49

SCHMIERER K, WHEELER-KINGSHOT CA, BOULBY PA, SCARAVILLI F, ALTMANN DR, BARKER GJ, TOFTS PS, MILLER DH (2007) Diffusion tensor imaging of post mortem multiple sclerosis brain. Neuroimage 35: 467-77

SCHONBERG T, PIANKA P, HENDLER T, PASTERNAK O, ASSAF Y (2006) Characterization of displaced white matter by brain tumors using combined DTI and fMRI. Neuroimage 30: 1100-11

SCHWARCZ A, BOGNER P, MERIC P, CORREZE J, BERENTE Z, PAL J, GALLYAS F, DOCZI T, GILLET B, BELOEIL J (2004) The existence of biexponential signal decay in magnetic resonance diffusion-weighted imaging appears to be independent of compartmentalization. Magn Reson Med 51: 278-285

SEN PB, BASSER PJ (2005) A Model for Diffusion in White Matter in the Brain. Biophys J 89: 2927-2938

SHEPERD T, WIRTH E, THELWALL P, CHEN H, ROPER S, BLACKBAND S (2003) Water diffusion measurements in perfused human hyppocampal slices undergoing tonicity changes. Magn Reson Med 42: 461-466

SHIMONY JS, MCKINSTRY RC, AKBUDAK E, ARONOVITZ JA, SNYDER AZ, LORI NF, CULL TS, CONTURO TE (1999) Quantitative diffusion tensor anisotropy brain MR imaging: normative human data and anatomic analysis. Radiology 212: 770-784

SONG SK, SUN SW, RAMSBOTTOM MJ, CHANG C, RUSSEL J, CROSS AH (2002) Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 17: 1429-1436

SOTAK C (2002) The role of diffusion tensor imaging in the evaluation of ischemic brain injury – a review. NMR Biomed 15: 561-569

STANISZ G, SZAFER A, WRIGHT G, HENKELMAN R (1997) An analytical model of restricted diffusion in bovine optic nerve. Magn Reson Med 37: 103-111.

STANISZ G, HENKELMAN R (1998) Diffusional anisotropy of T2 components in bovine optic nerve. Magn Reson Med 40: 405-410

STEJSKAL EO, TANNER JE (1965) Spin diffusion measurements: spin echoes in the presence of a time dependent field gradient. J Chem Phys 42: 288-292

SUN SW, LIANG HG, TRINKAUS K, CROSS AH, ARMSTRONG RC, SONG SK (2006) Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. Magn Reson Med 55: 302-308

SUSTANSKII A, YABLONSKIY D (2002) Effects of restricted diffusion on MR signal formation. J Magn Reson 157: 92-105

SYKOVA E (1997) The extracellular space in the CNS: its regulation, volume and geometry in normal and

pathological neuronal function. The Neuroscientist 3: 28-41 SZAFER A, ZHONG J, GORE J (1995) Theoretical model for water diffusion in tissues. Magn Reson Med 33: 697-712

TAKAHARA T, IMAY Y, YAMASHITA T, YASUDA S, NASU S, VAN CAUTEREN M (2004) Diffusion weighted whole body imaging with background body signal suppression (DWIBS): technical improvement using free breathing, STIR and high resolution 3D display. Radiat Med 22: 275–282

TAKAHASHI M, HACKNEY D, ZHANG G, WEHRLI S, WRIGHT A, O'BRIEN W, UEMATSU H, WEHRLI F, SELZER M (2002) Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord. Proc Natl Acad Sci USA 99: 16192-16196

THELWALL P, GRANT S, STANISZ G, BLACKBAND S (2002) Human erythrocyte ghosts: exploring the origins of multiexponential water diffusion in a model biological tissue with magnetic resonance. Magn Reson Med 48: 649-657

TOFTS P, LLOYD D, CLARK C, BARKER G, PARKER G, MCCONVILLE P, BALDOCK C, POPE J (2000) Test liquids for quantitative MRI measurements of self-diffusion coefficient in vivo. Magn Reson Med 43: 368-374

TOURNIER JD, CALAMANTE F, GADIAN DG. CONNELLY A, Direct estimation of the fiber orientation density function from diffusion-weighted MRI using spherical deconvolution (2004) NeuroImage 23: 1176-1185

TUCH DS (2004) Q-ball imaging. Magn Reson Med 52: 1358-72

WEDEEN VJ, REESE TG, TUCH DS, DOU J-G WEISSKOFF RM, CHESSLER D, (1999) Mapping fiber orientation spectra in cerebral white matter with Fourier-transform diffusion MRI, in Proceedings of the 7th ISMRM Scientific Meeting, Philadelphia, USA

WENG JC, CHEN JH, CHEN DY, TSENG WY(2005) Magnetic Resonance Diffusion Diffractogram in the Assessment of Microstructure Sizes of Rat Corpus Callosum during Brain Maturation. In Proceedings of the 13th ISMRM Scientific Meeting, Miami, USA, p. 841

YABLONSKIY D, BRETTHORST G, ACKERMAN J (2003) Statistical model for diffusion attenuated MR signal. Magn Reson Med 50: 664-669