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New Histological and Physiological Stains Derived from Diffusion-Tensor MR Images

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INTRODUCTION

The measurement of the self-diffusivity of water (and other solvents) using the phenomenon of nuclear magnetic resonance was first reported more than four decades ago.[1] Methodological improvements in these diffusion measurements[2] and the subsequent development of magnetic resonance imaging,[3] together created the possibility to measure diffusion properties of water in tissues on a voxel by voxel basis. Diffusion imaging (DI), which was first realized in 1985,[4–6] consists of measuring an apparent diffusion constant (ADC)[7] in each voxel. Both its theoretical underpinnings and its applications are well-known and are described in a number of excellent books and review articles.[7,8]

While in tissues such as brain gray matter the ADC measured by diffusion imaging is largely independent of the orientation of the tissue, in brain white matter the ADC depends strongly upon the orientation of the tissue.[9–16] Since the ADC characterizes molecular displacements in only one direction, it inherently does not provide enough information to describe the three-dimensional translational displacements of protons (or other labeled nuclei) necessary to characterize diffusion in brain white matter and other anisotropic media. This additional information, however, is provided by the effective or apparent diffusion *tensor* of water, $\underline{D}$, in each voxel. The measurement of $\underline{D}$ in each voxel and the analysis and display of the information derived from it is called diffusion tensor imaging (DTI).[17] Of particular interest are new scalar parameters that possess properties of a quantitative histological or physiological stain and can be displayed as images that elucidate intrinsic features or characteristics of diffusion in tissues. Examples include images or maps of the mean diffusivity and the degree of diffusion anisotropy.

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BACKGROUND

In diffusion imaging (DI), one measures a single scalar apparent diffusion constant (ADC) in each voxel from a series of diffusion-weighted images (DWIs). These are just conventional MRIs whose contrast is sensitized or weighted by the local diffusivity in each voxel. Specifically, from these DWIs, one uses linear regression of Eq. (1) below to estimate an ADC in each voxel:

$$\ln\left(\frac{A(b)}{A(0)}\right) = -\,b\,D = -\,b\,ADC, \tag{1}$$

where A(b) is the measured echo magnitude in each voxel; b is a constant called the b-value or b-factor, which is calculated for each gradient pulse sequence[18]; and A(0) is the echo magnitude without any applied diffusion gradients. Whether one is acquiring DWIs or maps of the ADC, DI is inherently a *one-dimensional* technique, that is, it can only meaningfully measure molecular displacements along one direction.

Diffusion tensor imaging (DTI)[17] is a new MRI modality that was developed to describe diffusion in anisotropic medium for which Eq. (1) is no longer valid. With DTI, one estimates an effective diffusion tensor, $\underline{D}$, from DWIs using a more general relationship between the measured echo magnitude in each voxel and the applied magnetic field gradient sequence[19–21]:

$$\ln\left(\frac{A(\underline{b})}{A(\underline{0})}\right) = -\sum_{i=1}^{3}\sum_{j=1}^{3} b_{ij}D_{ij} = -\,\text{Trace}\,(\underline{b}\,\underline{D})$$

$$= -\,(b_{xx}D_{xx} + 2b_{xy}D_{xy} + 2b_{xz}D_{xz} + b_{yy}D_{yy} + 2b_{yz}D_{yz} + b_{zz}D_{zz}) \tag{2}$$

Above, b_{ij} is a component of the symmetric b-matrix, $\underline{b}$, and $A(\underline{b})$ the echo magnitude for a gradient sequence whose b-matrix is $\underline{b}$. Whereas in DI a b-factor is usually calculated for a gradient sequence applied in one direction, in DTI the b-matrix is always calculated from all applied gradient sequences (including all imaging and diffusion gradient sequences).[19–21]

To understand the role of the b-matrix in DTI, it is useful to view the diffusion process in the principal frame of the anisotropic medium, by diagonalizing $\underline{D}$ as follows:

$$\underline{D} = \underline{E}\,\underline{\Lambda}\,\underline{E}^{T} \tag{3}$$

Above, $\underline{E}$ is the matrix whose columns are the eigenvectors or principal directions of $\underline{D}$: ϵ_1, ϵ_2, and ϵ_3; and $\underline{\Lambda}$ is the diagonal matrix whose diagonal elements are the corresponding eigenvalues or principal diffusivities of $\underline{D}$: λ_1, λ_2, and λ_3. Using Eq. (3), and the fact that Trace($\underline{M}\,\underline{N}$) = Trace ($\underline{N}\,\underline{M}$) for two

matrices $\underline{M}$ and $\underline{N}$, Eq. (2) can be rewritten as:

$$\ln\left(\frac{A(\underline{b})}{A(\underline{b}=\underline{0})}\right) = -\,\mathrm{Trace}(\underline{b}\,\underline{E}\,\underline{\Lambda}\,\underline{E}^{T}) = -\,\mathrm{Trace}(\underline{E}^{T}\,\underline{b}\,\underline{E}\,\underline{\Lambda})$$

$$= -\,\mathrm{Trace}\,(\underline{b}'\,\underline{\Lambda}). \qquad (4)$$

The matrix $\underline{b}' = \underline{E}^{T}\,\underline{b}\,\underline{E}$ is just the b-matrix in the principal frame of $\underline{D}$. The diagonal elements of $\underline{b}'$: b_{11}', b_{22}', and b_{33}' represent the projections of the original b-matrix (measured in the laboratory frame) along the principal directions of $\underline{D}$. Now, expanding Eq. (4), we obtain the following simple formula:

$$\ln\left(\frac{A(\underline{b}')}{A(\underline{b}'=\underline{0})}\right) = -\,b_{11}'\lambda_1 - b_{22}'\lambda_2 - b_{33}'\lambda_3 \qquad (5)$$

In the principal frame of the anisotropic medium, the contribution of each principal diffusivity on the echo attenuation is seen to be weighted by its corresponding b′-matrix element. Whereas in isotropic diffusion there is a single b-factor premultiplying the diffusion coefficient, in anisotropic diffusion there are three coefficients premultiplying each of the three principal diffusivities.

Once a b-matrix element has been calculated for each DWI, we estimate $\underline{D}$ from all the DWIs using multivariate linear regression[b] of Eq. (2). One requirement of DTI is that we apply diffusion gradients in a multiplicity (at least six) noncollinear direction.[21]

Diffusion tensor imaging subsumes diffusion imaging; the former reduces to the latter when the sample is isotropic. In such cases, it can be shown that Eq. (2) reduces to Eq. (1).

Diffusion of a Water in an Isotropic, Homogeneous Medium

Diffusion isotropy describes the case in which the translational mobility of the diffusing molecule is independent of the medium's orientation. Homogeneous diffusion refers to the case in which the translational mobility of the diffusing molecule is independent of the position within the medium. If a medium is both isotropic and homogeneous, then the translational displacement profile is given by[22]:

$$\rho(\mathbf{r}|\tau_d) = \frac{1}{\sqrt{(4\pi D\tau_d)^3}}\exp\left(-\frac{\mathbf{r}^{T}\mathbf{r}}{4D\tau_d}\right) = \frac{1}{\sqrt{(4\pi D\tau_d)^3}}\exp\left(-\frac{x^2+y^2+z^2}{4D\tau_d}\right)$$

[b]Multivariate linear regression is just one of a number of statistical techniques that could be used to estimate $\underline{D}$ from the echo data.

Above, $\rho(\mathbf{r}|\tau_d)$ is the probability that a particle located initially at position $\mathbf{r} = \mathbf{0}$ is located at position $\mathbf{r}$ at a later time τ_d. Surfaces of constant $\rho(\mathbf{r}|\tau_d)$ are concentric spheres ("diffusion spheres"), as we see by setting the exponent of Eq. (6) equal to a constant. When we choose the constant to be ½,

$$x^2 + y^2 + z^2 = (\sqrt{2D\tau_d})^2 = 2D\tau_d. \tag{7}$$

Then, the radius of the diffusion sphere, $\sqrt{2D\tau_d}$, is also the standard deviation of $\rho(\mathbf{r}|\tau_d)$, σ, defined by the well-known Einstein formula[22]:

$$\sigma = \sqrt{2D\tau_d} \tag{8}$$

Thus, the radius of this particular diffusion sphere has the physical interpretation of being the mean-squared displacement of a particle released at the center of the sphere at time τ_d. The translational displacement profile of water is spherically symmetric in this case, and is completely specified by a single scalar constant, D, the diffusion coefficient; and the diffusion time, τ_d.

Diffusion of a Water in an Anisotropic, Homogeneous Medium

Recall that diffusion anisotropy is a property of certain media in which the translational mobility of the diffusing molecule depends upon the medium's orientation. In biological tissues such as brain white matter, we can ascribe anisotropic diffusion (observed in MR spectroscopy or imaging studies) to spatial variations of molecular mobility (heterogeneity) at micron and submicron length scales. This phenomenon appears to be caused primarily by the spatial arrangement of macromolecular, membranous, and fibrous constituents and their interfaces. In such tissues, diffusion anisotropy can be characterized within a macroscopic voxel by an effective diffusion tensor, $\underline{D}$. The voxel-averaged displacement distribution is now slightly more complicated:

$$\rho(\mathbf{r}|\tau_d) = \frac{1}{\sqrt{|\underline{D}|(4\pi\tau_d)^3}} \exp\left(\frac{-\mathbf{r}^T\underline{D}^{-1}\mathbf{r}}{4\tau_d}\right). \tag{9}$$

Whereas in an isotropic medium D appears in the variance of the distribution [Eq. (6)], in an anisotropic medium $\underline{D}$ appears in the "matrix of variances and covariances"[23] [Eq. (9)]. Whereas D^3 appeared in the normalization factor of $\rho(\mathbf{r}|\tau_d)$ in Eq. (6), $|\underline{D}|$ (the determinant of $\underline{D}$) appears in its place in Eq. (9). When we construct surfaces of constant probability (again by setting the exponent of $\rho(\mathbf{r}|\tau_d)$ to a constant), we now obtain instead:

$$\begin{aligned}(D_{yy}D_{zz} - D_{yz}^2)x^2 + 2(D_{xz}D_{yz} - D_{xy}D_{zz})xy + (D_{xx}D_{zz} - D_{xz}^2)\,y^2 \\ 2(D_{xy}D_{yz} - D_{xz}D_{yy})xz + 2(D_{xy}D_{xz} - D_{xx}D_{yz})yz \\ + (D_{xx}D_{yy} - D_{xy}^2)z^2 = |\underline{D}|\tau_d,\end{aligned} \tag{10a}$$

which, rewritten in a more familiar form,

$$a\,x^2 + 2b\,xy + dy^2 + 2c\,xz + 2e\,yz + f\,z^2 = 1, \qquad \textbf{(10b)}$$

is easily recognized as the equation of a three-dimensional ellipsoid,[c] called the "diffusion ellipsoid."[17,24]

Clearly, in an anisotropic medium, six independent parameters (a—f in Eq. **(10b)** or equivalently the six independent coefficients of $\underline{D}$: D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz}, and D_{yz}) are required to describe the three-dimensional displacements of particles, whereas in an isotropic medium, only one parameter, D, is sufficient. These additional parameters are required because in anisotropic media, displacements generally appear to be *correlated* in both parallel and perpendicular directions, whereas in isotropic media they do not. In fact, the elements of the diffusion tensor represent the magnitude of the correlations between the translational displacements in parallel and perpendicular directions. Specifically, the diagonal elements of $\underline{D}$, D_{xx}, D_{yy}, and D_{zz} represent the strength of correlations between molecular displacements along the same directions (i.e., along x, y, and z, respectively), while its off-diagonal elements, D_{xy}, D_{xz}, D_{yz}, represent strength of correlations in molecular displacements along perpendicular directions (i.e., between x and y, x and z, and y and z, respectively). In anisotropic media the diagonal elements of the diffusion tensor are generally unequal, whereas in isotropic media they are all equal. Moreover, in anisotropic media the off-diagonal elements are generally non-zero and may be large (i.e., comparable in magnitude to the diagonal elements), whereas in isotropic media they all equal zero.

For an anisotropic medium, we can always find a preferred frame of reference, generally other than the laboratory frame, in which translational displacements in orthogonal directions appear to be uncorrelated. This is called the "principal frame." Thus, in this frame all off-diagonal elements of the diffusion tensor vanish. The new coordinate axes are now coincident with the principal axes of the diffusion ellipsoid, and the equation describing the diffusion ellipsoid, Eq. **(10)**, assumes a simpler, familiar form:

$$\left(\frac{x'}{\sqrt{2\lambda'_{xx}\tau_d}}\right)^2 + \left(\frac{y'}{\sqrt{2\lambda'_{yy}\tau_d}}\right)^2 + \left(\frac{z'}{\sqrt{2\lambda'_{zz}\tau_d}}\right)^2 = 1. \qquad \textbf{(11)}$$

Above λ_{xx}', λ_{yy}', and λ_{zz}' are the principal diffusivities along the three respective principal directions; and $\sqrt{2\lambda'_{xx}\tau_d}$, $\sqrt{2\lambda'_{yy}\tau_d}$, $\sqrt{2\lambda'_{zz}\tau_d}$, are the mean-squared displacements of a molecule along the (three principal) x′, y′, and z′ directions at time τ_d, respectively. The mean-squared displacements are represented as the lengths of the major and minor axes of the diffusion ellipsoid. It is important to note that in most MRI applications, the principal

[c]Both $\underline{D}$ and the coefficient matrix are positive definite.

axes of the diffusion ellipsoid are not known *a priori*, and generally do not coincide with the x-y-z laboratory axes.[20]

In summary, the diagonal and off-diagonal elements of $\underline{D}$ are essential in specifying the probability distribution in Eq. (9), and characterizing the *size, shape,* and *orientation* of the diffusion ellipsoid in the (x-y-z) laboratory coordinate frame. Below we will see that they are also required to calculate new MRI stains.

QUANTITATIVE DIFFUSION TENSOR IMAGING—DEVELOPING AND USING MRI "STAINS"

Characterizing Diffusion Isotropy

Moseley and colleagues discovered in animals[25–27] and Warach *et al.* later showed in humans[28,29] that a reduction in the ADC is a sensitive indicator of the onset and severity of a cerebral ischemic event. However, Moseley also showed that while in gray matter (where diffusion is approximately isotropic) the ADC is independent of the direction of the diffusion sensitizing gradients, in white matter this is not the case.[13] In white matter, the contrast of the DWI (or of the ADC) in a voxel also depends on the direction in which the diffusion sensitizing gradient is applied with respect to the direction of the white matter fiber tracts in that voxel. This introduces an additional source of image contrast which complicates the interpretation of diffusion images in anisotropic white matter. Why? In white matter, one cannot ascertain whether the measured image contrast results from a structural/physiologic change (brought on by the ischemic event itself), or arises from diffusion anisotropy in the tissue (i.e., the dependence of the ADC to the relative orientation of the applied diffusion gradient and the local fiber orientation). Clearly, in ischemia monitoring, diffusion anisotropy in white matter produces an unwanted artifact that complicates the interpretation of diffusion images.

Diffusion tensor spectroscopy[20,30] and diffusion tensor imaging[17,31] provided new imaging parameters that solved this and other vexing MR imaging problems. Associated with each diffusion tensor are scalar quantities known as *invariants* that are *intrinsic* to the medium. Specifically, these parameters (and functions of them) are independent of the orientation of the tissue structures, their relative orientation to the patient's body within the MR magnet, the direction of the applied imaging and diffusion sensitizing gradients, and the choice of the laboratory coordinate system (in which the components of the diffusion tensor and magnet field gradients are measured).[20,21] In 1992, the scalar invariants of $\underline{D}$ were first proposed as novel MR parameters and were shown experimentally to be independent of fiber tract direction in anisotropic skeletal muscle.[30]

The three fundamental scalar invariants of $\underline{D}$, I_1, I_2, and I_3, are the

coefficients of the characteristic equation of $\underline{D}$:

$$\lambda^3 - I_1\lambda^2 + I_2\lambda - I_3 = 0, \quad (12)$$

which is used to calculate the three principal diffusivities (λ_1, λ_2 and λ_3) of $\underline{D}$. I_1, I_2, and I_3 can be calculated directly from the diffusion tensor, or expressed in terms of λ_1, λ_2 and λ_3:

$$I_1 = \lambda_1 + \lambda_2 + \lambda_3; \quad I_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_1\lambda_3; \quad I_3 = \lambda_1\lambda_2\lambda_3. \quad (13)$$

Another desirable property of the scalar invariants is that each is independent of the assignment or order of the principal diffusivities or eigenvalues. Therefore, if we permute the subscripts of the eigenvalues, the value of a scalar invariant is unchanged. The same property holds for functions of the scalar invariants. Moreover, each scalar invariant has a distinct geometrical (and physical) interpretation. I_1 is proportional to the sum of the squares of the major and minor axes of the diffusion ellipsoid, I_2 is proportional to the sum of the squares of the areas of the three principal ellipses of the diffusion ellipsoid, and I_3 is proportional to the square of the volume of the diffusion ellipsoid.

The First Invariant—The Trace of the Diffusion Tensor

The first scalar invariants, I_1 can be written in several ways:

$$I_1 = \text{Trace}(\underline{D}) = D_{xx} + D_{yy} + D_{zz} = 3\langle D\rangle = \lambda_1 + \lambda_2 + \lambda_3 = 3\langle\lambda\rangle. \quad (14)$$

It is proportional to the orientationally averaged apparent diffusivity.[32] To see this, note that according to the Einstein equation, the mean-squared displacement in the i^{th} principal direction, $\langle r_i^2\rangle$, is given by:

$$\langle r_i^2\rangle = 2\lambda_i\tau \quad (15)$$

in a diffusion time τ, so the mean-squared displacement averaged along the three principal directions, $\langle\langle r^2\rangle\rangle$ is

$$\langle\langle r^2\rangle\rangle = \frac{\langle r_x^2\rangle + \langle r_y^2\rangle + \langle r_z^2\rangle}{3} = 2\frac{\lambda_1 + \lambda_2 + \lambda_3}{3}\tau = 2\langle\lambda\rangle\tau \quad (16)$$

This is the same result one obtains by averaging the mean-squared displacement uniformly over all directions.[32]

Characterizing Diffusion Anisotropy

Although $\text{Trace}(\underline{D}) = I_1$ characterizes the mean diffusion properties in a voxel, it provides no information about diffusion anisotropy within a voxel. However, the second and third invariants of $\underline{D}$ do. One potentially useful measure of diffusion anisotropy is their ratio, I_2/I_3, which can be interpreted as

the square of the surface-to-volume ratio of the diffusion ellipsoid. We would expect this quantity be a minimum in an isotropic medium in which the diffusion ellipsoid is a sphere, and to increase monotonically as the diffusion ellipsoid becomes more eccentric. However, the surface-to-volume ratio has units of inverse length. Since we prefer to have a non-dimensional measure of anisotropy, we can normalize it accordingly:

$$\text{"S-to-V"} = \frac{(2I_2)^{3/2}}{I_3} = \frac{(2(\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_1\lambda_3))^{3/2}}{\lambda_1\lambda_2\lambda_3} \tag{17}$$

Rotational invariance of "S-to-V" is assured because it depends solely on the ratio of two scalar invariants.

Another approach to characterizing diffusion anisotropy is to determine the magnitude of the anisotropic part of the diffusion tensor in each voxel. This can be done by decomposing $\underline{D}$ into its isotropic and anisotropic parts[33]:

$$\underline{D} = \underbrace{\langle D \rangle \underline{I}}_{\substack{\text{isotropic} \\ \text{tensor}}} + \underbrace{(\underline{D} - \langle D \rangle \underline{I})}_{\substack{\text{anisotropic} \\ \text{tensor}}}. \tag{18}$$

The isotropic part of the diffusion tensor is the familiar mean diffusivity, $\langle D \rangle$, multiplied by the identity tensor, $\underline{I}$, while the anisotropic part of $\underline{D}$ is what we call the "diffusion deviatoric" or "diffusion deviation tensor," $\underline{D}$[33]:

$$\underline{D} = \underline{D} - \langle D \rangle \underline{I} \tag{19}$$

The first invariant of $\underline{D}$, $I_1' = \text{Trace}(\underline{D})$ can be shown to be zero,[33] while the other scalar invariants of $\underline{D}$, I_2' and I_3', are simply related to I_1, I_2 and I_3 (e.g., see Ref. 34):

$$I_2' = I_2 - \frac{1}{3} I_1^2 \quad \text{and} \quad I_3' = I_3 - \frac{1}{3} I_1 I_2 + \frac{2}{27} I_1^3 \tag{20}$$

While in their present form I_2' and I_3' are not too informative, they can be rewritten[d] to reveal interesting features about tissue microstructure. In particular, I_2' can be shown to be proportional to the mean-squared deviation of the eigenvalues or principal diffusivities with respect to their mean value:

$$I_2' = \frac{(\lambda_1 - \langle\lambda\rangle)^2 + (\lambda_2 - \langle\lambda\rangle)^2 + (\lambda_3 - \langle\lambda\rangle)^2}{2} = \frac{3}{2}\,\text{Variance}(\lambda). \tag{21}$$

[d] I_2' and I_3' are easily expressed as functions of the form Trace ($\underline{D}^n$) (where $\underline{D}^n$ signifies multiplication of $\underline{D}$ by itself n times).

$$I_2' = \frac{1}{2}\text{Trace}(\underline{D}^2) \quad \text{and} \quad I_3' = \frac{1}{3}\text{Trace}(\underline{D}^3)$$

This method to generate scalar invariants is well-known in the continuum mechanics literature.[34]

It was recently proposed as a measure of diffusion anisotropy[35] as it (i) is a scalar invariant quantity and it (ii) measures the magnitude of the anisotropic part of the diffusion tensor, $\underline{D}$ in Eq. **(18)**.[33] In the context of the DTI experiment, I_2' can also be interpreted as being proportional to the sample variance of the *estimated* eigenvalues in each voxel.

While I_2' measures the amount by which the measured eigenvalues of $\underline{D}$ deviate from their sample mean, it does not reflect how they are distributed about it. Conturo *et al.*[36] recently intimated that higher moments could. When might this information be useful? Suppose diffusivity were large along one principal direction, and were much smaller in the two transverse directions, that is, $\lambda_1 \gg \lambda_2 \approx \lambda_3$. Then, the corresponding diffusion ellipsoid would be "cigar"-shaped. This shape has recently been observed in white matter fibers in the corpus callosum and in the pyramidal tract in monkeys[37] and in humans.[38] Now, suppose that $\lambda_1 \approx \lambda_2 \gg \lambda_3$. This corresponds to a diffusion ellipsoid that is "pancake"-shaped. While in general, I_2' cannot distinguish between these two cases, the third moment or skewness should be able to.

$$I_3' = \frac{(\lambda_1 - \langle\lambda\rangle)^3 + (\lambda_2 - \langle\lambda\rangle)^3 + (\lambda_3 - \langle\lambda\rangle)^3}{3} = \text{Skewness}(\lambda), \qquad (22)$$

For the cigar-shaped diffusion ellipsoid, the skewness of the estimated eigenvalues would be negative, while for the pancake-shaped ellipsoid, it would be positive (depending on whether $\langle\lambda\rangle$ is significantly greater than or less than λ_2). Higher moments of the eigenvalues of $\underline{D}$ may furnish additional information about their distribution, although they may be increasingly susceptible to noise (e.g., see Ref. 37).

Other potentially informative anisotropy indices are the ratios of the principal diffusivities.[17] These dimensionless ratios measure the relative effective diffusivities in the three principal directions. Effectively, they measure the prolateness or eccentricity of the diffusion ellipsoid, independent of its size and orientation. If we number the principal diffusivities in decreasing order, the dimensionless anisotropy ratio, λ_2/λ_3, then measures the degree of cylindrical symmetry (with $\lambda_2/\lambda_3 = 1$ indicating perfect cylindrical symmetry). To measure the relative magnitude of the diffusivities along the fiber-tract direction and the two transverse directions, we can calculate λ_1/λ_2 and λ_1/λ_3, or, as above, measure the eccentricities of the two remaining great ellipses obtained from the diffusion ellipsoid that also contain its major axis (i.e., the axis along the fiber tract direction).[e] Pierpaoli and Basser[37] recently showed that while in principle these quantities are physically meaningful, in practice they are highly susceptible to noise in the MRIs, which introduces a bias when the eigenvalues are sorted according to size.[37,39]

[e]While the ratios of the eigenvalues of $\underline{D}$ represent the ratios of its principal diffusivities, it may be preferable to measure the ratios of the mean squared diffusion distances. This can be done simply by taking the square roots of the ratios presented above, i.e. $\sqrt{\lambda_i/\lambda_j}$

In summary, diffusion anisotropy is an intrinsic feature of the tissue, so its measures should be independent of the sample's placement or orientation with respect to the (laboratory) x-y-z reference frame.[35] Characterizing the degree of diffusion anisotropy is tantamount to characterizing features of the shape of a three-dimensional diffusion ellipsoid, independent of its orientation, and size. Thus, it is easy to see that knowing only the diagonal elements of the diffusion tensor is not adequate to characterize diffusion anisotropy. One should know at least the three eigenvalues of the diffusion tensor, and preferably higher moments of their distribution. In most MRI applications we typically do not know the eigenvalues *a priori*. We generally calculate them from the estimated diagonal *and* off-diagonal elements of $\underline{D}$.

COMBINING STAINS OF ISOTROPIC AND ANISOTROPIC DIFFUSION

One way to display information simultaneously about isotropic and anisotropic diffusion using a single image is by representing the three (sorted) principal diffusivities, λ_1, λ_2, and λ_3, using red, green, and blue (R-G-B) intensities, respectively.[40] Ideally, isotropic regions should appear as a shade of gray, whereas anisotropic tissues should appear colored. However, this display method still requires sorting the eigenvalues in each voxel, (for example, in decreasing order), making it susceptible to the same bias that afflicts images of the ratios of the principal diffusivities.[37] Still, this color imaging scheme is superior to one proposed in which R-G-B colors are assigned to the DWIs measured in the x-, y-, and z- directions, respectively.[41,42] Latour's method[40] does not introduce an orientational artifact, i.e., a change in hue or intensity if the laboratory frame or the sample is rotated, whereas Nakada's method[41] does.

OTHER STAINS DERIVED FROM THE DIFFUSION TENSOR

One might think of a stain as a scalar quantity, but it does not have to be. The diffusion ellipsoid that we construct in each voxel is also an invariant quantity whose size, shape, and orientation do not vary with respect to translation or rotation of the laboratory coordinate system. The same holds for the eigenvectors of the diffusion tensor.

One of the most intriguing applications of diffusion tensor imaging is in developing MRI stains that reveal new *architectural* features of anisotropic structures such as fiber tract directions in brain and other tissues. So far, we have concentrated our efforts on developing MRI stains based upon diffusion tensors measured within each voxel. However, useful information also is found in the *pattern* of diffusion tensors or quantities derived from them, which could provide additional insights about tissue organization, structure, and function. For example, if we take the local nerve fiber tract direction in

each voxel (given by the eigenvector associated with the largest eigenvalue), we can surmise that the fiber-tract *pattern* or direction field contains useful biological and clinical information. Its temporal evolution from the embryonic to adult stages may be of interest in understanding dynamical processes in normal and abnormal brain development. Moreover, subsequent alterations may indicate degeneration, aging, or disease. The geometry of various cortical regions, in particular, the curving and twisting of fiber tracts may be useful in elucidating organizing principles of information processing within the brain.

While we have previously used tensor algebraic approaches to obtain information about the *pattern* of diffusion tensors in an image,[33] we can also apply concepts from differential geometry to identify new and useful features of the *diffusion tensor field*. One way to exploit constructs of differential geometry is to treat each diffusion tensor estimated in each voxel as a discrete, volume-averaged sample of a diffusion tensor field. In some cases, we can establish a correspondence between the three normalized orthogonal eigenvectors of the diffusion tensor: ϵ_1, ϵ_2, and ϵ_3, and the three orthogonal vectors that describe a space curve, $\mathbf{r}(s)$, in three dimensions: $\mathbf{t}(s)$, $\mathbf{n}(s)$, and $\mathbf{b}(s)$ (where s is the arc length). Above, $\mathbf{t}(s)$ is the unit tangent vector to the curve, $\mathbf{n}(s)$ is the principal normal vector, and $\mathbf{b}(s)$ is the binormal vector. Together, they constitute a "moving trihedron" that follows the space curve.[43] These vectors also define three mutually orthogonal planes, the normal plane, the rectifying plane, and the osculating plane which are normal to $\mathbf{t}(s)$, $\mathbf{n}(s)$, and $\mathbf{b}(s)$, respectively. If we assume that the fiber tracts are continuous from voxel to voxel, we can use the spatial variation of these vectors to characterize *intrinsic* local features of these curves, namely, their *torsion* and *curvature*. The curvature vector, $\mathbf{k}(s)$, is defined below as follows:

$$\mathbf{k}(s) = \frac{d\mathbf{t}}{ds}$$

and its magnitude is the curvature, $\kappa(s)$. The torsion, $\tau(s)$, is defined as

$$\tau(s) = -\frac{d\mathbf{b}}{ds} \cdot \mathbf{n}.$$

Once $\mathbf{t}(s)$, $\mathbf{n}(s)$, and $\mathbf{b}(s)$ are determined in each voxel, we can plot scalar functions of them, $\kappa(\mathbf{r})$ and $\tau(\mathbf{r})$ in each voxel so that now the curvature and torsion are displayed in each voxel. These intrinsic, rotationally and translationally invariant parameters specify new characteristics of the fiber-tract pattern within each voxel. (N.B.: In tissues like skeletal muscle and white matter, we can safely assign the tangent vector to be parallel to the eigenvector associated with the largest eigenvalue in a voxel. However, the eigenvectors are known to within a factor of -1. Therefore, a convention for determining a positive and negative fiber direction must be established. To this author's knowledge, developmental or histological reasoning do not suggest such a

convention at the present time. A right-handed coordinate system can then be constructed coincident with all three eigenvectors. The assignment of the **n** and **b** vectors could be performed on the basis of the relative magnitudes of the remaining eigenvalues.)

VECTOR CALCULUS OPERATIONS APPLIED TO THE FIBER DIRECTION FIELDS

Other vector operations applied to the diffusion tensor field should provide new information. The divergence of a vector field produces a scalar field that is rotationally and translationally invariant, like the other stains we have discussed so far. Such is the case if we compute the divergence of the tangent vector field given as a function of **r**, **t** (**r**), as well as for **n**(**r**), and **b**(**r**):

$$\nabla \cdot \mathbf{t}(\mathbf{r}); \quad \nabla \cdot \mathbf{n}(\mathbf{r}); \quad \nabla \cdot \mathbf{b}(\mathbf{r}); \tag{25a}$$

where

$$\nabla \cdot \mathbf{t}(\mathbf{r}) = \frac{\partial t_x(\mathbf{r})}{\partial x} + \frac{\partial t_y(\mathbf{r})}{\partial y} + \frac{\partial t_z(\mathbf{r})}{\partial z} \tag{25b}$$

The divergence of a vector field is often used to identify whether and where there are sources or sinks of a flowing quantity, such as charge or heat. In our application, it would be used to identify regions of convergence or divergence of the fiber tracts. In voxels where fiber tracts radiate or terminate, we expect $\phi(\mathbf{r}) = \nabla \cdot \mathbf{t}(\mathbf{r})$ to be non-zero. Peskin proposed that the direction field vector, **t**(**r**), describing the muscle fiber directions in the heart are divergence-free, i.e., $\nabla \cdot \mathbf{t}(\mathbf{r}) = 0$.[44] One would not expect this to apply in the brain, where nerve fiber tracts cross and terminate in certain regions. Using diffusion tensor imaging, one can, in principle, test these hypotheses directly.

Since we estimate a diffusion tensor in each voxel, we only obtain a discrete sample of the tensor field. Thus, we must calculate the gradients of the direction vectors **t**(**r**), **n**(**r**), and **b**(**r**) numerically. A reasonable approach is to use centered differences to obtain a discrete approximation to $\nabla \cdot \mathbf{t}(\mathbf{r})$:

$$\nabla \cdot \mathbf{t}(\mathbf{r}) = \frac{t_x(\mathbf{r} + \Delta x\mathbf{i}) - t_x(\mathbf{r} - \Delta x\mathbf{i})}{2\Delta x} + \frac{t_y(\mathbf{r} + \Delta y\mathbf{j}) - t_y(\mathbf{r} - \Delta y\mathbf{j})}{2\Delta y} + \frac{t_z(\mathbf{r} + \Delta z\mathbf{k}) - t_z(\mathbf{r} - \Delta z\mathbf{k})}{2\Delta z} \tag{26}$$

Another potentially revealing vector operation that produces a scalar invariant of a vector field is the magnitude of the curl or circulation of the direction vector field:

$$|\nabla \times \mathbf{t}(\mathbf{r})| \tag{27a}$$

where

$$\nabla \times \mathbf{t}(\mathbf{r}) = \mathbf{i}\left(\frac{\partial t_z(\mathbf{r})}{\partial y} - \frac{\partial t_y(\mathbf{r})}{\partial z}\right) + \mathbf{j}\left(\frac{\partial t_x(\mathbf{r})}{\partial z} - \frac{\partial t_z(\mathbf{r})}{\partial x}\right) + \mathbf{k}\left(\frac{\partial t_y(\mathbf{r})}{\partial x} - \frac{\partial t_x(\mathbf{r})}{\partial y}\right). \tag{27b}$$

If all the fibers in a local area were straight, $|\nabla \times \mathbf{t}(\mathbf{r})|$ would vanish; where they curl, $|\nabla \times \mathbf{t}(\mathbf{r})|$ is positive. Owing to previously acquired fiber maps in human and animal brains, it is reasonable to expect that this quantity will not be zero everywhere. We would expect it to be large in the cortical area of the brain, where there are many convolutions and U-fibers that have recently been made visible using diffusion tensor MRI methods.[38]

A discrete approximation to this expression is obtained by using the formula:

$$\begin{aligned}\nabla \times \mathbf{t}(\mathbf{r}) \approx\ & \mathbf{i}\left(\frac{t_z(\mathbf{r} + \Delta y\mathbf{j}) - t_z(\mathbf{r} - \Delta y\mathbf{j})}{2\Delta y} - \frac{t_y(\mathbf{r} + \Delta z\mathbf{k}) - t_y(\mathbf{r} - \Delta z\mathbf{k})}{2\Delta z}\right) \\ & + \mathbf{j}\left(\frac{t_x(\mathbf{r} + \Delta z\mathbf{k}) - t_x(\mathbf{r} - \Delta z\mathbf{k})}{2\Delta z} - \frac{t_z(\mathbf{r} + \Delta x\mathbf{i}) - t_z(\mathbf{r} - \Delta x\mathbf{i})}{2\Delta x}\right) \\ & + \mathbf{k}\left(\frac{t_y(\mathbf{r} + \Delta x\mathbf{i}) - t_y(\mathbf{r} - \Delta x\mathbf{i})}{2\Delta x} - \frac{t_x(\mathbf{r} + \Delta y\mathbf{j}) - t_x(\mathbf{r} - \Delta y\mathbf{j})}{2\Delta y}\right)\end{aligned} \tag{28}$$

One obvious problem with implementing differential geometric measures with real data is that they are inherently noisy. These measures require taking spatial derivatives of vectors, like local direction vectors, which are themselves random variables. Differentiation just amplifies the uncertainty. Filtering methods will undoubtedly have to be developed to obtain smoothed maps of these quantities. However, as the signal-to-noise ratio, quality, and acquisition rate in DWI increase, differential geometry–based measures should play an increasingly important role as MRI stains.

In summary, new invariant MR stains can also be derived from the diffusion tensor field *per se*, not just from the individual diffusion tensors measured in each voxel. In regions (e.g., along some white matter tracts and in the cortex) where this tensor field is expected to be continuous, invariant measures of fiber tract or sheet architecture (torsion, twisting, etc.) should be informative. Moreover, at interfaces between tissue types where we expect the diffusion tensor fields to be discontinous (e.g., at the boundary of white matter fiber tracts and CSF-filled ventricles), differential geometric approaches should aid in identifying these boundaries, both in normal and pathological tissues.

CONCLUDING REMARKS

Diffusion tensor MRI provides a new paradigm for probing tissue structure at different levels of hierarchical organization. While experimental diffusion times are consistent with measurements of molecular displacements on the order of microns, these molecular motions are ensemble-averaged within a voxel, and then subsequently assembled into multislice or 3-D images of tissues or organs. Thus, this single imaging method permits us to study and elucidate complex structural features spanning length scales from the macromolecular to the macroscopic!

If one is interested in using a scalar quantity to characterize an intrinsic feature of an anisotropic medium, such as its degree diffusion anisotropy, that parameter should be invariant to translation and rotation of the laboratory coordinate system. If, in addition, that parameter is physically meaningful, it should possess characteristics of a quantitative physiological or histological stain. Scalar invariants of the diffusion tensor and functions of them possess these desirable properties.

The development of fast, high-quality, high-resolution DWI sequences[38,45] and user-friendly software with which to estimate diffusion tensors and produce images of quantitative "stains" derived from them have greatly facilitated the clinical implementation of DT-MRI.

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DISCUSSION

QUESTION: In the diffusion tensor formulation that you referred to, a term that we will call "q" effectively introduces a spatial scaling in the measurement of the diffusion coefficient. It seems to me that that term might be an interesting parameter because in isotropic diffusion fields, there should be a scaling of the diffusion coefficient that reveals the size of the cell and could distinguish cases where fluid is transferred from inside to outside the cell in some swelling processes. Have you looked at that parameter?

BASSER: There is another approach, which is called displacement imaging, that addresses that issue directly. You can measure the proton displacements using much shorter-duration diffusion gradients that are much larger than what we used in this study, and then estimate a conditional probability distribution of particles being at certain places at certain times. In our particular imaging application, owing to the use of long diffusion times and of imaging gradients, we don't have the ability to measure that probability distribution directly. We are also spatially averaging over a very large number of structures, which are not necessarily homogenous. So we are performing a spatial homogenization as well. So your question is on target, but it is very difficult to make these measurements using clinical scanners in a clinical environment.