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## Liquid crystal phantom for validation of microscopic diffusion anisotropy measurements on clinical MRI systems

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### Abstract

**Purpose**—To develop a phantom for validating MRI pulse sequences and data processing methods to quantify microscopic diffusion anisotropy in the human brain.

**Methods**—Using a liquid crystal composed of water, detergent, and hydrocarbon, we designed a 0.5 L spherical phantom showing the theoretically highest possible degree of microscopic anisotropy. Data was acquired on the *Connectome* scanner using EPI signal read-out and diffusion encoding with axisymmetric *b*-tensors of varying magnitude, anisotropy, and orientation. The mean diffusivity (MD), fractional anisotropy (FA), and microscopic fractional anisotropy ( $\mu$ FA) parameters were estimated.

**Results**—The phantom was observed to have values of MD similar to brain tissue and relaxation times compatible with EPI echo times on the order of 100 ms. The estimated values of  $\mu$ FA were at the theoretical maximum of 1.0, whereas the values of FA spanned the interval from 0.0 to 0.8 due to varying orientational order of the anisotropic domains within each voxel.

**Conclusion**—The proposed phantom can be manufactured by mixing three widely available chemicals in volumes comparable to a human head. The acquired data is in excellent agreement with theoretical predictions, showing that the phantom is ideal for validating methods for measuring microscopic diffusion anisotropy on clinical MRI systems.

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

## Keywords

dMRI;  $q$ -trajectory; QTE; gamma model; powder averaging; microstructure

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## INTRODUCTION

MRI measurements of the directional dependence of water diffusion (1) offer the possibility of noninvasive investigations of the microstructure of anisotropic biological tissues, e.g., muscle (2,3), cartilage (4), and brain white matter (5–7). Diffusion tensor imaging (DTI) (8,9) relies on diffusion encoding with the Stejskal-Tanner sequence (10) over multiple directions to estimate maps of diffusion tensors and rotationally invariant scalar parameters such as the mean diffusivity (MD) and the fractional anisotropy (FA) (11,12). For brain white matter, these parameters are affected by the microstructural organization of the nerve fibers (13), and thus allow for inferring micrometer-scale tissue properties from images with millimeter-scale spatial resolution. Consequently, DTI and related methods have been extensively applied for studying the microstructure and connectivity of the human brain (14–17).

Because of the limited spatial resolution of the images, each voxel likely contains multiple tissue types or fiber populations (18), leading to ambiguities when interpreting the DTI parameters in terms of microstructural features (19,20). As an example, in order to correctly estimate the distribution of fiber orientations, it is necessary to first quantify the local diffusion characteristics within a single fiber bundle (21), which is influenced by the packing density, diameter, and myelination of the fibers. For locally anisotropic materials that exhibit no diffusion anisotropy on the macroscopic scale because of low orientational order, the microscopic diffusivities within the anisotropic domains can be estimated by detailed analysis of the signal decay as a function of the diffusion weighting variable  $b$ , as demonstrated for a wide range of biological and synthetic materials (22–30). This type of analysis relies on the assumption that all of the anisotropic domains have identical local diffusion properties and that orientation dispersion is the only mechanism contributing to the observed distribution of effective diffusivities. However, the presence of multiple water compartments with different isotropic diffusivities can give rise to signals that are virtually indistinguishable from the ones originating from compartments with microscopic anisotropy combined with orientation dispersion (31,32), and the analysis is fraught with ambiguity for materials that are heterogeneous on the voxel scale – brain tissue being a prime example.

Microscopic anisotropy and isotropic heterogeneity can be disambiguated with more advanced diffusion encoding schemes that are classified as double diffusion encoding (DDE) (33–36), triple diffusion encoding (TDE) (37), and  $q$ -trajectory encoding (QTE) (32,38,39) following the terminology suggested by Shemesh et al. (40). Within this naming convention, the Stejskal-Tanner experiment (10) is denoted single diffusion encoding (SDE). Lasi et al. (41) showed that the isotropic diffusion encoding (32,42–46) incarnation of QTE is instrumental for separating and quantifying the effects of isotropic heterogeneity, microscopic anisotropy, and orientational order, which are inextricably entangled in conventional diffusion MRI. Since the voxel-scale anisotropy from DTI is usually reported

in terms of the FA parameter, we have defined the microscopic fractional anisotropy ( $\mu$ FA) as a microscopic equivalent that is not affected by orientation dispersion (41,47). The definition of  $\mu$ FA is identical to the one for the fractional eccentricity (FE) of Jespersen et al. (36,40,48), but differs from the microscopic anisotropy (MA) index of Lawrenz et al. (49). Parameters for quantifying heterogeneity, anisotropy, and orientations are discussed in depth in recent reviews (50,51), as well as in a series of original research papers (39,41,52,53).

During our last few years of development of NMR and MRI methods for investigating the microstructure of heterogeneous anisotropic tissues, we have noticed the need for a standard substance or material to validate pulse sequences, data processing pipelines, and implementations on clinical MRI systems. DTI measurements are typically validated with isotropic liquids (54) and polymer solutions (55), as well as anisotropic physical phantoms made by microcapillaries (56–58) or wound fibers (59–66). An ideal phantom for validating microscopic anisotropy measurements should have a well-defined and uniform value of  $\mu$ FA, a value of MD similar to that observed for brain tissue in vivo, variable degrees of orientational order and values of FA, as well as nuclear relaxation properties allowing for spin-echo signal preparation and EPI read-out (67,68) that is ubiquitous for clinical diffusion MRI sequences. Additionally, the phantom should be inexpensive, stable over long periods of time, and simple to manufacture at sizes comparable to the relevant human anatomy. Previously introduced phantoms were made by randomly oriented microcapillaries (69), chemically fixated pig spinal cord (70), and asparagus puree (41), which all suffer from ill-defined quantitative values of the microscopic anisotropy. The microcapillary phantom of Komlosch et al. (69) has so far only been used on a vertical bore microimaging system, and the asparagus puree phantom of Lasi et al. (41) is susceptible to odorous biological degradation.

In the field of surface- and colloid science, there are numerous studies of diffusion anisotropy in lyotropic liquid crystals where self-assembled detergent aggregates hinder the translational motion of the water (24,34,71–89). For methods development on microimaging systems, we have used several liquid crystalline materials to demonstrate and validate new pulse sequences (32,37,41,52,53,90,91). Most of the previously used materials have severe drawbacks that prevent their use on clinical MRI systems. The non-ionic detergents used in Refs. (32,41,90) yield lamellar liquid crystals with temperature-dependent anisotropy and rapid transverse relaxation incompatible with single-shot EPI. The latter drawback also applies to the detergent sodium dioctyl sulfosuccinate (52), which is also known as Aerosol-OT, AOT, or docusate sodium, and will be referred to as AOT in the following text.

In Refs. (91) and (53), we added the hydrocarbon isooctane to the aqueous AOT system to induce the formation of a reverse hexagonal phase (92) as illustrated in FIG. 1. The three components AOT, isooctane, and water spontaneously assemble into an anisotropic structure where the water is confined in cylindrical channels with diameters of a few nanometers and lengths extending hundreds of micrometers or even millimeters (53). On the ~100 milliseconds observational time scale defined by the duration of the diffusion-encoding block in the MRI pulse sequence, the translational motion of the water is unhindered in the direction of the channels, leading to one-dimensional root-mean-square displacements of approximately ten micrometers. The low solubility of water in isooctane prevents

translational motion in the plane perpendicular to the direction of the channels. Consequently, we expect the structure in FIG. 1 to give rise to the largest possible microscopic anisotropy and a value of  $\mu\text{FA}$  equal to one. In case all anisotropic domains within a voxel have the same orientation, the values of FA and  $\mu\text{FA}$  are identical. Unless effort is made to align the domains, each voxel will contain domains of many different orientations, causing FA to be smaller than  $\mu\text{FA}$ . A quantitative relation between FA,  $\mu\text{FA}$ , and the orientational order parameter OP is given in Ref. (41).

The MRI signal from the phantom includes contributions from all  $^1\text{H}$ -containing species of the phantom, i.e. AOT, isooctane, and water, which all occur in a liquid state with molecular reorientation on the pico- to nanosecond time scale and translational diffusion similar to the values in the corresponding neat liquids. Despite the rapid reorientation and diffusion, the anisotropy of the liquid crystalline structure leads to non-complete averaging of the intramolecular  $^1\text{H}$ - $^1\text{H}$  dipolar couplings (93,94) and transverse relaxation times ( $T_2$ ) on the order of milliseconds for AOT and isooctane. The  $^1\text{H}$ - $^1\text{H}$  dipolar couplings of water are also averaged by chemical exchange of hydrogen atoms between neighboring water molecules, giving  $T_2$ -values of tens or hundreds of milliseconds. Consequently, we expect only water to contribute to the single-shot EPI signal.

In this paper, we use experimental methods from surface- and colloid science (92,95) to systematically explore the liquid crystal phase structure for the AOT-isooctane-water system as a function of chemical composition in order to maximize the water content of the reverse hexagonal phase and increase  $T_2$  to values allowing for single-shot EPI. Using this chemical composition, we scale up the manufacturing process and construct a phantom with dimensions appropriate to test MRI systems for human brain measurements. We demonstrate the phantom by validating our recent implementation of axisymmetric diffusion encoding tensors (52,96) with smoothly modulated gradient waveforms (53) and EPI signal read-out on the *Connectome* scanner at the Athinoula A. Martinos Center at Massachusetts General Hospital (97), as well as a protocol with numerically optimized waveforms (90,98) on a conventional scanner. In addition to conventional DTI processing to estimate FA, we analyze the data with the *gamma* model (41,99,100), giving quantitative estimates of the isotropic heterogeneity and, which is the focus of the present study, the microscopic anisotropy as quantified by  $\mu\text{FA}$  (41). Since the *gamma* model is valid only in the limit of low  $b$ -values, we also analyze the data with the *Pake* model of Eriksson et al. (52), which is valid for the full range of  $b$ -values in case all anisotropic domains within a voxel have the same local diffusion properties. Although the *gamma* model is more generally applicable to heterogeneous anisotropic materials such as brain tissue, the *Pake* model is better suited for verifying that the phantom has the expected reverse hexagonal structure and corresponding one-dimensional water diffusion. We show that the liquid crystal phantom has ideal properties for thorough testing of new MRI methods to quantify microscopic diffusion anisotropy on clinical MRI systems.

## METHODS

### Preparation of the liquid crystal phantom

Sodium dioctyl sulfosuccinate (AOT) (purum, 96%, TLC, Lot no. BCBQ6818V) and isooctane (anhydrous, 99.8%, Lot no. STBF6788V) were purchased from Sigma-Aldrich. The water used was purified with a Millipore-Q water system. Samples for assessment of the phase diagram were prepared from stock solutions of different proportions of AOT and isooctane, which were diluted with water in 4 mL glass vials to obtain 95 compositions within the region of interest, which was identified based on previously published phase diagrams (92,95). Note that the stock solutions are viscous and highly flammable liquids (101) that should not be exposed to open flames or electrical discharges. The addition of water to the stock solutions greatly reduces the flammability. All 95 samples were stored and investigated at 25 °C and six of the ones identified as having the reverse hexagonal structure were further studied as a function of temperature in the range from 5 to 40 °C.

The identity and compositional extension of the different phases were determined from characterization of the samples by visual inspection (for general appearance, viscosity, and possible birefringence), polarized light microscopy (for identification of characteristic birefringence patterns of the anisotropic phases), and for selected samples, small-angle X-ray scattering (SAXS; for identification of nanostructure). The SAXS experiments were performed on a SAXS Lab instrument from JJ X-ray (Denmark), equipped with a 100XL +microfocus X-ray tube (Rigaku, Texas) with Cu K $\alpha$  radiation of wavelength 1.542 Å, and a Pilatus detector (Dectris Ltd., Switzerland). The lattice spacing  $a$  of the reverse hexagonal phase in the phantom was calculated from the scattering vector  $Q_1$  of the first Bragg peak using the relation

$$a = \frac{2}{\sqrt{3}} \cdot \frac{2\pi}{Q_1} \quad [1]$$

For preparation of the 0.5 L phantom, isooctane was added to AOT and set to dissolve overnight. Water was added to the AOT-isooctane solution, giving a final composition of 44.12 wt% AOT, 13.94 wt% isooctane, and 41.94 wt% water, and the mixture was stirred on a magnetic stirrer at 40 °C. The melted liquid crystal was gently poured into a 0.5 L round-bottom glass flask, avoiding the formation of air bubbles. The liquid crystal was formed by cooling the phantom from 40 to 20 °C over a time period of 5 h.

### MRI measurements

Imaging was performed on the Magnetom 3T Skyra Connectome scanner (Siemens Healthcare, GmbH, Erlangen, Germany) at the Athinoula A. Martinos Center at Massachusetts General Hospital. The scanner is equipped with a gradient system capable of 0.3 Tm<sup>-1</sup> maximum amplitude and 200 Tm<sup>-1</sup>s<sup>-1</sup> slew rate, as well as a 64-channel receiver head coil array. The phantom was put on a cork stand in the head coil and anchored with soft foam pads. Before MRI measurements, the phantom was left within the bore of the magnet

for 30 min in order to reach the same temperature as its immediate surroundings (approximately 20 °C).

A spin echo EPI sequence was adapted to allow user-defined gradient waveforms to be played out before and after the refocusing pulse as shown in FIG. 2. An echo time (TE) of 140 ms allowed for diffusion encoding waveforms of duration  $\tau = 54.95$  ms before and after the 6.9 ms long block of spoiler gradients and the refocusing pulse. Images were acquired in 10 slices (7.5 mm thick) with EPI read-out at a matrix size of  $70 \times 70$ , field of view of  $280 \times 280$  mm<sup>2</sup>, and a spatial resolution of  $4.0 \times 4.0 \times 7.5$  mm<sup>3</sup>. The waveforms before and after refocusing were identical and self-balanced, meaning that the integral of the gradient on each side was zero. Using the explicit equations in the section below, we generated gradient waveforms  $\mathbf{G}(t)$  at diffusion encoding strengths  $b = 0.25, 0.5, 1, 2, 4,$  and  $8 \cdot 10^9$  sm<sup>-2</sup>, normalized anisotropy  $b = 0, 0.58, 0.82,$  and 1, along 20 encoding directions. In total, the acquisition generated 480 signal samples per voxel at a repetition time (TR) of 2000 ms, giving a total measurement time of 16 min. All data was corrected for motion and eddy-currents in elastix (102) using extrapolated reference images (103). Based on the repeated measurements at  $b = 0$ , the mean signal-to-noise ratio was estimated to 30 at the minimum  $b$ -value of  $0.25 \cdot 10^9$  sm<sup>-2</sup>.

### Gradient waveform design

We calculated  $\mathbf{G}(t)$  according to Ref. (53) by first specifying an axial waveform  $G_A(t)$  with total duration  $\tau$ , quarter-sine ramp up  $0.12\tau$ , and half-sine ramp down  $0.3\tau$  as shown in FIG. 2(b), top left panel. The  $q$ -vector magnitude  $q(t)$ , trace of the diffusion encoding tensor  $b$  (also known as the  $b$ -value), azimuthal angle  $\psi(t)$ , and complex radial waveform  $G_R(t)$  are given by, respectively,

$$q(t) = \gamma \int_0^t G_A(t') dt', \quad [2]$$

$$b = \int_0^\tau q(t)^2 dt, \quad [3]$$

$$\psi(t) = \frac{2\pi}{b} \int_0^t q(t')^2 dt', \text{ and} \quad [4]$$

$$G_R(t) = \left[ G_A(t) + \frac{2\pi q(t)^3}{\gamma b} i \right] \exp[i\psi(t)]. \quad [5]$$

Subsequently,  $\mathbf{G}(t)$  is obtained by

$$\mathbf{G}(t) = \begin{bmatrix} G_x(t) \\ G_y(t) \\ G_z(t) \end{bmatrix} = \begin{bmatrix} \operatorname{Re}[G_R(t)] \sin(\zeta) \\ \operatorname{Im}[G_R(t)] \sin(\zeta) \\ G_A(t) \cos(\zeta) \end{bmatrix}, \quad [6]$$

where the polar angle  $\zeta$  gives  $b$  through (52)

$$b_{\Delta} = P_2(\cos \zeta). \quad [7]$$

In Eq. [7],  $P_2(x) = (3x^2 - 1)/2$  is the 2<sup>nd</sup> Legendre polynomial.

For data evaluation purposes, the full diffusion encoding tensor  $\mathbf{b}$  was calculated with

$$\mathbf{b} = \int_0^{\text{TE}} \mathbf{q}(t) \mathbf{q}^T(t) dt \quad [8]$$

where

$$\mathbf{q}(t) = \gamma \int_0^t \mathbf{G}(t') dt' \quad [9]$$

and  $\mathbf{G}(t)$  is the explicit gradient waveform played out on the scanner, including the effects of rotation and amplitude scaling.

### Data analysis

For each voxel, the diffusion tensor  $\mathbf{D}$  was estimated by non-linear fitting of the DTI model (8,104,105)

$$S(\mathbf{b}) = S_0 \exp(-\mathbf{b}:\mathbf{D}) \quad [10]$$

to the experimental signal intensities  $S(\mathbf{b})$  using seven adjustable parameters, namely the initial signal  $S_0$ , the diffusion tensor eigenvalues  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$ , and the three Euler angles describing the tensor orientation. The tensor scalar product  $\mathbf{b}:\mathbf{D}$  is defined by

$$\mathbf{b}:\mathbf{D} = \sum_i \sum_j b_{ij} D_{ij} \quad [11]$$

and the tensor elements  $D_{ij}$  were evaluated from the eigenvalues and Euler angles using equations given in Ref. (106). For voxels containing a distribution of microscopic diffusion tensors, Eq. [10] is valid in the limit  $S(\mathbf{b})/S_0 \rightarrow 1$  (39,50). To minimize systematic errors caused by violating this condition, only data points fulfilling  $S(\mathbf{b})/S_0 > 0.5$  were included in the fitting procedure. After estimating  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$ , the values of MD and FA were calculated using (11)

$$\text{MD} = (\lambda_1 + \lambda_2 + \lambda_3) / 3 \quad [12]$$

and

$$\text{FA} = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \text{MD})^2 + (\lambda_2 - \text{MD})^2 + (\lambda_3 - \text{MD})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}, \quad [13]$$

respectively.

When estimating  $\mu\text{FA}$ , the full set of 480 experimental data points  $S(b, b, \theta, \phi)$  was “powder-averaged” over the 20 orientations (41,50), leaving a reduced set of  $6 \times 4$  points  $S(b, b)$ . This data was analyzed by assuming a gamma distribution of effective diffusivities  $P(D)$ , the Laplace transformation of which is given by (41,50,100)

$$S(b, b_\Delta) = S_0 \left( 1 + b \frac{V_{\text{iso}} + V_{\text{aniso}} b_\Delta^2}{\text{MD}} \right)^{-\frac{\text{MD}^2}{V_{\text{iso}} + V_{\text{aniso}} b_\Delta^2}}, \quad [14]$$

where  $V_{\text{iso}}$  and  $V_{\text{aniso}}$  are the isotropic and anisotropic contributions to the variance of  $P(D)$ . Eq. [14] was regressed onto the experimental data using  $S_0$ , MD,  $V_{\text{iso}}$ , and  $V_{\text{aniso}}$  as adjustable parameters. In the limit  $S(b, b)/S_0 \rightarrow 1$ , Eq. [14] is valid for any distribution  $P(D)$ . Following Ref. (41), only data points fulfilling  $\exp(-b\text{MD}) > 0.1$  were included in the analysis in order to alleviate systematic errors. The estimated values of MD and  $V_{\text{aniso}}$  were converted to  $\mu\text{FA}$  using the expression (41)

$$\mu\text{FA} = \sqrt{\frac{3}{2}} \sqrt{\frac{1}{2\text{MD}^2/5V_{\text{aniso}} + 1}}. \quad [15]$$

Values of  $\mu\text{FA}$  were also estimated by fitting



$$S(b, b_{\Delta}) = S_0 \exp(-bD_{\text{iso}}) \cdot \frac{\sqrt{\pi} \exp(bD_{\text{iso}}b_{\Delta}D_{\Delta})}{2 \sqrt{3bD_{\text{iso}}b_{\Delta}D_{\Delta}}} \operatorname{erf}\left(\sqrt{3bD_{\text{iso}}b_{\Delta}D_{\Delta}}\right) \quad [16]$$

to the experimental data using the adjustable parameters  $S_0$ , the isotropic diffusivity  $D_{\text{iso}}$ , and the normalized diffusion tensor anisotropy  $D$  (50,52). Eq. [16] is the Laplace transformation of a distribution  $P(D)$  corresponding to the powder pattern from an axially symmetric chemical-shift tensor in solid-state NMR spectroscopy (107), the functional form of which was originally derived by Pake (108). Unlike Eqs. [10] and [14], the expression in Eq. [16] is valid for any value of  $S(b, b_{\Delta})/S_0$ , but only as long as all sub-ensembles of water within the voxel have the same values of  $D_{\text{iso}}$  and  $D$ . Under these conditions  $\text{MD} = D_{\text{iso}}$  and  $V_{\text{iso}} = 0$ , and  $\mu\text{FA}$  can be calculated from Eq. [15] using the substitution (52)

$$V_{\text{aniso}} = \frac{4}{5} \langle (D_{\text{iso}}D_{\Delta})^2 \rangle. \quad [17]$$

For brevity, Eqs. [10], [14], and [16] will be referred to as the *DTI*, *gamma*, and *Pake* models, respectively. These models are included in the “Multidimensional Diffusion MRI” software package available at [github.com/markus-nilsson/md-dmri](https://github.com/markus-nilsson/md-dmri).

## RESULTS

### AOT-isooctane-water phase diagram

The results from the characterization of the AOT-isooctane-water samples are presented as a partial ternary phase diagram in FIG. 3. Although there are deviations in the extensions of the respective phase regions, the general features are consistent with those of a previously published phase diagram for the same system (95). The observed differences can tentatively be attributed to differences in the qualities of the used chemicals.

Samples with compositions at and around the value selected for the phantom (orange cross in FIG. 3) show the macroscopically homogeneous appearance of a stiff, optically clear (or slightly hazy) gel that is expected for a hexagonal liquid crystalline phase. Furthermore, investigation of these samples in polarized light microscopy reveals a birefringence pattern characteristic of a hexagonal phase (109), and the relative positions of the reflections in the SAXS profile obey the expected ratio of  $1:3^{1/2}$  (110), see inserts in FIG. 3. Inserting the value of  $Q_1$  for the first SAXS reflection into Eq. [1] yields a distance  $a = 7$  nm between the centers of neighboring water cylinders as indicated in FIG. 1(b).

The stability of the reverse hexagonal phase with respect to temperature is reported in Supporting Table S1. While the reverse hexagonal structure persists to the lowest investigated temperature 5 °C for all samples, it melts into an isotropic liquid at a temperature that depends on the chemical composition. For the composition chosen for the phantom, melting takes place at 31.0 °C.

## MRI measurements

Parameter maps and histograms for the liquid crystal phantom are shown in FIG. 4 and Supporting FIG. S1, and the estimated values of MD, FA, and  $\mu$ FA are compiled in Table 1. While the *DTI*, *gamma*, and *Pake* models give similar results for the  $S_0$  and MD parameters, the results are as expected radically different for FA from *DTI* and  $\mu$ FA from *gamma* and *Pake*. The relations between the appearance of the parameter maps, the assumptions of the various models, and the phantom structure is treated in detail in the Discussion section below.

The quality of the fit for the *gamma* and *Pake* models can be judged from the  $S(b,b)$  data shown for four voxels in FIG. 5. The root-mean-square deviation between data and fit is in the range between 0.01 and 0.02, and the deviation is about 40% higher for the *gamma* model consistent with the small but visually apparent discrepancies between the experimental data and the model fit at the highest  $b$ -value. The voxels are selected to include high and low values of both  $S_0$  and FA. Despite the differences in these parameters, the normalized signals  $S(b,b)/S_0$  are nearly identical for all voxels.

Additional data showing the reproducibility of the phantom with time and its applicability on a conventional scanner can be found in Supporting FIG. S2–S3.

## DISCUSSION

### Selecting the chemical composition of the phantom

Spin echo sequences with diffusion encoding gradients and EPI signal read-out require transverse relaxation times ( $T_2$ ) above ~50 ms. Increasing water content leads to larger values of  $T_2$ , and in order to obtain the highest possible value we chose to make the phantom using the composition 44.12 wt% AOT, 13.94 wt% isooctane, and 41.94 wt% water as indicated with a cross in FIG. 3. At this water content the region of stability of the pure reverse hexagonal phase is narrow with respect to changes in the AOT and isooctane concentrations, and the components have to be weighed with a precision of  $\pm 2\%$  in order to achieve a homogeneous sample with the desired anisotropic structure. At the selected chemical composition, the reverse hexagonal phase was observed to melt into a low-viscous isotropic liquid at 31 °C (see Supporting Table S1), and the equilibration, inspection, and analysis of the phantom consequently has to be done below this temperature.

### Appearance of the parameter maps

Despite the fact that the phantom is perfectly transparent and visually homogeneous as shown in the photo in FIG. 1(d), there is a pronounced inhomogeneity in the  $S_0$  maps in FIG. 4 on account of differences in the transverse relaxation time  $T_2$  and dephasing time  $T_2^*$ , which depend on the orientation of the liquid crystalline domains with respect to the main magnetic field as well as magnetic susceptibility differences between the phantom and the surrounding air. Orientational dependence of  $T_2$  and  $T_2^*$  is a common feature for anisotropic materials such as liquid crystals (22,111), cartilage (112,113), and brain tissue (114,115).

The MD maps in FIG. 4 are nearly homogeneous for all models, consistent with the expectedly uniform chemical composition and liquid crystalline phase structure illustrated in FIG. 1(b). The obtained values of approximately  $0.4 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$  are comparable to MD for white matter, which is typically in the range from  $0.8$  to  $1.0 \cdot 10^{-9} \text{ m}^2\text{s}^{-1}$  (20,116). A few voxels in the very bottom of the phantom display elevated values of MD, which we attribute to artifacts from less-than-perfect eddy current correction at high  $b$ -values.

As demonstrated experimentally in Ref. (53) and illustrated in FIG. 1(b), each voxel contains multiple anisotropic domains with nearly random distribution of orientations, giving rise to low anisotropy on the macroscopic scale despite the essentially one-dimensional water diffusion within each domain. Consequently, the  $\mu\text{FA}$  maps from the *gamma* and *Pake* model fits are uniform with values near unity, while the FA map from the *DTI* model features a broad range of values between 0 and 0.8, with values around 0.2 being most common. Since the microscopic anisotropy is constant, the FA map reflects the orientational order of the domains within each voxel (41). If desired, the *DTI*, *gamma*, and *Pake* maps can be converted to quantitative maps of the orientational order parameter (41) or the Saupe order tensor (53). Macroscopic alignment of the anisotropic domains can be induced by, e.g., the presence of glass surfaces or temperature gradients when forming the liquid crystal from the isotropic liquid (89). The hyperintense band in the FA map towards the bottom of the phantom can be attributed to such voxel-scale alignment of the anisotropic domains.

### Information gained by varying the $b$ -tensor anisotropy $b$

The  $S(b, b)$  data sets in FIG. 5 are displayed as semilogarithmic graphs of the signal  $S$  vs.  $b$  for the four values of  $b$ . The value  $b = 1$  corresponds to directional diffusion encoding as obtained with pulse sequences based on the conventional Stejskal-Tanner experiment (10) which are ubiquitous in diffusion MRI. The pronounced curvature indicates the presence of multiple water components having different values of the effective diffusivity  $D$ , giving rise to a broad distribution of effective diffusivities  $P(D)$ . Assuming that the distribution originates from an ensemble of randomly oriented axially symmetric domains, the values of the principal diffusivities  $D_{\parallel}$  and  $D_{\perp}$  can be estimated by analyzing the data with expressions corresponding to the  $b = 1$  version of the *Pake* model in Eq. [16]. Despite the widespread use of *Pake* ( $b = 1$ ) analysis in the literature (22–30), it is not appropriate for complex materials such as biological tissues since multiple microscopic structures, which are not necessarily anisotropic, can give rise to exactly the same distribution  $P(D)$  and signal  $S(b)$  (31,41).

Microscopic anisotropy as the underlying cause of the curvature of  $\log S$ -vs- $b$  can be proven by applying isotropic diffusion encoding, corresponding to  $b = 0$  (32). In case of anisotropy as the sole mechanism contributing to the curvature, a monoexponential decay is obtained when  $b = 0$  (32). Such behavior is indeed observed in FIG. 5. On the other hand, if the curvature were caused by isotropic mechanisms, then there would be no difference between the  $b = 0$  and 1 data (32). As shown in FIG. 5, the *Pake* model fits well to the experimental data for all values of  $b$ , from the marked multiexponentiality at  $b = 1$  to the monoexponential decay at  $b = 0$ , as expected for signal that originates from a single water

component with unique values of  $D_{\text{iso}}$  and  $D$  or, equivalently,  $D_{\parallel}$  and  $D_{\perp}$ . The excellent agreement between the experimental data and the *Pake* model, as well as the near-ideal values  $\mu\text{FA} = 0.99 \pm 0.01$ , verifies that the liquid crystal phantom has the appropriate properties for validating MRI methods for quantifying microscopic anisotropy.

### Parameter bias resulting from the model assumptions

For voxels containing several domain orientations and corresponding diffusion tensors, the *DTI* model is only valid in the limit  $S(\mathbf{b})/S_0 \rightarrow 1$  (51). Model fitting using data points outside the range of validity causes systematic underestimation of MD. Even the rather narrow range  $S(\mathbf{b})/S_0 > 0.5$  used here gives rise to a  $-20\%$  bias in the values of MD for the *DTI* model in comparison to the *gamma* and *Pake* models as shown in the MD histograms in FIG. 4.

Although the  $\mu\text{FA}$  maps from the *gamma* and *Pake* fits appear similar, closer scrutiny of the corresponding histograms in FIG. 4 reveals that the *gamma* and *Pake* models give values of, respectively,  $1.05 \pm 0.03$  and  $0.99 \pm 0.01$  (global mean  $\pm$  standard deviation). The *gamma* model is valid in the limit  $S/S_0 \rightarrow 1$  and, as suggested in Ref. (41), the model fitting procedure only included data points fulfilling the condition  $\exp(-b\text{MD}) > 0.1$ . Despite this restriction of the range of used  $b$ -values, the estimated parameters suffer from a small bias, giving rise to  $\mu\text{FA}$ -values exceeding the theoretical maximum of unity. Raising the threshold from the current value of 0.1 would reduce the bias, however at the expense of increasing the sensitivity to experimental noise.

### Implications for studies of heterogeneous tissues

Although the *gamma* model suffers from systematic errors, it is superior to the *Pake* model for heterogeneous materials with multiple values of  $D_{\text{iso}}$  and  $D$  within each voxel. The variance of isotropic diffusivities is captured in the parameter  $V_{\text{iso}}$  of Eq. [14], which has been shown to be non-zero in heterogeneous materials such as a yeast suspension with distinct differences in effective diffusivities of the intra- and extracellular water (41), brain parenchyma containing multiple tissue types (20), white matter with elevated levels of “free” water because of neuroinflammation or atrophy (39), and brain tumors with variable cell density (117). More detailed characterization of heterogeneous materials was recently demonstrated with exhaustive sampling of the  $(b, b)$  acquisition space on microimaging equipment and data analysis with a multi-component *Pake* model that yields the microscopic anisotropy for each component having a distinct value of  $D_{\text{iso}}$  (51,91). Two- or three-component analysis has also been applied to human brain data acquired with  $b = 0$  and 1, but has so far relied on narrow constraints on the allowed values of the component diffusivities to reduce the influence of experimental noise and the limited sampling of the  $(b, b)$  space (118). We anticipate that data acquired with the herein used protocol, having multiple values of  $b$ , will allow for relaxing the constraints of the multi-component data inversion, potentially giving information about heterogeneous tissues at an unprecedented level of detail.

## Limitations of the phantom

The experimental data in FIG. 4 and FIG. 5 demonstrates that the phantom has the desired properties regarding the values of MD, FA, and  $\mu$ FA. However, there are important precautions that must be taken during preparation, storage, and use of the phantom. The position and extension of the reverse hexagonal phase formed by AOT, isooctane, and water may be influenced by the presence of impurities in the used chemicals. Thus, the composition for obtaining a phantom with optimal properties can vary with the quality and purity of the used chemicals. Since the composition range for formation of a reverse hexagonal phase with high water content is rather narrow (see FIG. 3), it is preferable to prepare a small series of samples with compositions in the vicinity of the expected one, to verify homogeneity, before the phantom is produced.

At the chemical composition used for the scaled-up phantom, the liquid crystal melts into an isotropic phase at 31 °C, which puts an upper limit of the temperature in the scanner room. Because of the limited range of compositions where the reverse hexagonal phase is thermodynamically stable, it is important to avoid evaporation of water or isooctane that would lead to changes in composition and nanostructure. While glass is impermeable to both water and isooctane, plastic containers may be permeable to isooctane (119) and therefore ill-suited for prolonged storage. Vigorous shaking of the phantom will not affect the nanostructure, but could change the size and alignment of the anisotropic domains (120), as well as introduce air bubbles that give artifacts in images obtained with EPI read-out. If such bubbles have accidentally been formed, a simple remedy is to heat the phantom to above 31 °C, where the liquid crystal melts into a low-viscous isotropic solution, and let the bubbles disappear through the action of the buoyancy force before cooling down to room temperature. The heating-cooling procedure could also be applied to “reset” the phantom in case the anisotropic domains have been macroscopically oriented by, e.g., extended exposure to the aligning effect of the MRI magnet (121).

## CONCLUSIONS

We have shown that a mixture of the detergent AOT, hydrocarbon isooctane, and water, which are all commercially available, inexpensive, and reasonably harmless substances, form a thermodynamically stable liquid crystal with diffusion properties suitable for testing MRI methods to quantify microscopic diffusion anisotropy: MD comparable to that of brain tissue, arbitrary values of FA, and  $\mu$ FA equal to the theoretical maximum of unity. The mixture can conveniently be prepared in large volumes, melted into an isotropic liquid with low viscosity by mild heating, poured into a container with dimensions compatible with the chosen MRI system, and cooled to room temperature to reform the anisotropic liquid crystalline structure. The utility of the phantom was demonstrated by validating our recent implementation of smoothly modulated gradient waveforms (53) for  $q$ -trajectory diffusion encoding (32,38,39) on the *Connectome* scanner (97), giving excellent agreement between the experimental data and the *Pake* model (52). The herein used acquisition space of  $b$ -tensor magnitudes and anisotropies corresponds to those required for unconstrained estimation of diffusion tensor distributions using the multidimensional approach introduced in Ref. (91) and generalized in Ref. (51), thereby paving the way for in vivo mapping of,

e.g., fiber bundle orientation distributions and “free” water fractions without relying on poorly motivated constraints or priors.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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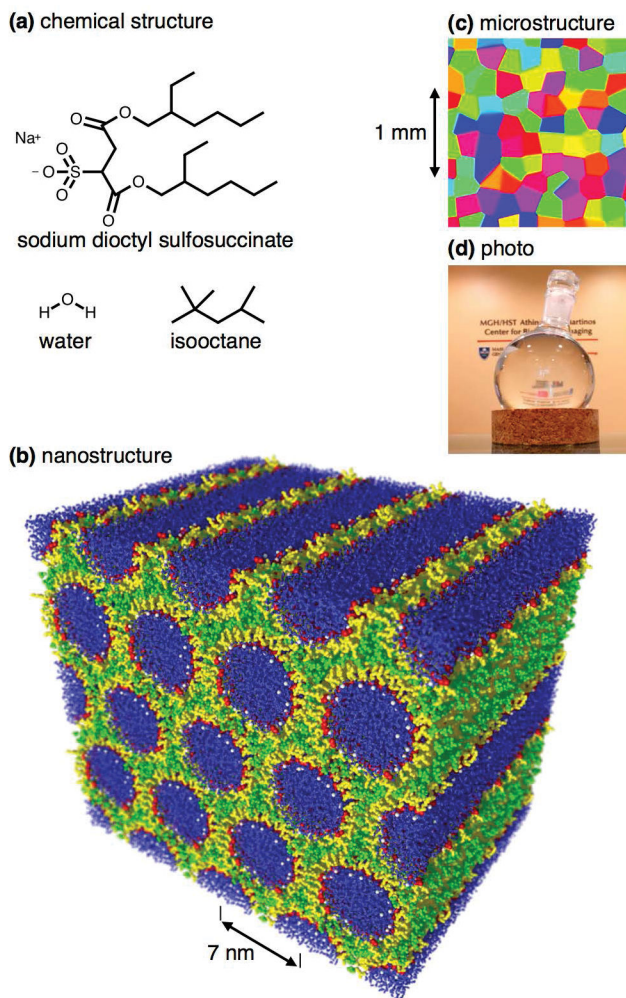


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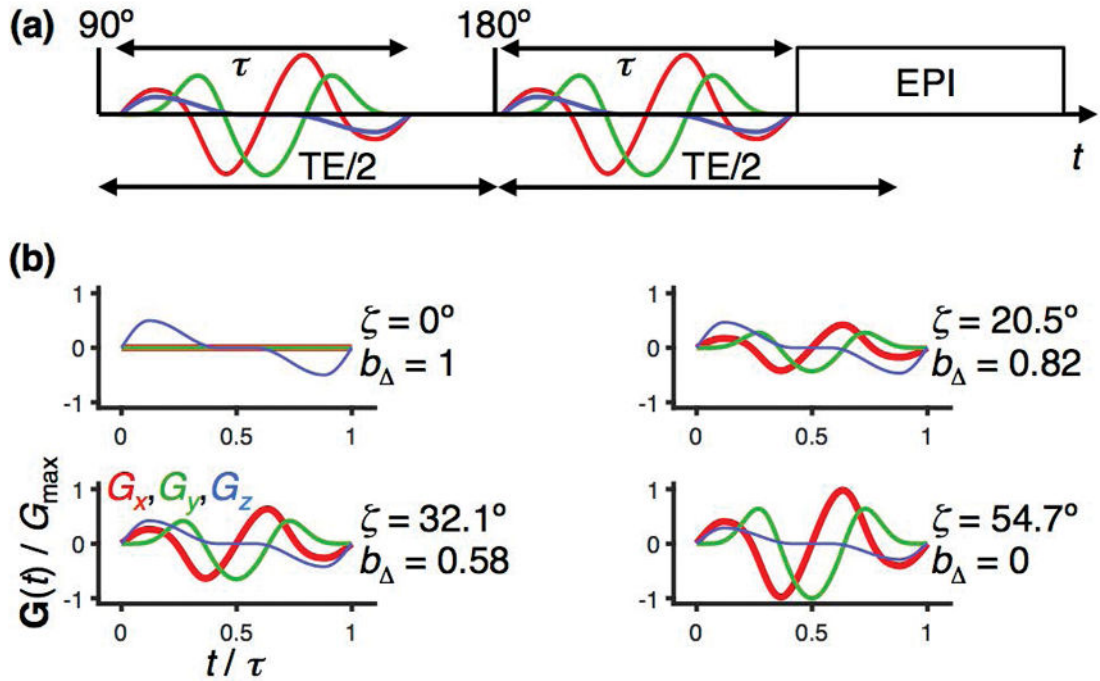
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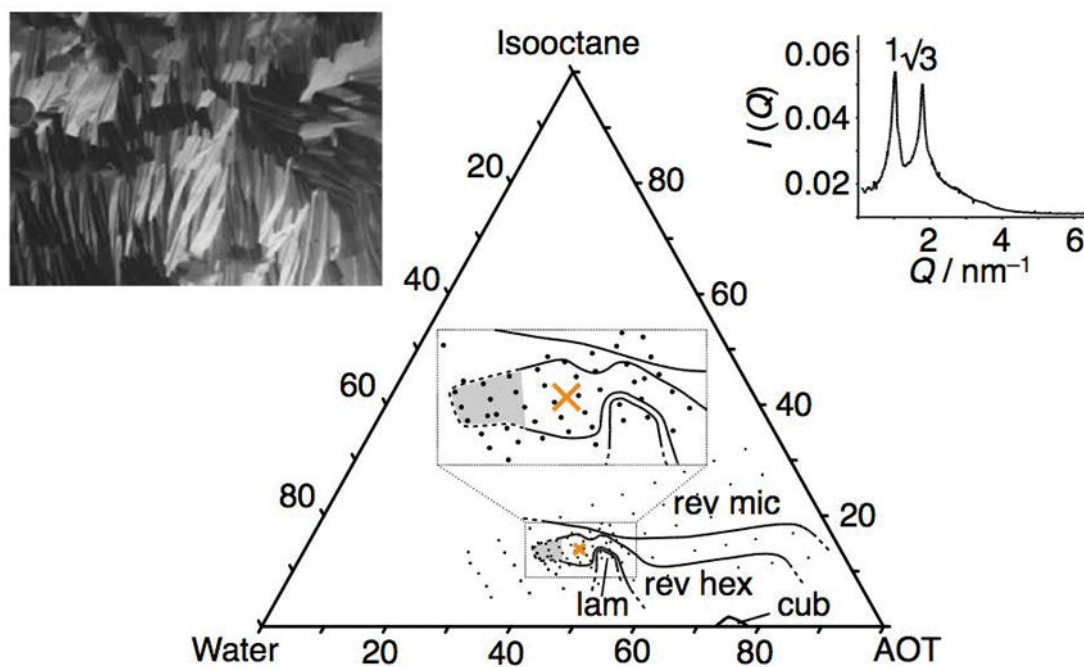
**FIG. 1.** Structure of the liquid crystal phantom. **(a)** Chemical structure of the detergent sodium dioctyl sulfosuccinate (trade name AOT used in the text), the hydrocarbon isooctane, and water. **(b)** Self-assembled nanostructure with AOT (white sodium ions, red hydrophilic headgroups, yellow hydrophobic tails) in the interface between a continuous matrix of isooctane (green) and hexagonally packed cylindrical rods of water (blue). The structure was generated with the molecular dynamics simulation package GROMACS (122) and rendered with POV-Ray (123). **(c)** Schematic microstructure with randomly oriented 100  $\mu\text{m}$ -scale anisotropic domains color-coded according to the direction of the six-fold symmetry axis as  $[x,y,z] = [\text{red}, \text{green}, \text{blue}]$ . **(d)** Photo of the phantom comprising the transparent liquid crystal in a 0.5 L round-bottom glass flask on a cork stand in front of the wall logo at the reception of the Athinoula A. Martinos Center at Massachusetts General Hospital (97).



**FIG. 2.**

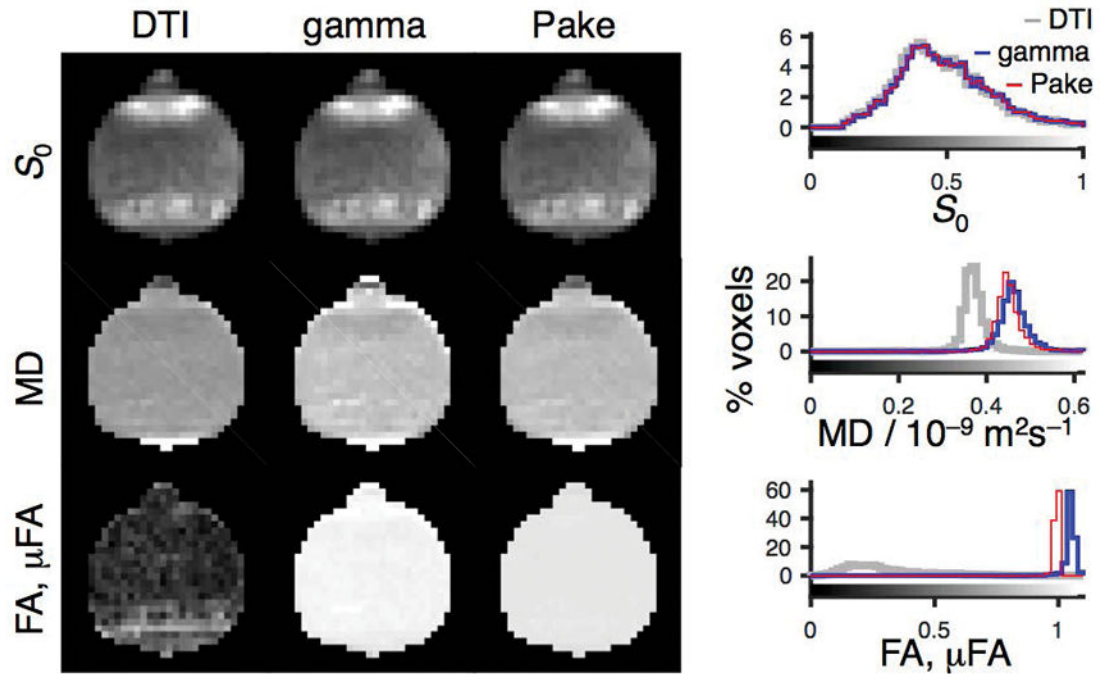
MRI pulse sequence for axisymmetric diffusion encoding (41,53). **(a)** Spin echo sequence with EPI image read-out (67,68) and a pair of modulated gradient waveforms with duration  $\tau$  (red, green, and blue lines) encoding the signal for translational motion. The 90° and 180° RF pulses generate a spin echo at the time TE. **(b)** Four examples of gradient waveforms  $G(t)$  calculated with Eqs. [2]–[6] for the angles  $\zeta = 0^\circ, 20.5^\circ, 32.1^\circ, 54.7^\circ$ . All examples have the same  $b$ -value, while the  $b$ -tensor anisotropy  $b$  is given by the value of  $\zeta$  through Eq. [7]. The global maximum gradient amplitude for the set of waveforms is  $G_{\max}$ .





**FIG. 3.**

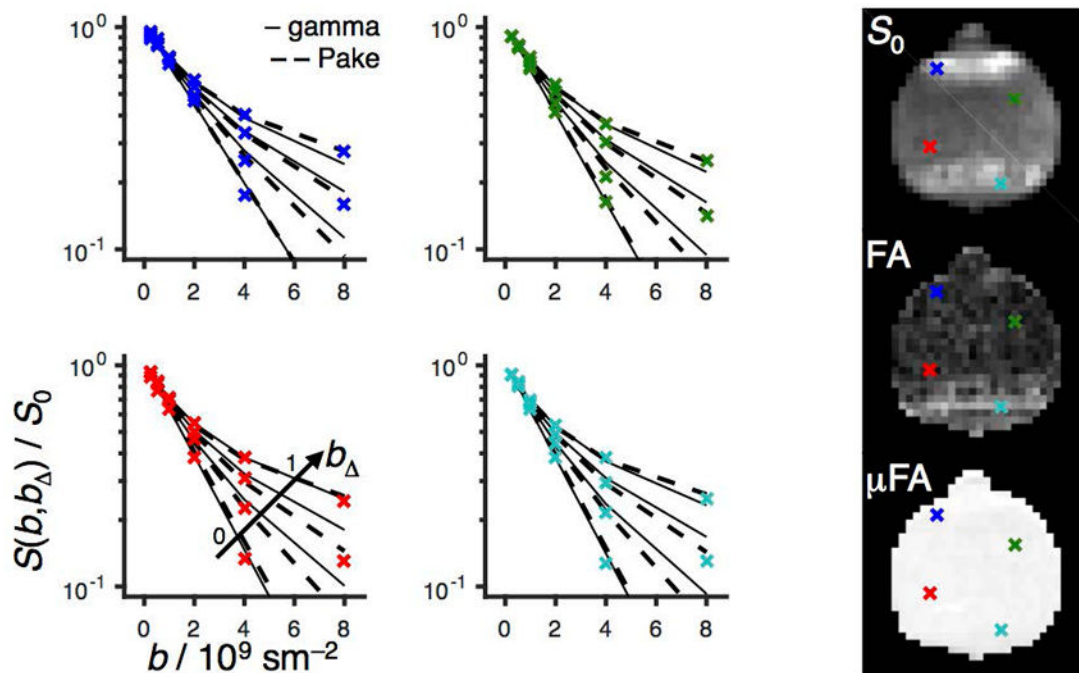
Ternary phase diagram of the AOT-isoctane-water system at 25 °C. The investigated part of the phase diagram comprises single-phase regions of lamellar liquid crystalline (LC) phase (lam), cubic LC phase (cub), reverse hexagonal LC phase (rev hex), and reverse micellar solution (rev mic). The main features of the diagram are consistent with the phase diagram of the same system presented in Ref. (95). Black dots show the compositions of the samples used in the present investigation and the orange cross indicates the composition selected for manufacturing of the phantom (44.12 wt% AOT, 13.94 wt% isoctane, and 41.94 wt% water). Samples with compositions within the shaded region were difficult to characterize unambiguously, but inspection in cross-polarized light revealed that they consist mainly of anisotropic phases (likely involving reverse hexagonal and/or lamellar). The insets display a polarized light microscopy image (left) and a plot of the SAXS intensity  $I$  vs. the scattering vector  $Q$  (right) for a sample with the composition used in the phantom, which both confirm that the sample shows hexagonal nanostructure.



**FIG. 4.**

MRI data acquired with the *Connectome* scanner using the liquid crystal phantom in FIG. 1 and the pulse sequence in FIG. 2. The data was analyzed with the *DTI* (column 1), *gamma* (column 2), and *Pake* (column 3) models, see Eqs. [10], [14], and [16], yielding parameter maps of the initial signal intensity  $S_0$  (row 1), the mean diffusivity MD (row 2), as well as the fractional anisotropy FA from *DTI* and the microscopic fractional anisotropy  $\mu$ FA from the *gamma* and *Pake* models (row 3). The 2D parameter maps show the axial slice through the center of the nearly spherical phantom (see Supporting FIG. S1 for all slices). The histograms (right) include data from all 4281 analyzed voxels throughout all slices of the 3D volume. The gray scales of the maps are given by the bars along the abscissas of the histograms. Note that while *DTI* largely fails to detect anisotropy, both *gamma* and *Pake* models yield  $\mu$ FA near the expected value of unity.





**FIG. 5.** Powder-averaged normalized signal intensity  $S(b, b_{\Delta})/S_0$  vs.  $b$  for four voxels indicated as blue, green, red, and turquoise crosses on the  $S_0$ , FA, and  $\mu$ FA parameter maps to the right. Experimental  $S(b, b_{\Delta})$  data points are shown as crosses;  $\gamma$  and Pake model fits are represented by solid and dashed lines for each value of the  $b$ -tensor anisotropy  $b_{\Delta}$  (0, 0.58, 0.82, and 1 from bottom to top as shown with the arrow in the bottom left panel).

**Table 1**Diffusion parameters (global mean  $\pm$  standard deviation) for the liquid crystal phantom.

	MD / $10^{-9} \text{ m}^2\text{s}^{-1}$	FA, $\mu\text{FA}$
<i>DTI</i>	$0.37 \pm 0.03$	$0.3 \pm 0.2$
<i>gamma</i>	$0.46 \pm 0.04$	$1.05 \pm 0.03$
<i>Pake</i>	$0.45 \pm 0.04$	$0.99 \pm 0.01$

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