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Multimodal integration of diffusion MRI for better characterization of tissue biology

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Abbreviations

MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
DTI	Diffusion tensor imaging
PDF	Probability density function
MAP	Mean apparent propagator
RTOP	Return-to-the origin probability
STI	Susceptibility tensor imaging
QSM	Quantitative susceptibility mapping
STIFT	Structure tensor informed fiber tractography
DDIF	Diffusion in the internal field
DR-COSY	Diffusion-relaxation correlation spectroscopy
CPMG	Carr-Purcell-Meiboom-Gill
ADC	Apparent diffusion coefficient
BOLD	Blood-oxygen-level-dependent
fMRI	Functional magnetic resonance imaging

Abstract

The contrast in diffusion-weighted MR images is due to variations of diffusion properties within the examined specimen. Certain microstructural information of the underlying tissues can be inferred through quantitative analyses of the diffusion-sensitized MR signals. In the first part of the paper, we review two types of approaches for characterizing diffusion MRI signals: Bloch's equations with diffusion terms and statistical descriptions. Specifically, we discuss expansions in terms of cumulants and orthogonal basis functions, the confinement tensor formalism, and tensor distribution models. Further insights of the tissue property may be obtained by integrating diffusion MRI with other techniques, which is the subject of the second part of the paper. We review examples involving magnetic susceptibility, structural tensors, internal field gradients, transverse relaxation and functional MRI. Integrating information provided by other imaging modalities (MR-based or otherwise) could be a key to improve our understanding of how diffusion MRI relates to physiology and biology.

1 Introduction

The diffusion of water molecules (or other molecules in general) can be measured by MRI, typically through pulsed field gradients. This measurement is possible because the random movement of water molecules in an inhomogeneous magnetic field results in a random accumulation of phase angles thus an irreversible loss of phase coherence within an ensemble. In the literature, two approaches are commonly used to analyze the effect of diffusion on MR signals: one by solving the Bloch-Torrey equations and the other by statistical analysis. The Bloch-Torrey equations extend the Bloch equations by adding a diffusion term which phenomenologically characterizes a signal decay rate given by Fick's second law. On the other hand, statistical approaches treat the molecular movement as a random process which accrues a random phase due to the random motion in an inhomogeneous field. The effect of diffusion is characterized by the transitional probability density function.

However, the complexity of biological tissues dictates that knowing the diffusion coefficients or the statistics of the diffusion process may not be fully satisfactory for understanding the underlying biological structures and processes. A better understanding of the diffusion properties measured by MRI can be supplemented by models of the underlying tissue microstructures and by other complementary tissue properties, for example, magnetic susceptibility, relaxivity, incoherent flow and magnetization transfer. Some of these properties may affect diffusion measurements directly while all reflect aspects of the underlying tissues that are measured by diffusion MRI. The purpose of this review is to summarize multimodal approaches for characterizing and utilizing diffusion MRI for quantifying tissue properties. The reviewed approaches include various models for describing diffusion MRI signal and

complementary MRI techniques that can be integrated with diffusion MRI techniques for improving the characterization of tissue properties. The scope is limited with the intention to complement other reviews within this special issue.

2 Approaches for analyzing diffusion MRI signal

The effect of translational molecular diffusion on MRI signal has been analyzed with various approaches including solving Bloch's equation with diffusion terms, stochastic ensemble averaging and microstructural modelling.

2.1 Bloch's equation with diffusion terms

In the case of spin echoes, the MR signals are not only attenuated by T1 and T2 relaxation, but also attenuated due to the diffusion of the molecules in inhomogeneous local magnetic field induced by internal magnetic susceptibilities or external field gradients. In the classical paper “spin echoes”, Hahn stated that “the effect of self-diffusion will be qualitatively accounted for by using Bloch's equations with a diffusion term added” (1). Torrey provided a general treatment of Bloch's equations with isotropic diffusion terms (2) while Stejskal extended the treatment to include anisotropic diffusion (3), now commonly written for spatially uniform diffusion tensor as (Fig. 1)

$$\frac{\partial \mathbf{M}}{\partial t} = \gamma \mathbf{M} \times \mathbf{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{M_z - M_0}{T_1} \hat{z} + \sum_{k=1}^3 \sum_{l=1}^3 D_{kl} \partial_k \partial_l \mathbf{M}. \quad [1]$$

Here $\mathbf{M}$ is the magnetization vector; t is time; γ is the gyromagnetic ratio; $\mathbf{B}$ is the magnetic flux; T1 and T2 are the longitudinal and transverse relaxation times, respectively; $\hat{x}$, $\hat{y}$ and $\hat{z}$ are the three unit-axis of the Cartesian coordinates; D_{kl} is the element of the diffusion tensor $\mathbf{D}$. The solution of Eq. [1] in the case of spin echo can be expressed as a linear relationship between the logarithm of the echo intensity and components of the diffusion tensor via (3)

$$\ln \left[\frac{s(\mathbf{b})}{s(0)} \right] = - \sum_{k=1}^3 \sum_{l=1}^3 b_{kl} D_{kl} = b_{kl} D_{kl} = \mathbf{b} : \mathbf{D}, \quad [2]$$

which forms the basis of diffusion tensor imaging (DTI) (4). Here, $s(\mathbf{b})$ is the echo amplitude with diffusion weighting and $s(0)$ is that without diffusion weighting; $\mathbf{b}$ is the $\mathbf{b}$ -matrix that describes the diffusion weighting in a pulse sequence.

Eq. [1&2] work well if the underlying diffusion process is Gaussian. However, in most biological tissues such as the brain, the microstructural complexity renders the molecular motion to be non-Gaussian. To accommodate the non-Gaussian diffusion effect observed on diffusion-weighted images of the brain, Liu et al. generalized Eq. [1] to include a series of higher-order tensors as follows (5–7)

$$\begin{aligned} \frac{\partial \mathbf{M}}{\partial t} = & \gamma \mathbf{M} \times \mathbf{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} - \frac{M_z - M_0}{T_1} \hat{z} + D_{i_1 i_2}^{(2)} \partial_{i_1} \partial_{i_2} \mathbf{M} + D_{i_1 i_2 i_3}^{(3)} \partial_{i_1} \partial_{i_2} \partial_{i_3} \mathbf{M} + \\ & D_{i_1 i_2 i_3 i_4}^{(4)} \partial_{i_1} \partial_{i_2} \partial_{i_3} \partial_{i_4} \mathbf{M} + \dots \end{aligned} \quad [3]$$

Here, $D_{i_1 i_2 \dots i_n}^{(n)}$ represents the elements of an n th-order diffusion tensor. This generalization is made by generalizing Fick's first law to include higher order partial differentiations of spin concentrations. Eq. [3] can be solved analytically for the pulsed-gradient spin echo sequence, and the echo amplitude, similar to Eq. [2], is given by (5,6)

$$\ln \left[\frac{s(\mathbf{b})}{s(0)} \right] = j^2 b_{i_1 i_2}^{(2)} D_{i_1 i_2}^{(2)} + j^3 b_{i_1 i_2 i_3}^{(3)} D_{i_1 i_2 i_3}^{(3)} + j^4 b_{i_1 i_2 i_3 i_4}^{(4)} D_{i_1 i_2 i_3 i_4}^{(4)} + \dots \quad [4]$$

where j is the imaginary unit and $b_{i_1 i_2 \dots i_n}^{(n)}$ represents the elements of an n th-order $\mathbf{b}$ -tensor determined by the amplitude and timing of the pulsed gradients. Eq. [4] shows that the odd-ordered diffusion tensors are encoded in the phase of signal while the even-ordered tensors are encoded in the magnitude. The series of higher-order tensors represents the covariance,

skewness and kurtosis etc. (8). The relationship is best understood using a statistical analysis of the signal.

Eq. [4] represents a higher-order-cumulant expansion of the relationship between diffusion-weighted signal and the statistical properties of the underlying diffusion process following Eq. [3]. In practice, the infinite series sum has to be truncated. The fourth-order truncation of the higher order tensor model has been popularly referred as diffusion kurtosis imaging (DKI) (9). However, Eq. [4] does not necessarily fully characterize the complex diffusion environment of biological tissues, for example, the time-dependence of the cumulants is not considered, similar to DTI and DKI. A more in-depth discussion of the limitations can be found in the effective medium theory of a diffusion-weighted signal as described by Novikov and Kiselev (10).

2.2 Statistical models

While Bloch's equations with diffusion terms provide a phenomenological approach to quantify the diffusion signal with a set of diffusion tensors, the meanings of the tensors are not immediately clear. One way to investigate their meanings involves analyzing the diffusion signal using statistical approaches, in which the signal is treated as an ensemble average of all spins within a voxel (11) (Fig. 2). The signal originating from each spin is expressed as a complex number with a unit amplitude and a phase term that is path dependent. The phase of a given spin n ($n = 1, 2 \dots N$) at time t can be written as a discrete summation of path-dependent phase accruals as

$$\Phi_n(t) = -\gamma \sum_{i=1}^K \mathbf{G}(t_i) \cdot \mathbf{r}_n(t_i) \delta t. \quad [5]$$

In generating the above summation, the time interval t is discretized into K small intervals with t_i representing the i -th interval. Each interval has a duration of δt . At a given time t_i , the position

of the spin is given by $\mathbf{r}_n(t_i)$ where spin experiences a local field gradient vector of $\mathbf{G}(t_i)$. This treatment is generally referred to as the random model. The accuracy of the model can be made arbitrarily high by increasing K , i.e. the number of time intervals.

For the pulsed gradient spin echo sequence, the summation of all spins gives the following echo amplitude

$$s = \frac{1}{N} \sum_{n=1}^N e^{j(\Phi_n^+ - \Phi_n^-)} = \frac{1}{N} \sum_{n=1}^N e^{j\Delta\Phi_n}, \quad [6]$$

where Φ_n^+ and Φ_n^- represent the total phase accumulation of the n -th spin before and after the 180° -refocusing pulse, and $\Delta\Phi_n$ is the difference between the two. Under an ergodic assumption, the ensemble average in Eq. [6] can be replaced by the expected value of the n -th spin as follows

$$s = \langle e^{j\Delta\Phi_n} \rangle = \langle e^{j\Delta\Phi} \rangle. \quad [7]$$

The subscript n was dropped without loss of generality. Denote the sequence of random positions of a given spin as $\{r_{2K}; r_{2K-1} \cdots r_{K+1}; r_K \cdots r_2; r_1\}$, where r_1 is the initial position, $\{r_K \cdots r_2; r_1\}$ are the positions before the refocusing pulse and $\{r_{2K}; r_{2K-1} \cdots r_{K+1}\}$ are the positions after the refocusing pulse. Further denote $p(r_{2K}; r_{2K-1} \cdots r_{K+1}; r_K \cdots r_2; r_1)$ as the joint probability density function (PDF) for the sequence of random positions, then the echo amplitude can be evaluated as follows

$$s = \int e^{j\Delta\Phi} p(r_{2K}; r_{2K-1} \cdots r_{K+1}; r_K \cdots r_2; r_1) dr_1 dr_2 \cdots dr_{2K}. \quad [8]$$

2.2.1 Cumulant expansion

Eq. [8] is essentially an integral over all particle trajectories—hence the phrase “path integral.” This expression can be evaluated analytically only in some special cases. In one case (12), the transition probability between two adjacent positions is treated as independent and

identically distributed (i.i.d.) Gaussian, in which case, a straightforward integration of Eq. [8] gives the exponential function shown in Eq. [2] where D is proportional to the covariance matrix of the Gaussian PDF. In another case, if the diffusion-encoding gradient pulses are of infinitesimally narrow width and have infinitely high amplitude (i.e., a delta function), then Eq. [8] simplifies to the q-space formula as (13–15)

$$s = \int e^{-j\gamma\delta\mathbf{G}\cdot\mathbf{r}}p(\mathbf{r})d\mathbf{r} = \int e^{-j2\pi\mathbf{q}\cdot\mathbf{r}}p(\mathbf{r})d\mathbf{r}, \quad [9]$$

where $p(\mathbf{r})$ denotes the probability distribution function (PDF) of net displacements, commonly referred to as the ensemble averaged propagator. For a general PDF, Eq. [9] can be expanded with a series of cumulant tensors (Fig. 3) which are shown to be proportional to the higher-order diffusion tensors (5,6) in the case of pulsed-gradient spin echo (PGSE) sequence (Fig. 4). The underlying ensemble averaged propagator can be approximated with Gram-Charlier expansion using Hermite tensors (5–7,16). A more general discussion of the cumulant expansion of diffusion signal can also be found in reference (17).

Eqs. [4&9] both show that the phase of the diffusion signal is needed to reconstruct an asymmetrical PDF or asymmetrical underlying diffusion boundaries (Fig. 5). However, the symmetry of the pulsed gradient spin echo sequence suggests that its signal does not have a non-trivial phase term. In some situations, the symmetry can however be broken. For example, spin exchange in and out of an imaging voxel would break the symmetry and introduce phase in the diffusion signal (5,6). The symmetry could also be broken with asymmetrical diffusion gradients. In one example, Laun et al. showed that the combination of long-narrow gradients was able to generate sufficient phase to reconstruct triangular-shaped pores (18,19).

2.2.2 Mean apparent propagator (MAP) MRI

An alternative representation of the MR signal attenuation is afforded by a series expansion of orthogonal basis functions. Similar to the cumulant expansion, this approach has its roots in statistics literature (20). Hermite functions, given by

$$\phi_n(u, q) = \frac{j^{-n}}{\sqrt{2^n n!}} e^{-2\pi^2 q^2 u^2} H_n(2\pi u q). \quad [10]$$

are particularly advantageous in this endeavor (21) as the first basis function is a Gaussian whose width, determined by the parameter u , can be chosen to match the Gaussian employed in DTI while the others are orthogonal to it. Being the eigenfunctions of the Fourier operator, it is possible to represent both the signal and the propagator in the same basis. Note that the odd ordered terms are imaginary, thus accounting for any asymmetry of the propagator to be reconstructed. This representation has been found quite powerful as it is capable of representing even challenging signal profiles at a wide range of q -values (22).

The three-dimensional generalization of this approach has led to the development of the mean apparent propagator MRI (MAP-MRI) framework (23). A collection of scalar indices has been formulated based on this representation. The ability of the method to capture the higher q -behavior has enabled the estimation of the return-to-the origin probability (RTOP). A non-Gaussianity metric has been formulated based on the dissimilarity of the real propagator to the Gaussian propagator, which is given simply by the first term in the series. The separability of the three-dimensional formulation into one-dimensional representations has been exploited to decompose the indices into orientations parallel and perpendicular to the principal diffusion orientation. The anisotropy of the propagator was also quantified, as well as the portion of anisotropy due solely to non-Gaussian terms.

The efficiency provided by the Hermite function basis has enabled the implementation of the technique on data acquired in clinical scanners (24). Figure 6 illustrates the MAP-MRI indices as well as some common DTI metrics on data acquired in vivo using a clinical scanner.

2.2.3 The confinement tensor model

In a recent work, Yolcu et al. (25) introduced a new model of diffusion anisotropy based on a diffusive process involving particles subject to a spatially-linear (Hookean) restoring force (26). This problem was studied using three techniques; analytically via path integral methods (as discussed above for Gaussian diffusion), using a semi-analytic method via modifying the Bloch-Torrey equation, and numerically via random walk simulations; the results obtained were found to be in agreement.

In this model, the steady state distribution of particles is a Gaussian as illustrated in Figure 7 for the one-dimensional case. In three-dimensions, a tensorial force constant is employed, which leads to an oriented Gaussian distribution, and eventually, the anisotropy of the diffusion process (25). The problem has significant similarities with the much more challenging problem of restricted diffusion. In fact, there are several observations, which justify employing the confinement tensor model as a model of restricted diffusion. First, the mean-squared displacements in the confinement tensor framework, when plotted against the diffusion time, reaches a plateau just like in the case of restricted diffusion. This is a manifestation of the restricted diffusion, which justifies the employment of the model for representing the microdomains making up a compartmentalized specimen. Second, the convergence toward Gaussianity is indeed a well-known statistical characteristic of restricted diffusion; as the diffusion gradient pulse duration is prolonged, the distribution of the centers of mass for particle trajectories, and hence the MR signal approach a Gaussian (27), which is always the case for the

confinement tensor model. Finally, the stochastic average effective force exerted by the walls diffusing in a restricted space has a linear dependence on the trajectories' center of mass at long durations. These observations have led to the identification of the confinement model as the “effective theory” of restricted diffusion for many acquisition scenarios (28).

2.2.4 Tensor distribution models

Yet another statistical approach involves considering the signal as the superposition of signal contributions originating from subdomains, each of which is represented by a diffusion tensor (29). Thus, the notion of a diffusion tensor distribution becomes relevant. In this picture, the detected signal is represented through a Laplace transform defined over the manifold of three-dimensional symmetric positive definite matrices $\mathcal{P}_3$:

$$S(\mathbf{b}) = S_0 \int_{\mathcal{P}_3} e^{-\text{tr}(\mathbf{b}\mathbf{D})} dF , \quad [11]$$

where dF is a probability measure, indicating the probability distribution of random diffusion tensors. Jian et al. (29) reported that when the ensemble of tensors is characterized by a central Wishart distribution, the signal expression can be evaluated analytically and is characterized by the expression

$$S(\mathbf{b}) = |\mathbf{I} + \mathbf{b}\mathbf{\Sigma}|^{-p} , \quad [12]$$

where $\mathbf{I}$ is the identity matrix, p is the degree of freedom and $\mathbf{\Sigma}$ is the scale matrix of the Wishart distribution. The expected value of the tensor distribution is just $p\mathbf{\Sigma}$. The above expression is the signal response for any gradient waveform. For the Stejskal-Tanner sequence, the signal is given by the Rigaut type asymptotic fractal expression. Remarkably, this expression had been successfully employed to represent the signal decay for various specimens (30). One important feature of such signal decay is its power-law tail (31), see Figure 8. In a recent work, the above

findings were extended to non-central Wishart distributions; the generalized version was shown to yield improved accuracy when applied to the problem of resolving distinct fiber populations (32).

The tensor distribution models have been employed widely in recent years in studies aiming to elucidate the tissue microstructure. Such studies typically employ a model of microstructure, in which the tissue is thought to comprise distinct subdomains represented by a set of diffusion tensors, and the mesostructure is introduced *via* an orientation distribution function (33). Clearly, such models are special cases of the integral in Eq. [11]. The diffusion tensor distributions have also been employed in studies employing generalized gradient waveforms. For example, q-space trajectory imaging was introduced that employs acquisitions with measurement tensors of all ranks and it was shown that the second order mean diffusion tensor as well as the fourth order covariance tensor can be computed simply from the first and second terms of the Taylor series expansion of the signal (34). Several indices quantifying the various parameters of the microstructure have been introduced. In another interesting approach, Eq. 11 was reduced to a two-dimensional integral by taking or assuming the measurement and diffusion tensors to be axisymmetric (35). The simplified equation has been inverted in similar spirit with that in solid-state NMR techniques, yielding joint distributions of axial and radial diffusivities within heterogeneous specimens.

2.3 Microstructural models

Biological tissues are generally compartmentalized in part due to cell membranes that are semipermeable to water molecules. Given the limited spatial resolution of MRI, the diffusion processes within an imaging voxel are spatially heterogeneous. While statistical approaches for

analyzing diffusion signals offer insights into the statistical properties of the diffusion process, inferring the microstructural components that give rise to the diffusion properties is not trivial. To address this limitation, various biophysical models have been developed by incorporating microstructural models of the underlying tissues (36–39). The general approach is to separate the tissue into multiple compartments with each compartment has distinctive diffusion properties, e.g. slow vs. faster diffusion, isotropic vs. anisotropic diffusion and unidirectional vs. dispersive diffusion. These compartments are generally derived based on the biological entities, e.g. in white matter, one may have myelin, axon and interstitial space. Details of these models are reviewed in other articles within this special issue.

3 Integrating diffusion MRI with other modalities

In this section, we review other imaging modalities as they relate to diffusion MRI. In many situations, these imaging techniques provide complementary information, thus enabling an improved delineation of the underlying tissue structure. For example, the capability of diffusion MRI in probing tissue microstructure is largely due to the existence of anisotropic diffusion arising from anisotropic tissue microstructures. These anisotropic tissue microstructures give rise not only to diffusion anisotropy but also other anisotropic properties such as structural tensor in anatomical images including light and electron microscopy, anisotropic relaxivities, internal magnetic field gradients and magnetic susceptibility anisotropy. Incorporating diffusion measurements into relaxation studies could provide more specificity on the distribution of microscopic compartments making up the complex specimen. As discussed below, diffusion MRI can also be employed as a functional imaging method with some potential for improving our ability to monitor physiological processes.

3.1 Magnetic susceptibility anisotropy and susceptibility tensor

3.1.1 Susceptibility tensor imaging

Recent studies have shown that magnetic susceptibility of biological tissues such as brain white matter (40,41), myocardium (42,43), kidney (44) and cartilage (45) is anisotropic, i.e. their susceptibility values depend on their orientations with respect to the B_0 field. These findings have led to the development of susceptibility tensor imaging which models the anisotropic magnetic susceptibility as a second-order tensor described as follows (40)

$$f(\mathbf{k}) = B_0 \left(\frac{1}{3} \hat{\mathbf{H}}^T \chi(\mathbf{k}) \hat{\mathbf{H}} - \hat{\mathbf{H}} \cdot \mathbf{k} \frac{\mathbf{k}^T \chi(\mathbf{k}) \hat{\mathbf{H}}}{k^2} \right). \quad [13]$$

Here, $f(\mathbf{k})$ is the Fourier transform of the frequency shift map; $\mathbf{k}$ is the $\mathbf{k}$ -space vector; B_0 is the strength of the applied magnetic flux density (field strength of the magnet); $\hat{\mathbf{H}}$ is the unit vector of the applied magnetic field vector; $\chi(\mathbf{k})$ is the Fourier transform of the susceptibility tensor.

The susceptibility tensor shown in Eq. [13] is typically calculated from gradient echo phase images. Gradient echo phase can vary with respect to the relative orientation between the object (e.g. the brain) and the B_0 field simply due to the dipole field pattern; it can also vary due to anisotropic structural arrangement (46) and anisotropic magnetic susceptibility (40,41). Estimating the susceptibility tensor using Eq. [13] requires a minimum of 6 B_0 field orientations, which is typically accomplished by rotating the object within the magnet. As a result, in vivo studies utilizing STI have been limited due to the requirement to rotate the object.

3.1.2 Susceptibility anisotropy vs. diffusion anisotropy in the brain

In some cases, susceptibility anisotropy shares common microstructural components that underlie diffusion anisotropy; in many other cases, however, susceptibility and diffusion anisotropy have distinctive properties. Magnetic susceptibility anisotropy of brain white matter originates predominately from the myelin sheath while myelin accounts for less than 20% of

diffusion anisotropy (47,48). In a study of the dysmyelinating shiverer mouse that was genetically modified by deleting the genes encoding myelin basic protein, it was found that both the contrast between gray and white matter and the susceptibility anisotropy vanished, while diffusion anisotropy still appeared strong (47) (Fig. 9). Biophysical modeling also showed that specifically the radially aligned myelin lipids are the source of the orientation dependence of macroscopic magnetic susceptibility in normal mouse brain ex vivo and human brain in vivo (48). While both susceptibility and diffusion generally show high anisotropy in the white matter, clear differences have also been reported. For example, Li et al. showed that there is a lack of correlation between susceptibility anisotropy and diffusion anisotropy in human brain white matter (49). In major white matter tracts, while STI-reconstructed fibers appeared similar to DTI-reconstructed fibers, there was clear divergence in fiber orientations and sensitivity (50) (Fig. 10a). The performance of STI and DTI fiber tracking has also been compared in the myocardium of mouse heart (42,43)(Fig. 10b).

3.1.3 Susceptibility anisotropy vs. diffusion anisotropy in the kidney

Xie et al. investigated the susceptibility and diffusion anisotropy of renal tubules in the mouse kidney (44). In this study, STI data were acquired using a gradient multi-echo sequence on perfusion fixed mouse kidney on a 9.4T scanner. Apparent magnetic susceptibility demonstrated an orientation dependence of susceptibility contrast in the tubules. One surprising finding was that, STI was able to detect tracks in the renal cortex and outer medulla where DTI failed (Fig. 10c). The study also found that STI eigenvector pointing along the tubule axis was the minor eigenvector instead of the major eigenvector as occurred in the brain, suggesting that there is a consistent diamagnetic content in the direction of the renal tubule long axis. The renal epithelial cells generally have large surface area – this is critical for fluid absorption and for increasing the

number of membrane proteins in the kidney as well as other organs. The surface amplification is achieved through densely arranged microvilli of the brush border on the luminal (apical) plasma membrane and basement infoldings on the basolateral membrane. It was reasoned that these structures significantly increase the percentage of lipids pointing along the tubule's long axis which gives rise to the observed susceptibility anisotropy. The reasons that DTI failed to reconstruct the tubules in the cortex and outer medullar are the short diffusion distance compared to the diameters of the tubules and the tortuosity of the tubules. For example, the expected diffusion displacement measured in the experiments was 5.2 μm , while in the outer medullar, the thin limbs had limited straight segments, and the tubules also had larger diameters up to 46.7 μm . As a result, water diffusion would appear to be isotropic, and DTI was not successful in representing the anisotropic structure in the outer medullar.

3.1.4 Susceptibility anisotropy vs. diffusion anisotropy in the cartilage

In certain cases, susceptibility anisotropy is demonstratively more sensitive to tissue microstructure than diffusion anisotropy. One recent study showed that susceptibility anisotropy within articular cartilage produces zone-specific magnetic susceptibility contrast (45). When collagen fibrils are mostly oriented perpendicular to B_0 field, the susceptibility values are more paramagnetic, e.g. in the superficial zone. When collagen fibrils are mostly parallel to B_0 field, the susceptibility values become more diamagnetic, e.g. in the deep zone. The macroscopic susceptibility anisotropy of collagen was believed to originate from the diamagnetic anisotropy of the peptide group at the molecular level (45,51). The collagen triple helix contains peptide group planes oriented at approximately 45° to the fibril axis. Because the most diamagnetic susceptibility is oriented normal to the peptide group plane, the net susceptibility of collagen fibrils is mostly diamagnetic in the direction parallel to the fibril axis. Cumulatively, these

organized peptide groups yield the greatest diamagnetism in the direction of the collagen molecule long axis. This trend is the opposite of the susceptibility anisotropy observed in brain white matter. Tractography results revealed that STI clearly depicted the collagen fibril orientation beyond the capability of diffusion tensor imaging (DTI) (51) (Fig. 10d).

3.2 Structure tensor

Structure tensor is a quantity used commonly in image texture analysis. Structure tensor $\mathbf{S}_w(\mathbf{r})$ of a 2D image $\mathbf{I}$ (or a function in general) is a matrix defined based on the spatial gradients of the image as follows (52)

$$\mathbf{S}_w(\mathbf{r}) = w(\mathbf{r}) * \mathbf{S}_0(\mathbf{r}), \quad [14]$$

$$\mathbf{S}_0(\mathbf{r}) = \begin{bmatrix} I_x(\mathbf{r})I_x(\mathbf{r}) & I_x(\mathbf{r})I_y(\mathbf{r}) \\ I_x(\mathbf{r})I_y(\mathbf{r}) & I_y(\mathbf{r})I_y(\mathbf{r}) \end{bmatrix}, \quad [15]$$

where “*” represents the convolution operation and $w(\mathbf{r})$ is a weighting function (e.g. Gaussian). Structure tensor can be used to identify the predominant directions of the gradient in a specified neighborhood of a voxel, and the level to which those directions are coherent. This can be achieved by eigenvalue decomposition of the structure tensor to derive local orientation, anisotropy, and intensity similar to what’s done in DTI. Some recent studies have used structure tensor analysis of histological slides as a means to validate DTI. In one study, anisotropy measured with structure tensor analysis of stained histological sections was found to be highly correlated with anisotropy measured by ex vivo DTI of the same brains ($R^2 = 0.92$) (53). The same group also applied the technique to study the pathological substrates underlying the significant DTI abnormalities following traumatic brain injury (54). Using a rat model of controlled cortical impact, the study attempted to identify the specific pathological features that give rise to the diffusion tensor imaging changes in subacute to chronic traumatic brain injury. Cortical anisotropy was found to be independently associated (standardized $\beta = 0.62$, $P = 0.04$) with the coherent organization of reactive astrocytes (i.e. gliosis) and was not attributed to axons. On the other hand, it was

reported that a decrease in white matter anisotropy ($P < 0.001$) was significantly related to demyelination ($\beta = 0.75$, $P = 0.0015$) and to a lesser extent, axonal degeneration ($\beta = -0.48$, $P = 0.043$). Gliosis within the lesioned cortex was also found to influence diffusion tensor tractography; it was argued that spurious tracts observed in the injured brain might not necessarily reflect continuous axons and might instead depict glial scarring.

Structure tensor has also been used to inform diffusion-based fiber tractography by combining diffusion-weighted images and gradient-echo images (55). This structure tensor informed fiber tractography (STIFT) takes advantage of the white matter and gray matter contrast shown in gradient echo images at high field. The study described a method to calculate a data-adaptive structure tensor from the gradient echo image to describe the morphology of fiber bundles. The structure tensor was then incorporated in a tractography algorithm to modify the diffusion-based tracking direction according to structure tensor properties. The study reported that this gradient-echo structure tensor was informative for tractography. In one example, reconstruction of the optic radiation with STIFT showed a larger anterior extent of Meyer's loop compared to a standard tractography alternative. In another example, STIFT in multifiber voxels yielded a reduction in crossing-over of streamlines from the cingulum to the adjacent corpus callosum, while tracking through the fiber crossings of the centrum semiovale was unaffected (55). More studies are needed to verify the improvements reported by STIFT.

3.3 Internal magnetic field gradient

While the pulsed-gradient spin echo sequence uses strong gradient pulses to encode diffusion and reduces the relative contributions from the internal gradients, one may also use the diffusion effect in the internal gradient field to infer microstructural properties. In highly porous structures such as the trabecular bone system, the largest internal gradients (and thus regions of fast signal decay) exist close to the trabecular surfaces that are perpendicular to the applied field. In one study, signal decay due to diffusion in the internal field (DDIF) contrast was investigated in two different water saturated bovine

trabecular bone samples (56). The results confirmed that the DDIF contrast is related to the surface-to-volume ratio. A clear correlation was reported between the maxima in the DDIF rate and the minima in the spin density at the trabecular rod/plate locations. The distributions of DDIF rates were reportedly to qualitatively mimic the shapes seen in the bulk sample results, but show a slight dependence on image slice orientation, which the authors attributed to variation in cross-term diffusion weighting between internal and externally applied field gradients. The study concluded that although DDIF may potentially be less sensitive, it may offer a more specific contrast for trabecular number tracking than the T_2' approach. In another example, the diffusion in the internal field was used to visualize internal magnetic field gradients arising from susceptibility contrast between an array of cylindrical glass tubes (solid matrix) and surrounding water (pore fluids) in a uniform applied magnetic field (57). A stimulated echo sequence was performed to image the decay rates due to diffusion in the internal magnetic field. The authors computed the interference pattern of the cross-terms between the internal and the applied field gradient and used that to extract the orientation of the internal field gradient. The experimental results were found to be consistent with the theoretical calculations.

Without relying on diffusion effect, the internal field gradients may also be mapped using gradient echo frequency shift. In the zeroth order approximation, the mean magnetic susceptibility or susceptibility tensor can be measured using quantitative susceptibility mapping (QSM) (58–63) or susceptibility tensor imaging (40) respectively. QSM and STI estimates the mean magnetic susceptibility of a whole voxel. To obtain higher-order information, specifically the distribution of magnetic field within a voxel, Liu and Li proposed to apply an external field gradient, before the gradient echo readout, which will interfere with the internal field gradient and affect the mean frequency shift of the voxel (64). Since the amplitude and direction of the external gradient can be controlled, this interference has been shown to be able to estimate the distribution of the internal field in some simulated cases and areas of mouse brains. However,

the contrast induced by the gradient interference is generally weaker than the zeroth order information, thus difficult to estimate accurately.

3.4 Diffusion-Relaxation Correlation

Heterogeneous specimens are often characterized by a distribution of MR related parameters. For example, different transverse relaxation rates can be associated with different compartments within the medium. Let $R_2=1/T_2$ denote the transverse relaxivity and $p(R_2)$ be the distribution of relaxivities. The MR signal collected at the echo time TE can be written through the expression

$$S(TE) = \int_0^\infty p(R_2) e^{-TE R_2} dR_2 . \quad [16]$$

In other words, the signal is simply the Laplace transform of the relaxivity distribution. Thus, repeating the acquisition with a number of echo times, and inverting the Laplace transform would provide an estimate of $p(R_2)$.

The same idea can be employed for the diffusive characteristics, which is often reduced to a scalar-valued diffusion coefficient for each compartment. The experimentally controlled parameter is the b-value, and the analogous relationship is

$$S(b) = \int_0^\infty p(D) e^{-bD} dD . \quad [17]$$

In many situations of practical interest, distribution of relaxation times or diffusion coefficients alone is not sufficiently descriptive of the specimen's structure. Probing the distribution of both characteristics would make it possible to distinguish separate compartments that share the same relaxivity or diffusivity value. Multi-dimensional techniques have been introduced precisely for this purpose. From a practical point of view, a two-dimensional D-TE "plane" is sampled at a large number of points, and a joint distribution $p(D, R_2)$ is computed by inverting the two-dimensional Laplace transform (65,66)

$$S(b, TE) = \int_0^\infty \int_0^\infty p(D, R_2) e^{-TE R_2} e^{-bD} dR_2 dD. \quad [18]$$

Note that $p(D)$ and $p(R2)$ are the one-dimensional projections of the joint density $p(R2, D)$. In NMR spectroscopy literature, this two-dimensional technique is referred to as diffusion-relaxation correlation spectroscopy (DR-COSY). Inverting the Laplace transform, whether one- or multi-dimensional is a challenging but necessary component of all these methods, and several algorithms have been developed in that literature over the years to address this important issue (67,68). Very recently, a novel pulse sequence featuring frequency-swept RF pulses along with gradients was devised that introduces different amounts of diffusion weightings at different z coordinates within the specimen (69). Thus, a one-dimensional “image” provides the entire set of data points along the b -axis. When this sequence is followed by a Carr-Purcell-Meiboom-Gill (CPMG) pulse train, the entire b -TE plane can, in principle, be sampled in one-shot, providing one to two orders of magnitude gain in total acquisition time.

The above-mentioned challenges are invariably intensified when one attempts to spatially localize the NMR signal as in imaging studies (70). Ever increasing demand for accurately characterizing tissue structure alongside technological advances that has improved data quality in clinical scanners has prompted some groups to incorporate two-dimensional relaxation-diffusion encoding into standard image acquisitions for biomedical applications (71). To overcome the said difficulties, Kim et al. (72) utilized a spatial regularization scheme in addition to the commonly used non-negativity constraint and employed their method on a mouse model of traumatic spinal cord injury. Benjamini and Bassler (73) introduced physical constraints to the problem based on the one-dimensional marginal distributions; they report a substantial reduction in the required number of data points thereby shortening the acquisition time. In a recent study (74), the same authors reported that the two-dimensional spectra revealed different tissue components such as axons, neuronal and glial soma, macromolecules within myelin, and interstitial space in ferret spinal cord.

3.5 Diffusion-based fMRI

Over the last three decades, there have been a number of attempts in employing the diffusion-attenuated MR signal as a marker for brain activation. The progress over the years has closely followed technological advances, most notably by introducing larger and larger diffusion weightings while maintaining an acceptable signal to noise ratio.

Le Bihan et al. (75) argued that DWIs at b-values less than 100 s/mm² would be sensitive to blood microcirculation linked to a hemodynamic response, like in T2*-weighted blood oxygenation level dependent (BOLD) functional MRI (fMRI) (76–78). However, the latter offered better sensitivity and reliability. Zhong et al. reported increased apparent diffusion coefficient (ADC) values due to visual stimulation at higher b-values (700 s/mm²) attributing the effect to changes in local magnetic field gradients (79). An opposite effect, i.e., a slight reduction in the ADC values, was observed at b-values of around 1500 s/mm² (80). The underlying factor was speculated to be not related to blood flow, but due to cortical cells undergoing a transient swelling.

The question of employing diffusion MRI as a functional imaging technique was revisited by performing measurements at even higher b-values (up to b=2400 s/mm²) (81). In this b-value range, a single signal decay rate (diffusion coefficient) is insufficient to represent the signal profile. Instead, the biexponential model, given by

$$S = S_0 f e^{-b D_{slow}} + S_0 (1 - f) e^{-b D_{fast}}, \quad [19]$$

was used, which enables one to compute two diffusion coefficients. Here, f represents the signal fraction corresponding to the slower diffusion component, i.e., the one with smaller diffusivity. As shown in Figure 11 (left), as more and more diffusion-weighting is introduced, the signal

increase is observed 2-3 seconds earlier than that in the BOLD fMRI response. The signal profiles appear qualitatively similar, though the initial signal undershoot is absent in the case of diffusion-weighted signals. Moreover, the percentage signal increase is comparable to that in BOLD fMRI at larger b-values. The underlying mechanism of such changes have been proposed to be cell swelling, which is thought to alter the membrane surface area, thus changing the fraction of molecules bound to the membranes. These findings have prompted a number of studies aiming to reproduce the results and assess the possible underlying mechanisms.

Tsurugizawa et al. (82) reported that employing nitroprusside (a neurovascular coupling inhibitor) removed the hemodynamic response as expected. However, the local field potentials and diffusion response were maintained for the most part, suggesting that the underlying mechanisms of the signal changes observed via BOLD fMRI and diffusion fMRI could be different. More recent findings (83) based on ethanol injection into rat brains suggest that in the presence of complications due to vasoactive drugs, the diffusion fMRI signal outperforms the BOLD fMRI signal as an indicator of neuronal activity.

Miller et al. (84) performed measurements of mild hypercapnia, which alters the blood flow without affecting neuronal firing. Their findings suggested a signal change, which is similar to the trend in diffusion fMRI signal profile, i.e., the signal increases as b-value is increased. Moreover, the diffusion fMRI response did not appear to be substantially earlier than the T2*-weighted BOLD response. These observations lead to the conclusion that a substantial part of the diffusion fMRI signal change could be of vascular origin. A similar conclusion was reached in a recent study (85) performed on organotypic cortex cultures examined simultaneously via calcium fluorescence imaging and diffusion MR acquisitions performed using a one-sided desktop MR

system (86). Using tissue cultures is advantageous in that neuroactivity can be manipulated and monitored without the confounding effects such as hemodynamic changes. The authors observed an increase in diffusion-weighted signal coupled to the prolonged depolarization of neurons. However, when neuronal activity is suppressed, e.g., via introducing tetrodotoxin, the diffusion-weighted MR signal and the signal fraction for slow diffusion did not appear to change (Fig. 12) suggesting the lack of correlation with spontaneous neuronal activity.

The above findings are largely contradictory especially with regards to the timing of the diffusion-weighted signal change and also to its biophysical underpinnings. It should be noted however, that the biological models, as well as the experimental framework to investigate them, substantially differ in these studies. For reliably utilizing diffusion MRI as a functional imaging technique, there appears to be a need for establishing its feasibility and sensitivity in various situations. Poorer signal to noise ratio afforded by high b-value diffusion weighted acquisitions calls for the development of a robust and reliable diffusion fMRI protocol as well as an optimized data processing pipeline for clinical studies (87). Last but not least, a correct interpretation of the results would be achieved after a better understanding of the mechanisms that lead to the observed signal changes.

4 Discussions and Conclusions

Molecular diffusion has become one of the most widely used contrast in MRI, both for clinical applications and basic research. While the fundamental principle, i.e., random motion in inhomogeneous field inducing phase dispersion, has been understood since the early days of NMR, the understanding of diffusion signal measured by MRI in biological tissues, especially the brain, remains incomplete. The complexity of this relationship is a result of the complexity of

physiology and the extreme sensitivity of diffusion to tissue cellular structure. The relationship between diffusion MRI signal and tissue structure and function remains a highly active area of research. Integrating other information provided by MR or non-MR imaging modalities might be a key to improve our understanding of how diffusion MRI relates to physiology and biology.

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7 Figures

Figure 1. Overview of Bloch's equations with diffusion terms for a pulsed-gradient spin echo sequence. The diffusion terms are highlighted in red.

Ensemble Average of Spins with Random Phase

Figure 2. Overview of signal evaluation by ensemble average of spins with random phase accumulation due to diffusion in a gradient field.

Figure 3. Comparison of higher order statistical properties of Gaussian vs. non-Gaussian diffusion processes. Left shows three examples of probability distribution function and corresponding excess kurtosis; right shows a table of standardized cumulants and moments of Gaussian and non-Gaussian distributions. Notice that the Gaussian distribution has non-zero higher-order (higher than 2) standardized moments but not cumulants.

Generalized Bloch-Torrey	Statistical Approach
$\frac{\partial m}{\partial t} = -j\gamma(\mathbf{G} \cdot \mathbf{r})m + D_{i_1 i_2}^{(2)} \partial_{i_1} \partial_{i_2} m + D_{i_1 i_2 i_3}^{(3)} \partial_{i_1} \partial_{i_2} \partial_{i_3} m + \dots$	$\langle s \rangle = \int e^{-i2\pi \mathbf{q} \cdot \mathbf{r}} p(\mathbf{r}) d\mathbf{r}$
$\frac{m}{m_0} = \exp \left(\sum_{n=2}^{\infty} j^n b_{i_1 i_2 \dots i_n}^{(n)} D_{i_1 i_2 \dots i_n}^{(n)} \right)$	$\langle s \rangle = \exp \left(\sum_{n=2}^{\infty} (-j)^n \frac{K_{i_1 i_2 \dots i_n}^{(n)} q_{i_1} q_{i_2} \dots q_{i_n}}{n!} \right)$
$D_{i_1 i_2 \dots i_n}^{(n)}$: n -th order diffusion tensor	$K_{i_1 i_2 \dots i_n}^{(n)}$: n -th order cumulant tensor of $\mathbf{r}$
$K_{i_1 i_2 \dots i_n}^{(n)} = (-1)^n n! D_{i_1 i_2 \dots i_n}^{(n)} \left(\Delta - \frac{n-1}{n+1} \delta \right)$ <p>Higher order diffusion tensors are measures of variance, skewness, kurtosis etc.</p>	

Figure 4. Diffusion signal equation derived from the Bloch-Torrey equation and statistical approaches. Comparing the two results, one may derive the relationship between cumulants and higher order diffusion tensors.

$$\langle s \rangle = \exp \left(\sum_{n=2}^{\infty} (-j)^n \frac{K_{i_1 i_2 \dots i_n}^{(n)} q_{i_1} q_{i_2} \dots q_{i_n}}{n!} \right) \Rightarrow \text{Asymmetrical structure requires odd-order cumulants (} n \text{ is odd), thus phase in DWI.}$$

Figure 5. The need of phase information to reconstruct an asymmetrical structure.

Figure 6. DTI-based parameters of mean diffusivity (MD), fractional anisotropy (FA), and direction-encoded color (DEC) maps are given on the left column. The remaining images are derived through the MAP-MRI framework. On the top row are the return-to-the-origin probability (RTOP), and its variants for diffusion parallel and perpendicular to the principal diffusion orientation—the return-to-the-plane probability (RTPP), and the return-to-the-axis probability (RTAP), respectively. The second row includes the propagator anisotropy (PA) measure followed by a comparable quantity for its Gaussian part. The enhancement of anisotropy due to non-Gaussian diffusion is illustrated via the rightmost map in the second row. Finally, on the last row are the non-Gaussianity (NG) index followed by its decomposition into directions parallel and perpendicular to the principal diffusion orientation. Adapted from Avram et al (24) with permission.

Figure 7. The restricted diffusion process (top, left) can be restated as diffusion taking place within a potential energy profile, which takes a constant value within the pore space, and diverges at the walls. The equilibrium density is then constant within the pore, and vanishes at the walls (bottom, left). Many of the features of the restricted diffusion process are captured when one assumes a Gaussian equilibrium distribution (bottom, right). This distribution emerges for the problem involving particles subject to a Hookean restoring force (top, right). Here the potential is quadratic as a function of the distance (x) from the attractive center of the pore. The confinement value is defined to be related to the force constant by a factor of $k_B T$, where k_B is the Boltzmann constant and T is the absolute temperature (25). Both models represent restricted diffusion in the sense that the mean squared displacements are bounded. Under certain experimental conditions, the Gaussianity inherent to the confinement model is approached by the restricted diffusion process (28).

Figure 8. A simulation of the normalized signal intensity. The signal for only one compartment is a Gaussian. We gradually add the contributions from compartments with decreasing diffusivity. The power-law tail is obtained when all 10,000 tensors drawn from a Wishart distribution are included. Adapted from Jian et al. (29).

Figure 9. Dysmyelination resulting in total loss of susceptibility contrast between gray and white matter but a small loss of diffusion anisotropy. Adapted from Liu et al (47) with permission.

Figure 10. Comparisons of STI and DTI fiber tracking in (a) mouse brain, (b) mouse heart, (c) mouse kidney and (d) pig cartilage.

Figure 11. Left: The signal change associated with functional activation in diffusion-weighted acquisitions with varying diffusion sensitivity. The signal increase was assessed via statistical *t* test by pairwise comparisons of the signal at different *b* values and found to be significant. The marks *, **, and *** correspond to cases $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Right: The black rectangular line indicates the time interval during which visual stimulus was induced by showing the patient the projection of a flickering dartboard. The red and blue curves depict the time courses of the slow diffusion phase (SDP) obtained from the diffusion signal values and the same for the BOLD fMRI signal, respectively. Figure adopted from Le Bihan et al. (81) with permission.

Figure 12. Neuronal activity is suppressed via TTX as shown by the calcium fluorescence signal on the top. The arrow on the left indicates the start of the TTX perfusion while the one on the right shows the washout with normal artificial cerebrospinal fluid (ACSF). The simultaneous measurement of the slow diffusion component of the signal is shown in the second row. The red curve is obtained by performing the average of six nearest data points. Figure adapted from Bai et al. (85) with permission.