Published in final edited form as:

Magn Reson Med. 2019 January ; 81(1): 533–541. doi:10.1002/mrm.27419.

In vivo microscopic diffusional kurtosis imaging with symmetrized double diffusion encoding EPI

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Abstract

Purpose—Diffusional kurtosis imaging (DKI) measures the deviation of the displacement probability from a normal distribution, complementing the data commonly acquired by diffusion MRI. It is important to elucidate the sources of kurtosis contrast, particularly in biological tissues where microscopic kurtosis (intrinsic kurtosis) and diffusional heterogeneity may co-exist.

Methods—We have developed a technique for microscopic kurtosis MRI, dubbed microscopic diffusional kurtosis imaging (μ DKI), using a symmetrized double diffusion encoding (s-DDE) echo planar imaging (EPI) sequence. We compared this newly developed μ DKI to conventional DKI methods in both a triple compartment phantom and in vivo.

Results—Our results showed that whereas conventional DKI and μ DKI provided similar measurements in a compartment of monosphere beads, kurtosis measured by μ DKI was significantly less than that measured by conventional DKI in a compartment of mixed Gaussian pools. For in vivo brain imaging, μ DKI showed small yet significantly lower kurtosis measurement in regions of the cortex, cerebral spinal fluid (CSF), and internal capsule compared to the conventional DKI approach.

Conclusions—Our study showed that μ DKI is less susceptible than conventional DKI to sub-voxel diffusional heterogeneity. Our study also provided important preliminary demonstration of

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our technique in vivo, warranting future studies to investigate its diagnostic utility in examining neurological disorders.

Keywords

microscopic DKI; double diffusion encoding; microscopic kurtosis; diffusional heterogeneity; neurological disorders

Introduction

Diffusion measures microscopic molecular displacement (1,2). The ability to noninvasively image diffusion in vivo has been transformative for biomedical applications of MRI (3,4). Specifically, diffusion-weighted imaging (DWI), which can detect signal change within minutes following ischemia, and its adoption in the emergency setting has tremendously enhanced diagnosis and management of acute stroke patients (5,6). The mismatch between perfusion and diffusion lesions, thought to approximate the salvageable ischemic tissue, has been used as a guide for late thrombolytic therapy (7–9). However, it has been noted that metabolic injury within the DWI lesion is heterogeneous (10–12), and a portion of the DWI lesion may be reversible (13,14). As such, the assumption that the DWI lesion identifies the ischemic core is oversimplified, pointing to a need for further development and refinement of diffusion MRI (15).

Diffusional kurtosis imaging (DKI) has been introduced to measure the degree of non-Gaussian displacement in disorders such as Parkinson's disease, epilepsy, traumatic brain injury, and stroke (16–24). In particular, emerging evidence has suggested that the kurtosis lesion likely provides greater specificity for the infarction core than the routine DWI lesion (21,25). However, kurtosis can arise either directly, from restricted diffusion (microscopic kurtosis), or indirectly, by averaging across multiple microscopic compartments that exhibit diffusional heterogeneity and that in isolation would show Gaussian diffusion (26,27). It is important to elucidate the origin of kurtosis contrast, particularly in biological tissues where microscopic kurtosis and diffusional heterogeneity may coexist (16). In studies aiming to address this need, it has been shown that techniques that correlate diffusion over multiple displacements can detect features such as microscopic anisotropy that intra-voxel averaging may obscure (28). Briefly, double diffusion encoding (DDE), also referred to as double pulsed field gradient (double-PFG), MRI may yield microstructural information beyond conventional single diffusion encoding MRI (29–33). We have developed a symmetrized DDE (s-DDE, previously referred to as sd-PFG) nuclear magnetic resonance (NMR) sequence that isolates microscopic kurtosis signal from diffusional heterogeneity, and demonstrated its use in porous media (27). We refer to our new technique, which combines s-DDE with echo planar imaging (EPI), as microscopic DKI (μ DKI). In the present study, we tested μ DKI in phantoms and conducted a preliminary evaluation in rodent brains, laying important groundwork for eventual translation of μ DKI to neurological disorders such as acute stroke.

Theory

For a sample with considerable diffusional heterogeneity, the conventional diffusion MRI signal can be described as a sum of multiple diffusion components (assuming the diffusion components have the same kurtosis):

$$\frac{E(b)}{E_0} = \int f(D) dD \exp[-bD + b^2 \bar{D}^2 K/6] \sim \exp[-\bar{D}b + \frac{1}{2}(\bar{D}^2 - \bar{D}^2)b^2 + b^2 \bar{D}^2 K/6] \quad (1)$$

where b is the diffusion weighting, $f(D)$ is the diffusion constant distribution, $\bar{D}$ and $\bar{D}^2$ are averages over the distribution, and K is kurtosis. The second term (i.e., $\frac{1}{2}(\bar{D}^2 - \bar{D}^2)b^2$) behaves as a deviation from the Gaussian diffusion (i.e., $-\bar{D}b$) and results in an apparent kurtosis term that is not related to microscopic kurtosis. As a result, conventional DKI measures both the intrinsic kurtosis and diffusional heterogeneity.

The DDE sequence has been developed to probe diffusion in porous media (34). The DDE sequence includes two pairs of diffusion gradients that can be sampled in a number of ways, including fully independently or by varying the relative orientation of the gradients (34–40). For sequences that vary the angle between gradients, the signal of a single component can be described as (41):

$$\log \frac{E(q)}{E_0} \sim -q^2(2\tilde{D}\Delta - Q\cos\theta) + \frac{q^4 K_{zzzz}}{12} + \dots \quad (2)$$

where q is the diffusion wave vector number, $\tilde{D}$ is the average of the diffusion tensor, Q is the average of displacement correlation tensor, and K_{zzzz} is the symmetric kurtosis tensor. The signal of the angular DDE experiment with large diffusion encoding has a q^4 (i.e., $\sim b^2$) term due to the distribution of the diffusion coefficient (e.g., diffusion anisotropy, μA), thus resulting in an inaccurate measurement of kurtosis.

Of importance, the s-DDE sequence is sensitive to microscopic kurtosis (27,42). In the s-DDE scheme, the two pairs of diffusion-sensitive gradients (Figure 1, g_1 :black and g_2 :gray) are orthogonal to one another, with their relative strength modulated according to:

$$g_1 = g \cdot \cos(\phi), g_2 = g \cdot \sin(\phi) \quad (3)$$

The ϕ -dependent oscillations in the s-DDE signal can separate contributions from the diffusional heterogeneity and microscopic kurtosis (27). Note that the angle ϕ denotes the amplitude modulation of the two diffusion gradients. For isotropic samples, the s-DDE signal is defined by

$$\ln \left\{ \frac{E(q, \phi)}{E_0} \right\} = -\Delta q^2 D_\Delta - \frac{q^4}{4!} \left[\frac{3 + \cos(4\phi)}{4} K_{sd, \Delta} \right] \quad (4)$$

where $q = |\vec{q}|$, and $\vec{q} = \gamma g \delta (\cos(\phi) \vec{e}_1 + \sin(\phi) \vec{e}_2)$ is the diffusion vector, in which γ is the gyromagnetic ratio, $\vec{e}_1$ and $\vec{e}_2$ represent two orthogonal directions, and g and δ are the diffusion gradient amplitude and duration, respectively. The equation uses the same tensor notation as that of Jespersen et al. for the cumulants that characterize the fourth moment of displacement (41). $K_{sd, \Delta}$ is the mean of the fourth power of displacement over the diffusion time Δ , defined as

$$K_{sd, \Delta} = \langle R_x^4 \rangle - 3 \langle R_x^2 \rangle^2 \quad (5)$$

The displacement can also be defined along the y-z and x-z planes. The $\cos(4\phi)$ modulation is specific to kurtosis because the diffusional heterogeneity does not contribute to the same modulation. The signal can be given by

$$\ln \left\{ \frac{E(q, \phi)}{E_0} \right\} = E_{c, \tilde{0}} + E_{c, \tilde{1}} \cos(\phi) + E_{c, \tilde{2}} \cos(2\phi) + \dots + E_{s, \tilde{1}} \sin(\phi) + \dots \quad (6)$$

where $E_{c, \tilde{n}}$ and $E_{s, \tilde{n}}$ are the cosine and sine coefficients for an n-cycle modulation of the exponent, respectively,

$$E_{c, \tilde{n}} = \sum_i \ln \left\{ \frac{E(q, \phi_i)}{E_0} \right\} \cos(n\phi_i) / \sum_i \cos(n\phi_i)^2 \quad (7)$$

$$E_{s, \tilde{n}} = \sum_i \ln \left\{ \frac{E(q, \phi_i)}{E_0} \right\} \sin(n\phi_i) / \sum_i \sin(n\phi_i)^2 \quad (8)$$

where i is the index of the sampled gradient pairs. It requires a minimum of 180° sampling along ϕ to isolate the microscopic kurtosis ($E_{c, \tilde{4}}$) and average diffusion term ($E_{c, \tilde{0}}$) from the term $E_{c, \tilde{2}}$ (due to diffusion coefficient anisotropy). Higher order terms (i.e., q^6 and above) are negligible given that their amplitudes decay rapidly.

μDKI Analysis and its Relationship to DKI

When kurtosis is dominated by microscopic kurtosis, kurtosis measurements from conventional DKI and μDKI should be equivalent. Kurtosis can be parameterized as a dimensionless parameter (i.e., K) as (16,43,44)

$$\ln \left\{ \frac{E(b)}{E_0} \right\} = -bD + \frac{1}{6}(bD)^2 K + \dots \quad (9)$$

The conventional DKI measurement is equivalent to the μDKI results at $\phi = 0$, where the second diffusion weighting is null, $g_2 = 0$. In this case, $\tilde{E}_{c,4}$ can be directly related to K as

$$K = \frac{4!E_{c,4}}{(bD)^2} \quad (10)$$

These contributions can be accounted for by including the term of the zero-cycle modulation, $E_{c,0}$, which is sensitive to both kurtosis and diffusion.

$$D = \frac{E_{c,0} - 3E_{c,4}}{-b} \quad (11)$$

where D is the diffusion coefficient obtained from μDKI when kurtosis contribution has been accounted for. Therefore, kurtosis can be obtained as

$$K = \frac{4!E_{c,4}}{(E_{c,0} - 3E_{c,4})^2} \quad (12)$$

without referring to the diffusion weighting strength (b) or diffusion coefficient.

Methods

Phantom

We engineered a triple-compartment phantom. One compartment comprised acrylic beads with 1% agarose gel, the second, a mixture of 40% sucrose and 1% agarose, and the third consisted of 1% agarose gel alone. For the bead compartment, we mixed 20 μm monosphere acrylic beads (MX-2000, Esprix Technologies, Sarasota, FL) with 1% low-gelling point agarose; we transferred the mixture into a centrifuge tube and centrifuged at 2000 rounds per minute (i.e., $22.36 \times g$, Eppendorf centrifuge 5804 R, Hauppauge, NY) for 2 minutes. For the second compartment, we mixed sucrose and agarose with deionized water; once heated

to dissolution, this mixture was transferred into a second centrifuge tube. The two centrifuge tubes were inserted into a 50-ml Falcon tube, which we then filled with 1% agarose gel, forming the third compartment. The phantom solidified at room temperature prior to imaging experiments.

Rodents

In vivo experiments were approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital. Five healthy adult male Sprague Dawley (Harlan/Envigo, Indianapolis, IN) were anesthetized with 1.5–2.0% isoflurane in air during the experiment. Respiration and temperature were monitored online (SA instruments, Stony Brook, NY), and body temperature was maintained by a circulating warm water jacket positioned around the animal's torso (Stryker Temperature Therapy Pad, Kalamazoo, MI).

Phantom MRI

All images were obtained using a 4.7 Tesla small-bore Bruker Biospec MRI scanner (Bruker Corporation, Billerica, MA). Fast DKI (45) and μ DKI were acquired with a field of view (FOV) of $50 \times 50 \text{ mm}^2$ and 8 mm slice thickness (Matrix size = 64×64), with gradient pulse duration and diffusion time of 4/17.5 ms, respectively (repetition time (TR)/echo time (TE) = 3000/76 ms). The fast DKI was acquired with 4 b-values (0, 1000, 1500 and 2500 s/mm^2), and number of average (NAE) = 4 along nine directions (scan time = 7 min). The μ DKI was acquired with 4 b-values (0, 1000, 1500 and 2500 s/mm^2), 26 modulation angles ϕ spanning $[0^\circ, 360^\circ]$ with g_1/g_2 in the x–y plane (NAE = 4 and scan time = 11 min). While the μ DKI sequence used diffusion times of $\delta/ = 4/17.5 \text{ ms}$, which match those of fast DKI, the total diffusion time for μ DKI was twice that of the conventional DKI due to the use of double diffusion encoding. Note that we matched the echo time for the fast DKI and μ DKI sequences.

in vivo MRI

For in vivo experiments, we obtained single slice scans with conventional DKI (fast DKI and tensor-based DKI) and μ DKI (FOV = $20 \times 20 \text{ mm}^2$, Matrix = 64×64 , slice thickness = 2 mm). For the tensor-based DKI protocol, diffusion images of two b-values of 1,000 and $2,500 \text{ s/mm}^2$ were collected along 30 diffusion directions in addition to a single reference image of $b=0$ ($\delta/ = 4/17.5 \text{ ms}$, TR/TE = 3000/76 ms, NAE = 4, and scan time = 10 min 50 s) (46). We also performed fast DKI ($b=0$, 1000 and 2500 s/mm^2 , $\delta/ = 4/17.5 \text{ ms}$, TR/TE = 3000/75.8ms, NAE = 4, and scan time = 4 min 36 s) (45,47). In addition, μ DKI was acquired ($b=0$, 1000 and 2500 s/mm^2 , 26 modulation angles ϕ spanning $[0^\circ, 360^\circ]$) with gradient g_1/g_2 in the x–y, x–z and y–z planes, respectively (NAE = 8, TR/TE = 3000/76 ms, and scan time = 66 min). Note that the echo times for all DKI sequences were carefully matched.

Data Processing

All data were processed in MATLAB (Mathworks, Natick, MA). For conventional DKI (i.e., tensor-based and fast DKI) diffusion and kurtosis were calculated based on established routines (45,46,48,49). For μ DKI, we first calculated the natural logarithm of the MRI signal, and obtained the cosine coefficients, $E_{c,\hat{p}}$ according to Eq. 7. Diffusion and kurtosis

were calculated from least-squares fitting (Eqs. 10–12). Because in vivo μ DKI was measured with a total of 78 modulation angles ϕ through the x–y, x–z, and y–z planes, the diffusion and kurtosis metrics were denoted as average apparent microscopic diffusion (average μD_{app}) and average apparent microscopic kurtosis (average μK_{app}), respectively. In addition, the diffusional kurtosis tensor W_{ijkl} was calculated from the tensor-based DKI approach, which was used to calculate apparent diffusion (D_{app}) and apparent kurtosis (K_{app}) along six directions ($\pm x$, $\pm y$ and $\pm z$). The average values of D_{app} and K_{app} along these directions are denoted as average apparent diffusion (average D_{app}) and average apparent kurtosis (average K_{app}), respectively. Moreover, regions of interests (ROIs) were manually outlined in the cortex, cerebral spinal fluid (CSF), thalamus, corpus callosum, and internal capsule, and their diffusion and kurtosis measurements were compared using one-way ANOVA with Bonferroni correction. P values less than 0.05 were considered statistically significant.

Results

The μ DKI pulse sequence consists of s-DDE preparation followed by EPI readout (Fig. 1). No slice-selective gradient was applied during the s-DDE preparation. After the s-DDE period, the magnetization is stored along the z-axis by a 90-degree RF pulse followed by EPI readout, and as such can be extended for multi-slice coverage after a single s-DDE preparation. A phase cycling scheme was also applied to eliminate artifacts.

The μ DKI sequence was first evaluated using the triple-compartment phantom (Fig. 2A). Although agarose gel alone and the mixture of sucrose and agarose gel exhibit Gaussian diffusion independently, when combined, there was noticeable diffusional heterogeneity (50). To demonstrate this, a single slice was positioned so the left ROI incorporated both the agarose alone and mixture of sucrose and agarose compartments, and the right ROI included the acrylic bead compartment. Fig. 2B shows the signals from both ROIs as a function of ϕ for diffusion b values of 1500 and 2500 s/mm². For the left ROI (two components of agarose gel alone and the mixture of sucrose and agarose gel), the signal shows little oscillation with respect to the angle ϕ . In comparison, the signal from the right ROI (bead compartment) displays a prominent 4ϕ oscillation pattern, indicating intrinsic kurtosis.

Fig. 3 compares diffusion and kurtosis images obtained by fast DKI and our new μ DKI method. In the bead compartment (right ROI), the kurtosis measurements from μ DKI and fast DKI agreed with one another reasonably well (0.42 ± 0.03 vs. 0.43 ± 0.01). However, kurtosis measured by μ DKI in the left ROI was close to zero (0.01 ± 0.01), significantly lower than that measured by fast DKI (0.39 ± 0.04 , $P < 0.05$). Because there is no diffusion restriction in the left ROI, we concluded that the kurtosis measured by conventional DKI was due to diffusional heterogeneity. Note that we matched the TE for fast DKI and μ DKI to minimize a potential confounding effect of TE.

We further evaluated μ DKI in normal rodent brains (Fig. 4). The MD_{tensor} , MD_{fast} , and average D_{app} maps from conventional DKI appeared similar to the μD_{app} map from μ DKI. Conversely, the average μK_{app} map appeared to differ from the MK_{tensor} , MK_{fast} , and average K_{app} maps. We evaluated diffusion and kurtosis measurements from regions of the cortex, thalamus, corpus callosum, internal capsule, and CSF (Table 1). Although the

average μD_{app} was about equal to the MD_{tensor} , MD_{fast} , and average D_{app} images acquired in the cortex, thalamus, and corpus callosum, μD_{app} was slightly but significantly higher in the CSF (Table 1). Of importance, the average μK_{app} (0.32 ± 0.12) is significantly lower in CSF regions compared to MK_{tensor} , MK_{fast} , and average K_{app} , which measured 0.68 ± 0.17 , 0.68 ± 0.52 and 0.70 ± 0.18 , respectively. Because CSF is composed largely of body fluid with relatively low concentration of proteins and semisolid macromolecules, its diffusion should be reasonably Gaussian, and hence exhibit low intrinsic kurtosis. In addition, both the conventional DKI and μ DKI sequences captured hyperintense kurtosis in the corpus callosum, where the average μK_{app} (0.82 ± 0.12) is about equal to MK_{tensor} and average K_{app} (0.81 ± 0.06 and 0.84 ± 0.11), and only marginally higher than MK_{fast} (0.79 ± 0.09). In the cortex, the average μK_{app} (0.49 ± 0.09) was significantly lower than MK_{fast} and average K_{app} (0.57 ± 0.09 and 0.57 ± 0.10). In the thalamus and internal capsule regions, the average μK_{app} was not significantly different from MK_{tensor} , MK_{fast} , and average K_{app} (Table 1).

Discussion

We combined s-DDE with fast EPI readout to develop and test μ DKI in a triple-compartment phantom and in vivo. The phantom data showed that μ DKI measures microscopic kurtosis (intrinsic kurtosis) that is not susceptible to diffusional heterogeneity. In addition, in vivo results demonstrated that compared to conventional DKI, μ DKI captures a small yet significant difference in the kurtosis map in regions of the cortex, CSF, and internal capsule, warranting further investigation of μ DKI for examination of neurological disorders such as acute stroke and epilepsy.

Our phantom study used fast DKI, which provides expedient DKI measurement (45,51). Because water diffusion in the phantom is isotropic, we acquired μ DKI in the x-y plane only. As the compartment containing acrylic beads exhibits little diffusional heterogeneity, kurtosis measurements acquired with conventional DKI and μ DKI were in close agreement, as expected. Conversely, μ DKI showed little kurtosis in the presence of diffusional heterogeneity. These data suggest that compared to conventional DKI, μ DKI is more specific to intrinsic kurtosis. It is also necessary to point out that MD and MK are rotationally invariant metrics. For a good comparison with metrics from μ DKI, we derived the average D_{app} and K_{app} along six diffusion directions ($\pm x$, $\pm y$ and $\pm z$). Our in vivo study showed that the average D_{app} and K_{app} measures along these six diffusion directions are reasonably close to the rotationally invariant MD_{tensor} and MK_{tensor} derived from the tensor-based DKI approach, suggesting that the greatest difference between average μK_{app} and MK is not attributable to the limited sampling directions. We would also point out that μ DKI revealed a regional kurtosis difference compared to the conventional DKI method, especially in the CSF region, suggesting that kurtosis measurements by conventional DKI include both diffusional heterogeneity and intrinsic kurtosis. Indeed, the MK_{tensor} , MK_{fast} , and average K_{app} maps exhibit complex gradations across the brain, indicating a mixed effect of diffusion coefficient and heterogeneity. In comparison, the average μK_{app} map is relatively uniform across gray matter regions, with sharp enhancement in white matter regions.

It is important to mention that other DDE-derived measurements have been studied to characterized microstructure; for example, compartment shape anisotropy (52), compartment

shape eccentricity (53), microscopic fractional anisotropy (54), and microscopic anisotropy (28,55). One common characteristic of these effects is diffusion distribution within the voxel, such as one due to angular microscopic diffusion anisotropy (μA) (29). The μA is different from the standard macroscopic fractional anisotropy (FA) in DTI (56,57) in that it characterizes the microscopic structures within the voxel. Kurtosis differs from μA as it characterizes diffusion dynamics within a single pore, whereas μA measures different pores. Nevertheless, both kurtosis and μA are closely related to the tissue microstructure, as shown by Hui and Jensen (32).

μDKI has lower signal-to-noise ratio (SNR) because it requires twice the diffusion time of conventional DKI. It is important to note that the rat brain is heterogeneous, with different characteristic T_2 -components (58). To eliminate the potential confounding T_2 effect, we matched the echo time in all DKI sequences. We also collected fast in vivo DKI at different TEs to further illustrate the TE dependence of conventional DKI (Supporting Information Figure S1). Although the error bar in MK measurement increases with TE, MD and MK showed relatively little change as a function of TE.

Also important to mention is that because diffusion encoding is performed with non-slice selective pulses, the sequence can be expanded to include multi-slice acquisition for each s-DDE preparation. However, μDKI requires four more RF pulses, which makes μDKI more sensitive to field inhomogeneity. Although our study showed no such artifacts, a composite pulse can be applied if needed to mitigate such effects. Furthermore, shortening μDKI scan time may be attainable by optimizing the choice of diffusion b-values and the range and sampling density of s-DDE angles. Our present study of μDKI sampled diffusion through three planes (x-y, x-z and y-z), and future experiments will develop accelerated and fully rotationally invariant kurtosis sampling schemes.

Conclusions

By combining s-DDE with EPI we have developed a new technique for microscopic diffusional kurtosis imaging, μDKI . We have demonstrated that μDKI provides reliable kurtosis measurement in a phantom containing monosphere beads, in good agreement with conventional DKI. We have also demonstrated that μDKI is noticeably less sensitive to diffusional heterogeneity than conventional DKI. Our preliminary in vivo study of μDKI revealed interesting features when compared to conventional DKI methods, suggesting that advanced DKI sequences may provide important information to complement conventional DKI, to better elucidate tissue microstructure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by research grants from the National Institutes of Health, R21NS085574 and R01NS083654. The authors thank Ms. Nichole Eusemann for editing the manuscript, and Esprix Technologies for providing us with acrylic beads used in this study.

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Fig. 1.

The proposed μ DKI sequence includes symmetrized double diffusion encoding (s-DDE) followed by EPI readout. Spins are stored along the z-axis after diffusion encoding, and crusher gradients are applied to remove undesired coherence pathways. Phase cycling is also used to eliminate artifacts.

Fig. 2.

Evaluation of DKI in a triple-compartment phantom. **A)** A schematic illustration of the triple-compartment phantom. **B)** μ DKI signal as a function of ϕ . In the left ROI, which exhibits diffusional heterogeneity, the μ DKI signal shows little change as a function of the angle (red circle, $b=1500$ s/mm² and green circle $b=2500$ s/mm²). In comparison, in the right ROI, μ DKI shows a prominent 4ϕ modulation pattern for $b=1500$ s/mm² (blue circle) and 2500 s/mm² (pink circle).

Fig. 3.

Comparison of μ DKI and conventional fast DKI in a triple-compartment phantom. Although the two DKI techniques provide similar kurtosis values for the bead compartment (right ROI), they showed significantly different kurtosis values for incorporation of both the agarose alone and mixture of sucrose and agarose compartments (left ROI), due to its diffusional heterogeneity.

Fig. 4.

Comparison of diffusion and kurtosis brain images from conventional DKI and μ DKI in a representative rat. The MD_{tensor} , MK_{tensor} , MD_{fast} , and MK_{fast} maps were obtained from the tensor-based DKI and fast DKI methods, respectively. In addition, the average D_{app} and K_{app} were obtained from six diffusion directions ($\pm x$, $\pm y$ and $\pm z$). The average μK_{app} map appeared different when compared with the MK_{tensor} , MK_{fast} , and average K_{app} maps.

Comparison of in vivo conventional DKI and uDKI measurements in Sprague Dawley rat brains. Means $\pm$ standard deviation in different brain regions are shown. Repeated measures of one-way ANOVA with Bonferroni multiple comparison analyses were performed. As noted in the key below, the letters in superscript (e.g., a, b, c...i) indicate statistically significant difference between two metrics.

Table 1

	Cortex	CSF	Thalamus	Corpus Callosum	Internal Capsule
Diffusion ($\mu\text{m}^2/\text{ms}$)	MD _{tensor}	1.95 $\pm$ 0.61 ^a	0.89 $\pm$ 0.10	0.91 $\pm$ 0.13	0.81 $\pm$ 0.06
	MD _{fast}	0.81 $\pm$ 0.09	0.94 $\pm$ 0.15	1.04 $\pm$ 0.35	0.82 $\pm$ 0.06
	average D _{app}	0.84 $\pm$ 0.15	0.89 $\pm$ 0.10	0.91 $\pm$ 0.13	0.81 $\pm$ 0.06
	average μD_{app}	0.85 $\pm$ 0.12	2.15 $\pm$ 0.50 ^{abc}	0.92 $\pm$ 0.23	0.78 $\pm$ 0.10
Kurtosis	MK _{tensor}	0.55 $\pm$ 0.08	0.64 $\pm$ 0.05	0.81 $\pm$ 0.06	0.81 $\pm$ 0.06
	MK _{fast}	0.57 $\pm$ 0.09 ^d	0.68 $\pm$ 0.06	0.79 $\pm$ 0.09	0.84 $\pm$ 0.07 ⁱ
	average K _{app}	0.57 $\pm$ 0.10 ^e	0.70 $\pm$ 0.18 ^h	0.84 $\pm$ 0.11	0.77 $\pm$ 0.07
	average μK_{app}	0.49 $\pm$ 0.09 ^{de}	0.32 $\pm$ 0.12 ^{gh}	0.82 $\pm$ 0.12	0.71 $\pm$ 0.10 ^j

^aStatistically significant difference between MD_{tensor} and average μD_{app} in CSF region ($p<0.05$).
^bStatistically significant difference between MD_{fast} and average μD_{app} in CSF region ($p<0.05$).
^cStatistically significant difference between average D_{app} and average μD_{app} in CSF region ($p<0.05$).
^dStatistically significant difference between MK_{fast} and average μK_{app} in Cortex region ($p<0.05$).
^eStatistically significant difference between average K_{app} and average μK_{app} in Cortex region ($p<0.05$).
^fStatistically significant difference between MK_{tensor} and average μK_{app} in CSF region ($p<0.05$).
^gStatistically significant difference between MK_{fast} and average μK_{app} in CSF region ($p<0.05$).
^hStatistically significant difference between average K_{app} and average μK_{app} in CSF region ($p<0.05$).
ⁱStatistically significant difference between MK_{fast} and average μK_{app} in Internal Capsule region ($p<0.05$).