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Noam Shemesh

`noam.shemesh@neuro.fchampalimaud.org`

Champalimaud Foundation <https://orcid.org/0000-0001-6681-5876>

Rafael Neto Henriques

Rita Alves

Lisa Novello

Patricia Das Neves Borges

Rui Simoes

Tania Carvalho

Thorsten Feiweier

Andrada Ianus

Adriana Sanchez Danes

Sune Nørhøj Jespersen,

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Biophysical Mechanisms of Microscopic Diffusional Kurtosis in diffusion MRI

Rafael Neto Henriques^{1,2*}, Rita Alves¹, Lisa Novello^{3,4}, Patrícia Das Neves Borges¹,
Rui Vasco Simões^{5,6}, Tânia Carvalho¹, Thorsten Feiweier⁷, Andrada Ianus^{8,1,9},
Adriana Sánchez-Danés¹, Jorge Jovicich³, Sune Nørhøj Jespersen^{10,11}, Noam
Shemesh^{1,12*}

¹Champalimaud Research, Champalimaud Foundation, Lisbon, Portugal

²Institute of Biophysics and Biomedical Engineering, Faculty of Science of the University of Lisbon, Lisbon, Portugal

³Center for Mind/Brain Sciences – CIMEC, University of Trento, Rovereto, Italy

⁴Fondazione Bruno Kessler, Trento, Italy

⁵Institute for Research & Innovation in Health (i3S), University of Porto, Porto, Portugal

⁶Porto Comprehensive Cancer Center Raquel Seruca, Porto, Portugal

⁷MR Research & Clinical Translation, Siemens Healthineers AG, Erlangen, Germany

⁸Imaging Physics and Engineering Research Department, School of Biomedical Engineering and Imaging Sciences, King's College London, London, United Kingdom

⁹Algarve Biomedical Center, University of Algarve, Faro, Portugal

¹⁰Center of Functionally Integrative Neuroscience (CFIN) and MINDLab, Clinical Institute, Aarhus University, Aarhus, Denmark.

¹¹Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark

¹²Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot, Israel

*Corresponding authors:

rnhenriques@fc.ul.pt

noam.shemesh@weizmann.ac.il

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Abstract

The Standard Model of diffusion MRI represents diffusion in tissue using multiple Gaussian components, an approximation that misses key non-Gaussian effects from intra-compartmental structural disorder and/or intercompartmental water exchange. These biophysical mechanisms are conflated in conventional measures such as diffusional kurtosis, and exchange is commonly described using the Kärger model. Here, we develop a Correlation Tensor MRI (CTI) framework that derives the full three-dimensional microscopic-kurtosis tensor and related directional metrics that separate disorder- and Kärger exchange effects. Monte Carlo simulations demonstrate robust disentanglement of these features. In vivo human CTI revealed dominant radial $K_{\mu}^{\perp}$ in white matter, consistent with structural disorder. In a rodent ischemic stroke model, elevated axial $K_{\mu}^{\parallel}$ in lesions corresponded to neurite beading, confirmed histologically. In medulloblastoma, reduced directional CTI metrics reflected infiltration by uniformly shaped tumor cells, while in glioma, phenotypes with distinct cellular anisotropy and packing were resolved. These findings show that microscopic kurtosis cannot be neglected under most biological scenarios, and that structural disorder dominates non-Gaussian diffusion, with disease-specific alterations reflecting underlying pathology. Consequently, Kärger exchange alone is unlikely to be an adequate biophysical model in tissues. Our work provides unravels biophysical mechanisms of diffusional kurtosis biology, from humans to animal models.

Introduction

Magnetic Resonance Imaging (MRI) has transformed our ability to map organs in vivo, non-invasively, and longitudinally, making it a critical modality for both research and clinical applications. Diffusion MRI (dMRI) offers unique sensitivity to the microstructural scale, reporting on barriers and compartments orders of magnitude smaller than the conventional MRI imaging resolution (1, 2). This sensitivity to the cellular-scale dimensions has been harnessed for understanding health and disease, from neurodevelopment and plasticity to neurodegeneration, cancer, and stroke (3–6).

To interpret dMRI signals in brain tissue, biophysical models have generally relied on the so-called Standard Model (7, 8), which represents tissue as a mixture of Gaussian diffusion components typically assigned to putative cellular compartments. This framework underpins most high-b signal analysis, supports compartment modeling, and forms the basis of many microstructural imaging techniques (9–13). Within the Standard Model, deviations from a single Gaussian diffusion component arise naturally from multiple orientations and/or multiple components and are captured by higher-order cumulants of the diffusion signal. The lowest such deviation, diffusional kurtosis, has been observed in nearly every biological context and the ensuing Diffusional Kurtosis Imaging (DKI) technique has become one of the most widely used extensions of Diffusion Tensor Imaging (DTI) (14, 15), consistently improving sensitivity beyond conventional tensor measures and revealing strong effects in e.g. aging, plasticity and disease (16–20).

Under the assumption of multiple Gaussian components, multidimensional diffusion encoding (MDE) approaches have been developed to separate kurtosis contributions arising from microscopic anisotropy versus those from multiple components (21–23), with significant implications for mapping disease, e.g. (24–26). However, more recently, by harnessing diffusion correlation tensors estimated using Double Diffusion Encoding (DDE) (27–30), it was shown that another source, namely, microscopic kurtosis (K_μ), is a significant contributor to the total diffusional kurtosis, alongside the anisotropic and multicomponent sources (31, 32). Correlation Tensor MRI (CTI) revealed that microscopic kurtosis is non-negligible in healthy human brain, and in healthy and stroked rodent brains, in vivo and ex vivo (32–34). These findings violate the central Gaussian assumption of the Standard Model, yet the underlying biophysical mechanisms underpinning them remain unknown. In particular, two fundamental processes: (a) diffusion in structurally disordered compartments (35–39); and (b) exchange of water across compartments, almost ubiquitously modeled through the Kärger model (14, 40–43). While both processes generate non-Gaussian contributions and leave measurable imprints in microscopic kurtosis, the current scalar CTI metrics cannot determine which one dominates in healthy tissue or how each shift in healthy or diseased tissue.

Here, we present a novel framework for investigating the biophysical origin of microscopic kurtosis by deriving its full three-dimensional tensor W^μ and respective rotationally invariant metrics. The functional dependence of these ensuing metrics can separate disorder- from Kärger exchange-driven contributions, thereby offering unique biophysical insights into the nature of diffusion in tissues. Simulations establish discriminative power, and applications across healthy human brains and multiple disease models, including stroke and three distinct cancer models, reveal a consistent biological picture: microscopic kurtosis predominantly reflects structural disorder in tissues, suggesting an oversimplification of the Kärger exchange model in most relevant biological scenarios. These results provide a long-sought biophysical

interpretation of diffusional kurtosis and establish a foundation for future modeling approaches in which microscopic kurtosis must be explicitly incorporated.

Theory

The cumulant expansion of DDE signals (31, 44) offers correlation tensors that are harnessed by CTI in the long mixing time regime ($\tau_m \gg L^2/2D_0$, where L is the characteristic length and D_0 the intrinsic diffusivity) to extract the powder-averaged scalar metrics total kurtosis ($\bar{K}_t$) anisotropic kurtosis ($\bar{K}_{ani}$), isotropic kurtosis ($\bar{K}_{iso}$), and microscopic kurtosis ($\bar{K}_\mu$). Employing Einstein summation convention, these quantities can be calculated using the following expression:

$$\bar{K}_t = \frac{W_{iijj}}{5} + \frac{2D_{ij}D_{ij}}{5\bar{D}^2} - \frac{6}{5}, \quad (1)$$

$$\bar{K}_{ani} = \frac{6(C_{ijij} + D_{ij}D_{ij}) - 2(C_{iijj} + D_{ii}D_{jj})}{15\bar{D}^2}, \quad (2)$$

$$\bar{K}_{iso} = \frac{C_{iijj}}{3\bar{D}^2}, \quad (3)$$

and

$$\bar{K}_\mu = \bar{K}_t - \bar{K}_{ani} - \bar{K}_{iso}. \quad (4)$$

where D_{ij} is the diffusion tensor, W_{ijkl} is the total kurtosis tensor, C_{ijkl} the diffusion covariance tensor, and $\bar{D}$ is the mean diffusivity ($\bar{D} = \frac{\text{trace}(\mathbf{D})}{3}$). Hence, kurtosis sources are resolved but the biophysical origin of microscopic-kurtosis still mixes exchange and disorder effects (34, 42). We here hypothesized that by resolving the 3D profile of directional kurtosis sources, one could access more directly the underlying biophysical mechanism. For an arbitrary spatial direction $\mathbf{n}$, the projection of the total kurtosis $K_t(\mathbf{n}) = \frac{\bar{D}^2}{D(\mathbf{n})^2} n_i n_j n_k n_l W_{ijkl}$ (14), where $D(\mathbf{n}) = n_i n_j D_{ij}$, depends on the sum of two contributions:

- 1) Directional variance-kurtosis ($K_v(\mathbf{n})$): kurtosis that rises from the variance of directional diffusivities $D_c(\mathbf{n})$ across individual compartments (containing information from both $\bar{K}_{ani}$ and $\bar{K}_{iso}$):

$$K_v(\mathbf{n}) = \frac{3\text{Var}(D_c(\mathbf{n}))}{D(\mathbf{n})^2}; \quad (5)$$

- 2) Directional microscopic-kurtosis ($K_\mu(\mathbf{n})$): kurtosis that rises from non-Gaussian diffusion effects $K_c(\mathbf{n})$ within individual compartments:

$$K_\mu(\mathbf{n}) = \frac{\langle D_c(\mathbf{n})^2 K_c(\mathbf{n}) \rangle}{D(\mathbf{n})^2}. \quad (6)$$

The directional dependence from these two kurtosis contributions can be represented by their own fully symmetric kurtosis tensors, which are here referred to as the variance- and microscopic-kurtosis tensors (W_{ijkl}^v and W_{ijkl}^μ) respectively:

$$K_v(\mathbf{n}) = \frac{\bar{D}^2}{D(\mathbf{n})^2} n_i n_j n_k n_l W_{ijkl}^v, \quad (7)$$

and

$$K_\mu(\mathbf{n}) = \frac{\bar{D}^2}{D(\mathbf{n})^2} n_i n_j n_k n_l W_{ijkl}^\mu. \quad (8)$$

From CTI, the variance-kurtosis tensor (W_{ijkl}^v) can be computed from the symmetrized covariance tensor $C_{(ijkl)}$ as this tensor is independent of microscopic-kurtosis effects:

$$W_{ijkl}^v = \frac{3C_{(ijkl)}}{\bar{D}^2}. \quad (9)$$

The microscopic kurtosis tensor W_{ijkl}^μ can then be estimated by subtracting the total kurtosis from the variance kurtosis tensor:

$$W_{ijkl}^\mu = W_{ijkl} - W_{ijkl}^v \quad (10)$$

Note that any rotational invariants previously defined for the total kurtosis W_{ijkl} can also be applied to tensors W_{ijkl}^v and W_{ijkl}^μ , e.g. (14, 45–47). In this study, we use the analogous axial and radial kurtosis quantities defined by Hansen and colleagues (45). Specifically, the mean/radial/axial variance-kurtosis ($\bar{K}_v/K_v^\perp/K_v^\parallel$) and mean/radial/axial microscopic-kurtosis ($\bar{K}_\mu/K_\mu^\perp/K_\mu^\parallel$) are computed using the following expressions:

$$\bar{K}_v = \frac{1}{5} (W_{xxxx}^v + W_{yyyy}^v + W_{zzzz}^v + 2W_{xxyy}^v + 2W_{xxzz}^v + 2W_{yyzz}^v) \quad (11)$$

$$K_v^\perp = \frac{3}{8} \frac{\bar{D}^2}{D_\perp^2} (\tilde{W}_{xxxx}^v + \tilde{W}_{yyyy}^v + 2\tilde{W}_{xxyy}^v) \quad (12)$$

$$K_v^\parallel = \frac{\bar{D}^2}{D_\parallel^2} \tilde{W}_{zzzz}^v \quad (13)$$

$$\bar{K}_\mu = \frac{1}{5} (W_{xxxx}^\mu + W_{yyyy}^\mu + W_{zzzz}^\mu + 2W_{xxyy}^\mu + 2W_{xxzz}^\mu + 2W_{yyzz}^\mu) \quad (14)$$

$$K_\mu^\perp = \frac{3}{8} \frac{\bar{D}^2}{D_\perp^2} (\tilde{W}_{xxxx}^\mu + \tilde{W}_{yyyy}^\mu + 2\tilde{W}_{xxyy}^\mu) \quad (15)$$

$$K_\mu^\parallel = \frac{\bar{D}^2}{D_\parallel^2} \tilde{W}_{zzzz}^\mu \quad (16)$$

where $D_\perp$ and $D_\parallel$ are the total radial and axial diffusivities, and $\tilde{W}_{ijkl}^v$ and $\tilde{W}_{ijkl}^\mu$ are the variance- and microscopic-kurtosis tensors rotated to the principal coordinate frame from the diffusion tensor eigenvectors ($\hat{z} = \mathbf{v}_1, \hat{y} = \mathbf{v}_2, \hat{x} = \mathbf{v}_3$) (45, 46). Note that while the mean quantities $\bar{K}_v = \bar{K}_{ani} + \bar{K}_{iso}$ and $\bar{K}_\mu$ can be computed from the quantities in equations 1-4, the radial and axial quantities ($K_v^\perp, K_v^\parallel, K_\mu^\perp$, and $K_\mu^\parallel$) in equations 11-16 correspond to novel information explored in this work.

Results

Monte Carlo Simulations reveal that directional CTI metrics produce distinct signatures for disorder and exchange

To evaluate how 3D kurtosis metrics behave under different microstructural conditions, we simulated three ground-truth scenarios (**Fig. 1B**): (B1) impermeable cylinders with uniform radius (radius $r_0=1 \mu\text{m}$); (B2) impermeable structures with caliber variation (relative radius standard deviation $SD(r)/r_0=0.42$ (33, 48)), expected to induce intra-compartmental kurtosis effects from structural disorder; and (B3) permeable cylinders modeled using the anisotropic Kärger exchange model with an exchange rate $k = 0.3/\Delta \text{ ms}^{-1}$ (where Δ is the diffusion time). All simulated random walks were convolved with a Watson fiber orientation distribution function (ODF), concentration parameter $c = 5.24$ ($FWHM \approx 18^\circ$) (49), to account for macroscopic orientation dispersion.

First, scalar CTI-derived metrics ($\bar{K}_t$, $\bar{K}_{ani}$, $\bar{K}_{iso}$, and $\bar{K}_\mu$) for all scenarios are presented in **Fig. 1C**. As expected, $\bar{K}_{ani}$ dominates in impermeable cylinders with uniform radius (Fig. 1C1), whereas $\bar{K}_\mu$ is negligible. In contrast, $\bar{K}_\mu$ becomes a non-zero component for structures with caliber-variation (Fig. 1C2) or permeability (Fig. 1C3). These results demonstrate, however, that $\bar{K}_\mu$ alone does not differentiate whether the underlying kurtosis rises from structural disorder or exchange.

We next examined 3D directional profiles of total-kurtosis $K_t(\mathbf{n})$ (13, 39, 41). All scenarios produced similar directional profiles for this conventional metric (**Fig. 1D**). In contrast, the profiles of the new quantities $K_v(\mathbf{n})$ and $K_\mu(\mathbf{n})$ reveal markedly different profiles across scenarios. While $K_v(\mathbf{n})$ only shows reduced contributions in the permeable scenario (**Fig. 1E**), $K_\mu(\mathbf{n})$ reveals axial peaks in the presence of caliber variation and radial peaks under permeability-driven exchange (**Fig. 1F**).

To further quantify these effects, we computed the radial and axial components of the variance-kurtosis ($K_v^\perp$ and $K_v^\parallel$) and microscopic-kurtosis ($K_\mu^\perp$ and $K_\mu^\parallel$). In impermeable structures, $K_\mu^\parallel$ shows the greatest sensitivity to increasing caliber variation (**Fig. 1G**). Under exchange (**Fig. 1H**), both $K_\mu^\perp$ and $K_\mu^\parallel$ show a non-monotonic response - they initially increase, peak at $k\Delta \approx 1.2$, and then gradually decline at higher exchange rates. Notably, as exchange increases, the ratios of microscopic to total kurtosis ($K_\mu^\perp/K_t^\perp$ and $K_\mu^\parallel/K_t^\parallel$) approach unity (Fig. 1H3), while the ratio between variance- and total-kurtosis components ($K_v^\perp/K_t^\perp$ and $K_v^\parallel/K_t^\parallel$) converges toward zero. This indicates that, in strongly exchanging systems described by the Kärger model, kurtosis is predominantly driven by microscopic-kurtosis contributions. These ratios are directionally independent, in line with our analytical predictions of CTI applied to the Kärger model (c.f. equations 28-29 below) which revealed isotropic relative kurtosis contributions.

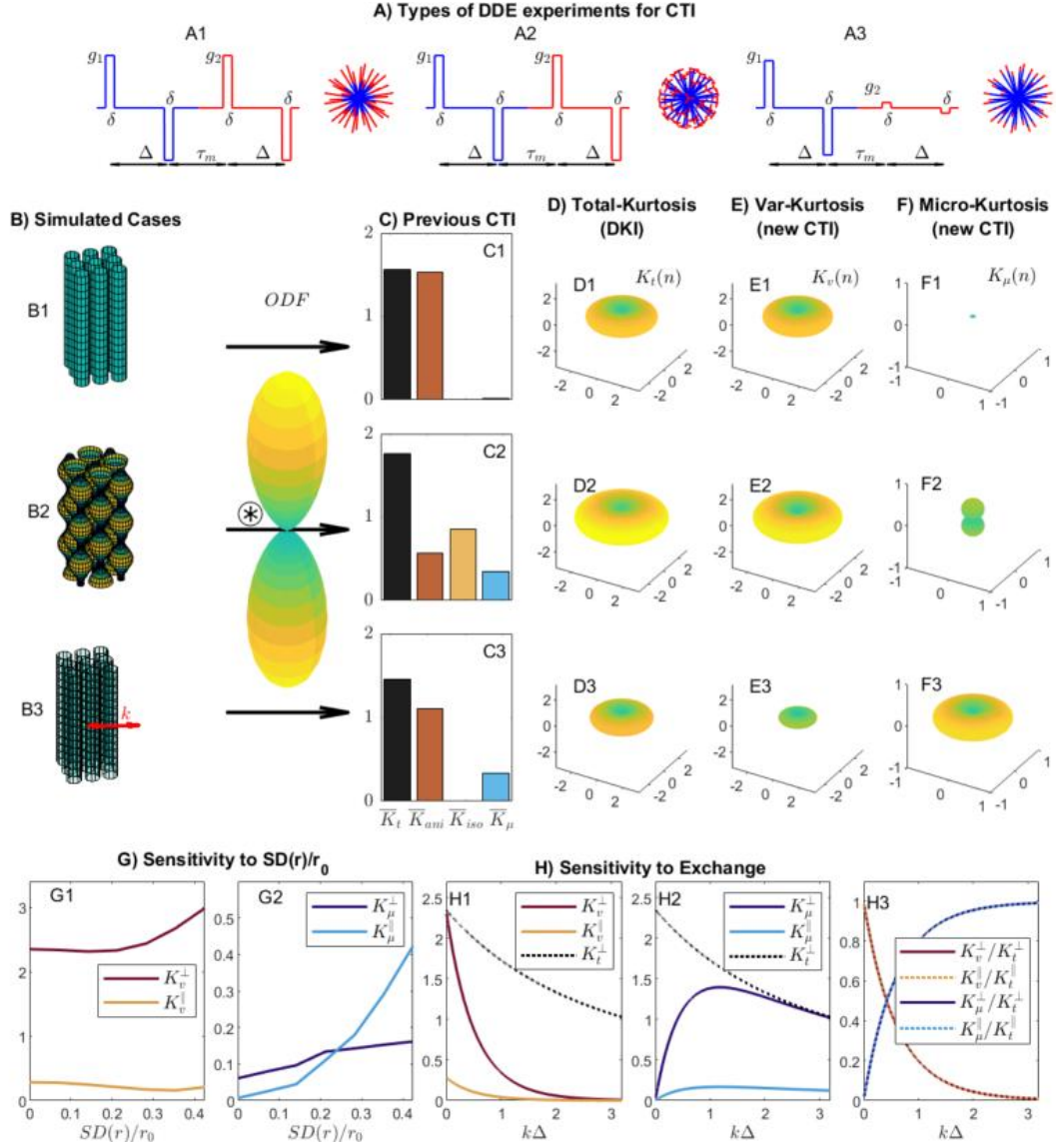


Figure 1 – Schematic of DDE acquisitions required for CTI and illustration of CTI-derived effects across different simulated microstructural scenarios. (A) In addition to acquisitions with at least two non-zero total b-values, CTI requires DDE signals obtained with: symmetric gradient intensities for both parallel (A1) and perpendicular (A2) directions, and asymmetric gradient intensities for parallel directions (A3). (B) Illustration of the three different geometries used for simulations: (B1) impermeable cylinders with uniform radius ($r_0=1 \mu\text{m}$); (B2) impermeable structures with caliber variation (relative radius standard deviation $SD(r)/r_0=0.42$); and (B3) permeable cylinders modeled using the Kärger exchange model (exchange rate $k = 0.3/\Delta \text{ ms}^{-1}$). Note that, to account for tissue dispersion, random walks for each scenario were convolved with a Watson fiber orientation distribution function (ODF). (C) Previous CTI's mean kurtosis quantities for the three simulated scenarios: mean total kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); and microscopic kurtosis ($\bar{K}_\mu$). (D) 3D directional profile of total-kurtosis $K_t(\mathbf{n})$ (information that could be resolved using the standard DKI approach), showing elevated values radially in all scenarios. (E) 3D directional profile of the variance-kurtosis $K_v(\mathbf{n})$, showing reduced contributions only in the presence of Kärger exchange. (F) 3D directional profile of the microscopic-kurtosis $K_\mu(\mathbf{n})$, showing residual values in uniform impermeable cylinders (F1), axial peaks in structures with caliber variation (F2), and maximum radial values in permeable cylinders (F3). (G) Directional kurtosis source sensitivity to cross-sectional variance: (G1-2) Radial/axial variance-kurtosis ($K_v^\perp$ and $K_v^\parallel$) and microscopic-kurtosis ($K_\mu^\perp$ and $K_\mu^\parallel$) as function of $SD(r)/r_0$ in impermeable structures. (H) Directional kurtosis source sensitivity to permeability: (H1-2) $K_v^\perp/K_t^\perp$, $K_v^\parallel/K_t^\parallel$, $K_\mu^\perp/K_t^\perp$, and $K_\mu^\parallel/K_t^\parallel$ (and $K_t^\perp$ for reference) as function of $k\Delta$ in permeable cylinders; and (H3) ratios $K_v^\perp/K_t^\perp$, $K_v^\parallel/K_t^\parallel$, $K_\mu^\perp/K_t^\perp$, and $K_\mu^\parallel/K_t^\parallel$ as function of $k\Delta$. Clear signatures for Kärger exchange vs. structural disorder can be discerned.

CTI in healthy human brain reveals that structural disorder dominates microscopic-kurtosis

Fig. 2 presents CTI-derived metrics from human brain data, obtained from 10 healthy volunteers. **Fig. 2A** shows the different CTI quantities for a representative slice from the group-level template. Consistent with previous literature (34, 45, 46), large total kurtosis values are observed radially to white matter (WM) structures (Fig. 2A5) and CTI's mean microscopic-kurtosis estimates shown to be non-negligible in healthy human volunteers (Fig. 2A10). The new metrics afforded by our approach reveal that this radial dominance is primarily driven by variance-kurtosis components (Fig. 2A8). Although the microscopic-kurtosis components exhibit lower overall values than variance-kurtosis, they consistently show non-zero positive contributions, particularly in radial directions (Fig. 2A11).

Fig. 2B quantifies the radial and axial components of kurtosis across selected WM regions of interest (ROIs) (Fig. 2B1-2), ordered according to their mean values $K_\mu^\perp$. Higher $K_\mu^\perp$ values are found in compact and coherent regions such as the anterior and posterior portions of the Internal Capsule, Corpus Callosum Genu and Splenium, and External Capsule (Fig. 2B2). To assess whether these larger $K_\mu^\perp$ values can be explained solely by inter-compartmental exchange effects, the ratio between microscopic and total kurtosis in radial ($K_\mu^\perp/K_t^\perp$) and axial ($K_\mu^\parallel/K_t^\parallel$) directions are plotted in Fig. 2B3. Significant differences between the radial and axial ratios are observed across most ROIs (controlled for multiple comparisons using FDR correction), suggesting the Kärger exchange model is insufficient to fully explain non-Gaussian diffusion effects in healthy WM tissue.

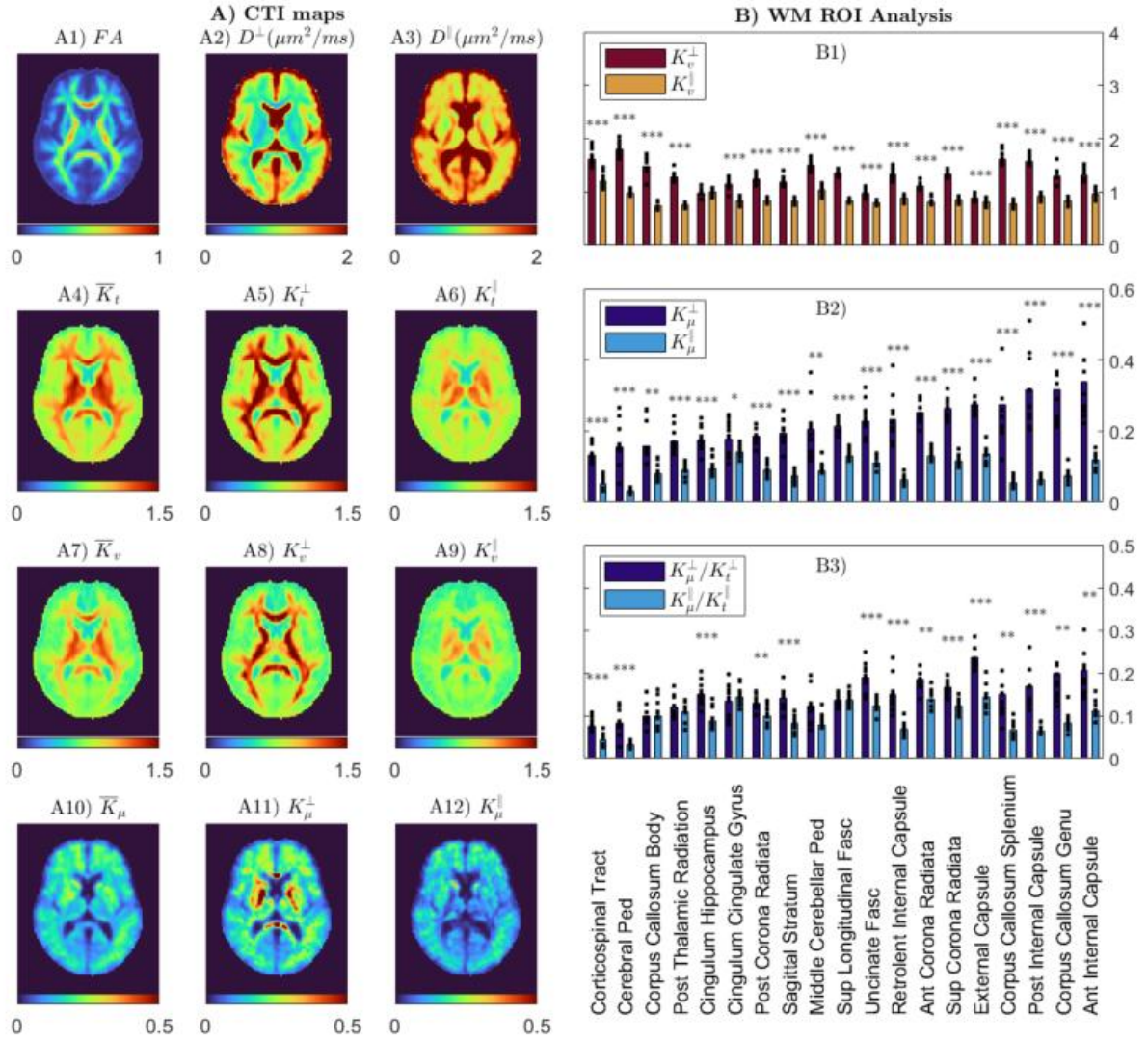


Figure 2 – CTI results obtained from the human dataset. (A) CTI maps from the template averaged data, including: fractional anisotropy (FA), radial diffusivity ($D^{\perp}$), axial diffusivity ($D^{\parallel}$), mean total-kurtosis ($\overline{K}_t$), radial total-kurtosis ($K_t^{\perp}$), axial total-kurtosis ($K_t^{\parallel}$), mean variance-kurtosis ($\overline{K}_v$), radial variance-kurtosis ($K_v^{\perp}$), axial variance-kurtosis ($K_v^{\parallel}$), mean microscopic-kurtosis ($\overline{K}_{\mu}$), radial microscopic-kurtosis ($K_{\mu}^{\perp}$), axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). (B) Kurtosis values for different white matter ROIs ordered to their mean values $K_{\mu}^{\perp}$: (B1) averaged radial and axial variance-kurtosis ($K_v^{\perp}$ and $K_v^{\parallel}$); (B2) averaged radial and axial microscopic-kurtosis ($K_{\mu}^{\perp}$ and $K_{\mu}^{\parallel}$); (B3) ratios between radial microscopic- and total-kurtosis ($K_{\mu}^{\perp}/K_t^{\perp}$) and between axial microscopic- and total-kurtosis ($K_{\mu}^{\parallel}/K_t^{\parallel}$). In panels B1–B3, bars represent values from the template-averaged data, while overlaid black dots represent values from individual subjects. Significant differences between radial and axial values are indicated by *, **, and *** for p-values < 0.05, 0.01, and 0.001, respectively (paired t-test, p-values adjusted for multiple comparisons using FDR correction). Note that in nearly all areas Kärger exchange signatures are not observed, while structural disorder signatures are. This supports a biophysical interpretation of structural disorder as the dominant source of microscopic kurtosis in the human brain.

Increased structural disorder in stroke emerges from beading effects

To gain insight into biophysical interpretations of microscopic kurtosis in pathological conditions, we analyzed preclinical CTI data acquired 3 hours following a photothrombotic stroke in the barrel cortex of 10 adult male C57BL/6J mice (33). **Fig. 3A** displays CTI parameter maps from a representative animal, highlighting the expected diffusion and kurtosis abnormalities within the ischemic lesion, such as decreased radial and axial diffusivities ($D^\perp, D^\parallel$) and increased mean, radial and axial total-kurtosis ($\bar{K}_t, K_t^\perp, K_t^\parallel$). In the newly derived kurtosis source metrics, clear increases are observed in both variance-kurtosis ($\bar{K}_v, K_v^\perp, K_v^\parallel$) and microscopic-kurtosis ($\bar{K}_\mu, K_\mu^\perp, K_\mu^\parallel$) components within the stroke-affected region.

For quantitative comparison, ROIs were manually delineated in the ischemic corpus callosum using FA maps (c.f. Supporting Information Fig. S2), with contralateral ROIs defined symmetrically. Averaged mean total-kurtosis values ($\bar{K}_t$) and their respective sources ($\bar{K}_{ani}, \bar{K}_{iso}, \bar{K}_\mu$) are presented in **Figs. 3B1**, while radial ($K_t^\perp, K_v^\perp, K_\mu^\perp$) and axial ($K_t^\parallel, K_v^\parallel, K_\mu^\parallel$) kurtosis quantities are presented in **Figs. 3B2–3** respectively. These results confirm that all directional kurtosis metrics are significantly elevated in ischemic ROIs compared to their contralateral counterparts. However, when relative changes were expressed as $(M_s - M_c)/M_c$, where M_s and M_c represent the average metric values in stroke and contralateral ROIs respectively (**Fig. 3B4**), the axial microscopic-kurtosis component ($K_\mu^\parallel$) exhibited the largest relative difference. This disproportionate rise aligns with a dominant contribution from intra-compartmental effects, such as axonal or neurite beading.

To support this interpretation, histochemical analysis was performed in ipsilesional and contralesional regions from the corpus callosum (**Figs. 3C1–2**). For this purpose, we induced a photothrombotic stroke in a transgenic Thy1-ChR2-YFP mouse, allowing for enhanced neurite visualization via Anti-GFP Alexa Fluor 488 staining. The stroke-affected hemisphere exhibited widespread neurite beading. Additional histochemical analysis in cortical layers 2/3 of the ischemic barrel cortex and the corresponding contralateral cortex reveals beading also in gray matter **Figs. 3D1–2**).

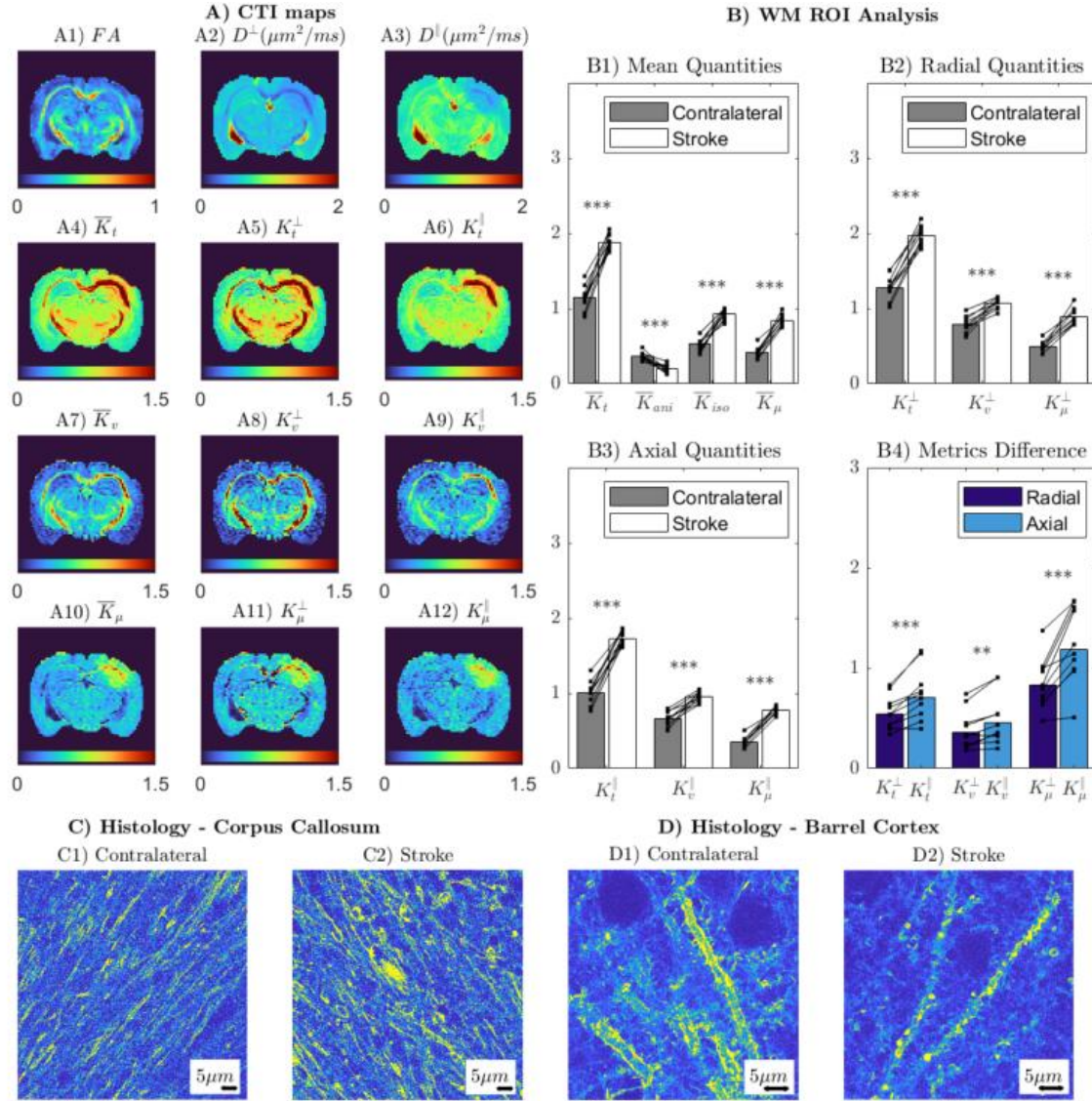


Figure 3 – CTI and histological results in a mouse model of acute ischemic stroke. (A) CTI maps from a representative mouse 3 hours after photothrombotic stroke induction, showing typical diffusion abnormalities in the ischemic lesion. These include reduced fractional anisotropy (FA), radial diffusivity ($D^{\perp}$), and axial diffusivities ($D^{\parallel}$) along with elevated mean, radial and axial total-kurtosis ($\bar{K}_t$, $K_t^{\perp}$, $K_t^{\parallel}$). Marked increases are also observed in the newly derived variance-kurtosis ($\bar{K}_v$, $K_v^{\perp}$, $K_v^{\parallel}$) and microscopic-kurtosis ($\bar{K}_{\mu}$, $K_{\mu}^{\perp}$, $K_{\mu}^{\parallel}$) metrics within the affected region. (B) Quantitative analysis of averaged CTI metrics extracted from the delineated WM ROIs (c.f. Supporting Material Figure S.2, for more information about these ROI). (B1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_{\mu}$). (B2) Radial components: radial total-kurtosis ($K_t^{\perp}$); radial variance-kurtosis ($K_v^{\perp}$); radial microscopic-kurtosis ($K_{\mu}^{\perp}$). (B3) Axial components: axial total-kurtosis ($K_t^{\parallel}$); axial variance-kurtosis ($K_v^{\parallel}$); axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). (B4) relative differences for all directional kurtosis quantities, calculated as $(M_s - M_c)/M_c$, where M_s and M_c represent average metric values in stroke and contralateral ROIs, respectively. The largest relative increase is observed in axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). In panels B1–B4, bars represent group-averaged values across 9 mice; overlaid black dots show individual animal data. Significant differences between stroke and contralateral ROIs (B1–B3) and between radial and axial values are indicated by *, **, and *** for $p < 0.05$, 0.01 , and 0.001 , respectively (paired t-test, FDR corrected). (C–D) Confocal histological analysis of white matter (corpus callosum, CC) and cortex (barrel cortex, BC, layers 2-3) in a Thy1-ChR2-YFP mouse (40 $\times$ magnification): (C1) contralateral CC; (C2) Stroke-affected CC; (D1) contralateral BC; (D2) Stroke-affected BC. Anti-GFP Alexa Fluor 488 was used to amplify endogenous YFP fluorescence, enabling high-resolution neurite visualization. Note that widespread neurite beading is evident in stroke-affected regions, supporting the biophysical interpretation of microscopic kurtosis as emerging from structural disorder but not Kärger exchange.

Microscopic kurtosis reflects homogeneous cell infiltration in medulloblastoma

To demonstrate the generalizability of CTI-derived biophysical interpretation across distinct pathological conditions, we next analyzed preclinical dMRI data from a genetically engineered mouse model of Sonic Hedgehog (SHH) medulloblastoma driven by constitutive activation of the hedgehog signaling pathway in granule neuron precursors located in the external granule layer of the cerebellum. Tumors were induced at postnatal day 3 (P3) through tamoxifen administration leading to the expression of the oncogenic hit, an active form of the Smoothed gene (SmoM2), in the granule neuron precursors using the Math1 promoter (Math1-CreERT2/R26-SmoM2) (50).

Histopathological analysis confirmed that, five weeks upon tamoxifen administration SHH medulloblastomas were present in Math1-CreERT2/R26-SmoM2 mice (N=5), whereas cerebella from littermate controls (Math1-CreERT2 mice) remained presented normal cerebellar anatomy (N=6) (**Fig. 4A-B**). Control cerebella displayed well-organized folia with intact layering and uniform cellularity (Fig. 4A1–A2). In contrast, medulloblastoma-bearing mice showed marked cerebellar expansion by a mass that distorted the parenchyma and caused cerebellar coning (Fig. 4B1, arrow). High-power views revealed a dense cellular neoplasm infiltrating the molecular layer (Fig. 4B2, arrows) and compressing the underlying Purkinje cell layer, granule layer, and white matter, resulting in widespread architectural disorganization.

Representative CTI maps at the level of the cerebellar arbor vitae from control and tumor-bearing animals are shown in **Figs. 4C** and **4D**. FA and $\bar{K}_{ani}$ maps were elevated in tumor-bearing mice (Fig. 4C1-2 vs Fig. 4D1-2), consistent with expected effects of elongated tumor cell infiltration and compression of surrounding layers. In the microscopic-kurtosis maps ($\bar{K}_\mu$, $K_\mu^\perp$, $K_\mu^\parallel$), higher values were still predominantly observed in the white matter in both groups.

Group-level comparisons of total and directional kurtosis components are shown in **Fig. 4E**. Compared to controls, tumor-bearing cerebella exhibited significantly reduced microscopic kurtosis, particularly in $K_\mu^\parallel$ components ($p < 0.05$, two-sample t-test, Fig. 4E3). This pattern contrasts with the stroke model, where elevated $K_\mu^\parallel$ was associated with neurite beading. Here, the opposite effect is consistent with tumor infiltration producing structures with more uniform cross-sectional size variances than normal neural tissue.

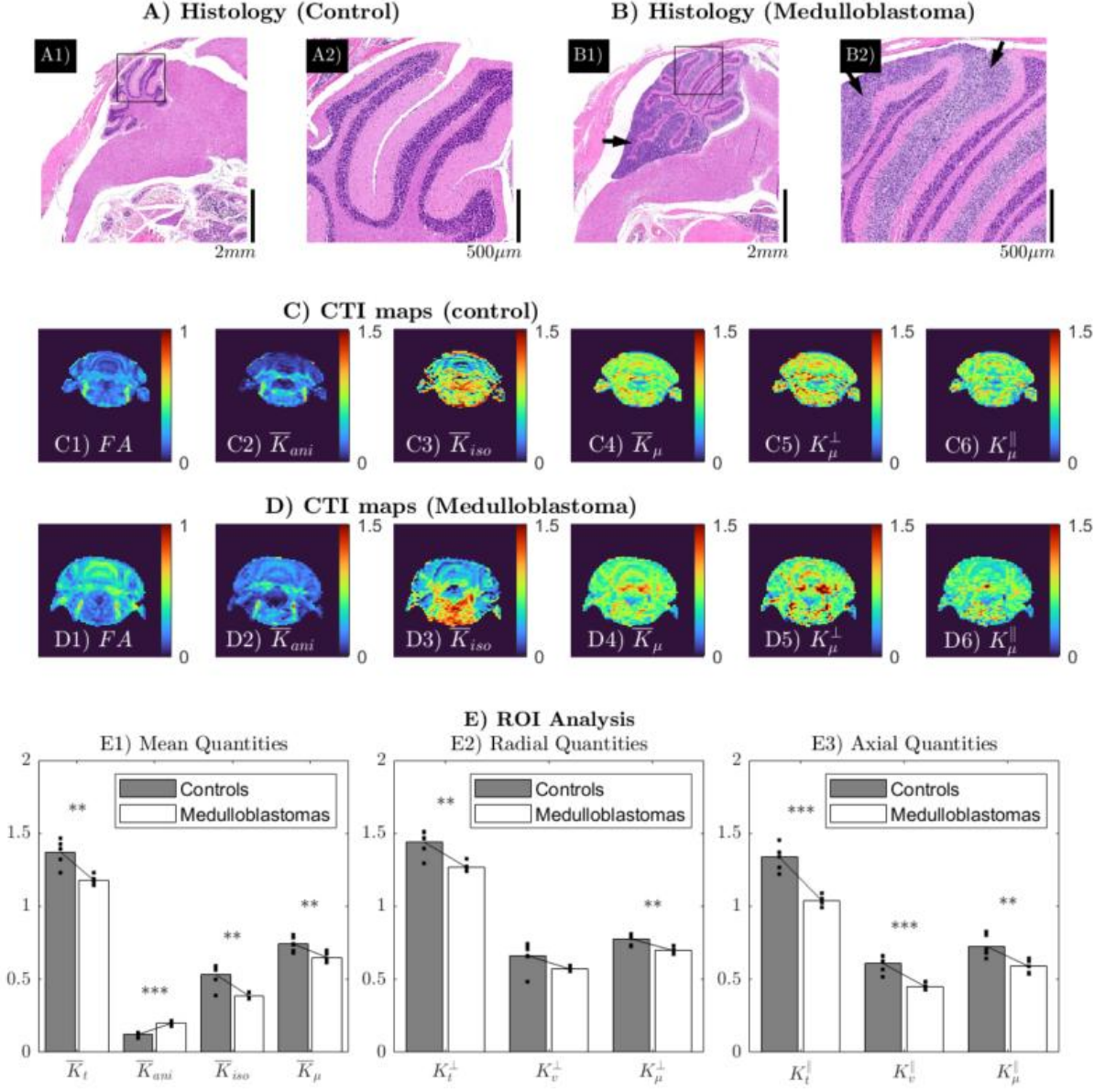


Figure 4 – Histological and CTI results in a mouse model of medulloblastoma. (A-B) Haematoxylin-eosin-stained histological sections from the cerebellum of littermate controls (A) and Math1-CreERT2/R26-SmoM2 (B) mice five weeks upon tamoxifen administration: lower (A1/B1) and higher (A2/B2) magnification. Control mice show well-organized cerebellar folia, with intact layering and uniform cellularity (A). In tumor-bearing mice, the cerebellum is significantly expanded by a mass that overlies and distorts the parenchyma, with cerebellar coning (B1, arrow). High-power view (B2) reveals a densely cellular neoplasm infiltrating the molecular layer (arrows) and compressing the Purkinje cell layer, granule layer, and white matter. (C-D) Representative CTI-derived maps at the level of the cerebellar arbor vitae for control and tumor-bearing animals, showing: (C1/D1) fractional anisotropy (FA); (C2/D2) anisotropic kurtosis ($\bar{K}_{ani}$); (C3/D3) isotropic kurtosis ($\bar{K}_{iso}$); (C4/D4) mean microscopic-kurtosis ($\bar{K}_{\mu}$); (C5/D5) radial microscopic-kurtosis ($K_{\mu}^{\perp}$); and (C6/D6) axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). (E) Group-level quantification of CTI-derived kurtosis metrics across manually segmented cerebellums. (E1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_{\mu}$). (E2) Radial components: radial total-kurtosis ($K_t^{\perp}$); radial variance-kurtosis ($K_v^{\perp}$); radial microscopic-kurtosis ($K_{\mu}^{\perp}$). (E3) Axial components: axial total-kurtosis ($K_t^{\parallel}$); axial variance-kurtosis ($K_v^{\parallel}$); axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). In panels E1-E3, bars represent group-averaged values; overlaid black dots indicate individual animal values. Significant group differences are denoted by *, **, and *** for $p < 0.05$, 0.01, and 0.001, respectively (two-sample t-test, FDR corrected).

Increased structural disorder revealed by kurtosis sources in different glioma phenotypes

We next analyzed data from two mouse models of glioma: CT2A (N = 3) and GL261 (N = 3), which are known to exhibit distinct histological features encompassing higher orientational disorder for the latter (51, 52). As confirmed by our own histochemistry analysis, CT2A tumors are composed of densely packed spindle-shaped cells and sparse, small-caliber vessels (**Fig. 5A**), while GL261 tumors are characterized by poorly cohesive polygonal cells, marked anisocytosis, multinucleated giant cells, and disorganized, tortuous vasculature with disrupted endothelial lining (**Fig. 5B**).

Representative CTI maps for FA, the mean kurtosis components ($\bar{K}_{ani}$, $\bar{K}_{iso}$, $\bar{K}_{\mu}$), and directional microscopic-kurtosis metrics ($K_{\mu}^{\perp}$, $K_{\mu}^{\parallel}$) are shown in **Figs. 5C–D**. In line with histological findings, CT2A tumors displayed higher FA and $\bar{K}_{ani}$ values than GL261, consistent with increased microscopic cell shape anisotropy (Fig. 5C1-2 vs Fig. 5D1-2). The $\bar{K}_{ani}$ maps, which are independent of orientation dispersion, provide enhanced specificity to this cellular anisotropy difference compared to FA. Both tumor models exhibited reduced microscopic kurtosis ($\bar{K}_{\mu}$) compared to healthy tissue, suggesting a lack of increased permeability and limited cellular caliber variation. Relative to the novel directional kurtosis quantities, a trend of larger $K_{\mu}^{\perp}$ values was observed for the CT2A tumors, consistent with residual alignment and increased cellular packing observed in histology. For a quantitative analysis of the observed differences between CT2A and GL261, averaged CTI-derived quantities derived from segmented tumors are shown in Fig. 5E.

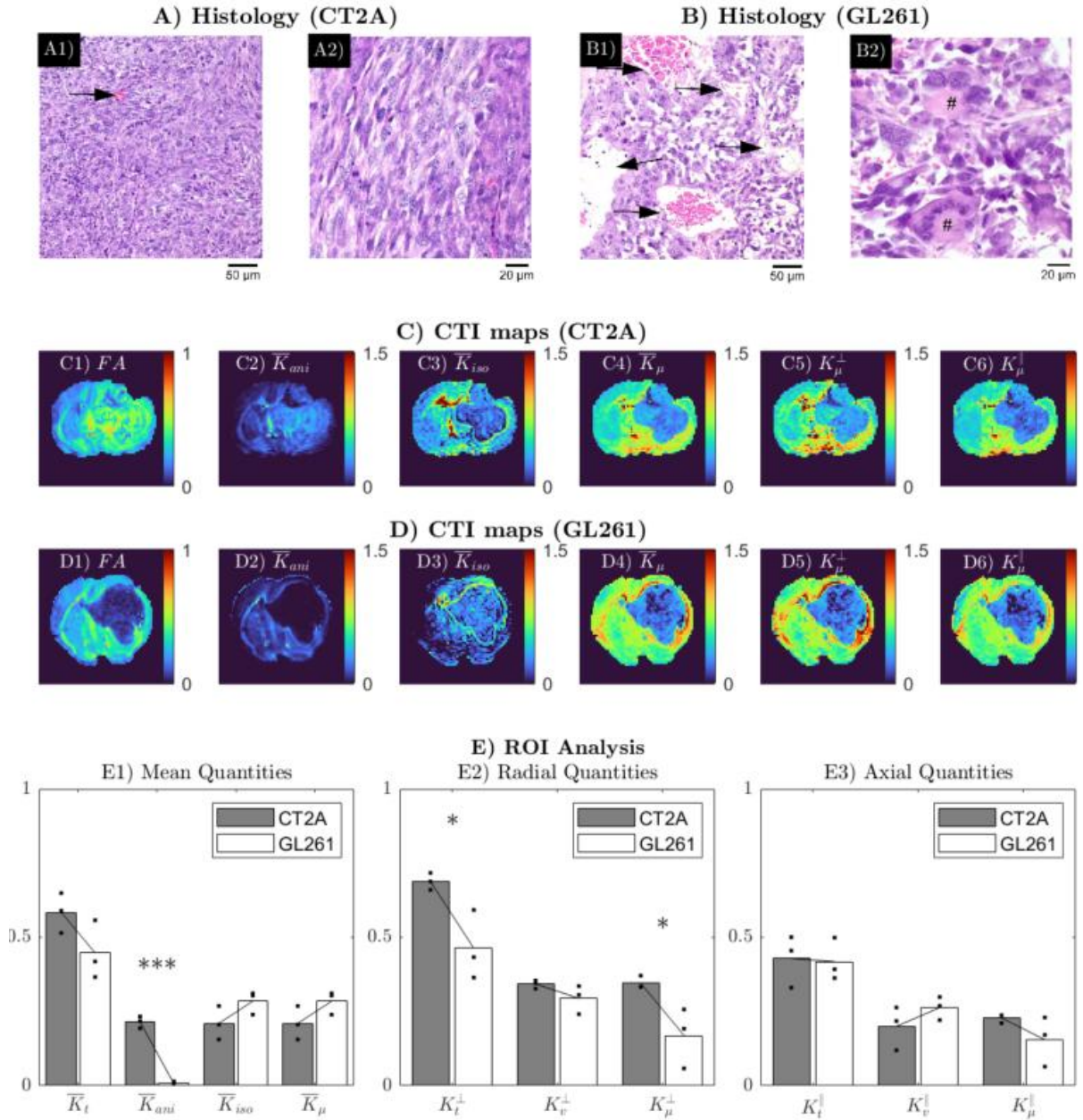


Figure 5 – Histological and CTI results in two glioma mouse models. (A-B) Hematoxylin-eosin-stained histological images acquired using bright-field microscopy of representative CT2A and GL261 tumors: lower (A1/B1) and higher (A2/B2) magnification (arrow points to tumor vessels, while cardinal points to multinucleated tumor cells). (C-D) Representative CTI-derived maps for CT2A and GL261 tumors, showing: (C1/D1) fractional anisotropy (FA); (C2/D2) anisotropic kurtosis ($\bar{K}_{ani}$); (C3/D3) isotropic kurtosis ($\bar{K}_{iso}$); (C4/D4) mean microscopic-kurtosis ($\bar{K}_{\mu}$); (C5/D5) radial microscopic-kurtosis ($K_{\mu}^{\perp}$); and (C6/D6) axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). (E) Group-level quantification of CTI-derived kurtosis metrics across manual segmented tumors. (E1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_{\mu}$). (E2) Radial components: radial total-kurtosis ($K_t^{\perp}$); radial variance-kurtosis ($K_v^{\perp}$); radial microscopic-kurtosis ($K_{\mu}^{\perp}$). (E3) Axial components: axial total-kurtosis ($K_t^{\parallel}$); axial variance-kurtosis ($K_v^{\parallel}$); axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). In panels E1-E3, bars represent group-averaged values; overlaid black dots indicate individual animal values. Significant group differences are denoted by *, **, and *** for $p < 0.05$, 0.01, and 0.001, respectively (two-sample t-test, no multiple comparison is here performed to highlight $K_{\mu}^{\perp}$ trends).

Discussion

Non-invasive to tissue microstructure assessment is a central goal of modern neuroimaging, as cellular-scale reorganization underlies brain development, plasticity, and disease. Diffusion MRI is uniquely suited to probe these processes in vivo, because non-Gaussian water diffusion, quantified by diffusional kurtosis, arises naturally from microscopic heterogeneity and complexity in cellular environments. Conventional diffusion models, including techniques based on the Standard Model (7, 8), interpret non-Gaussian diffusion as a consequence of diffusivity variance between a small number of compartments. This assumption has guided most microstructural imaging techniques (9–13) but is limited, as non-Gaussianity can also arise from structural disorder (35–39) and water exchange, typically analyzed via the Kärger model (14, 40–43). Disentangling these fundamentally different sources of kurtosis is therefore essential if diffusion MRI is to serve as a meaningful, non-invasive marker of microstructural change.

Correlation Tensor Imaging (CTI) was originally proposed to resolve the sources of kurtosis (31) – anisotropic, isotropic, and microscopic – through scalar quantities. While previous studies using this approach have successfully shown the relevance of microscopic-kurtosis both in animal models and humans (32–34), its biophysical origins remained unclear (42). The novel CTI approach developed here addressed this gap by exploiting the directional information of variance- and microscopic-kurtosis to distinguish the two fundamental effects: (a) diffusion in structurally disordered compartments (35–38); and (b) Kärger exchange of water across compartments (14, 40–42).

Indeed, Monte Carlo simulations extending standard-model assumptions show that these mechanisms produce different directional behaviors. In impermeable geometries, caliber variation leads to increased $K_\mu(\mathbf{n})$ with the largest effects along the principal (axial) direction $K_\mu^\parallel$ (Fig. 1G2). In contrast, Kärger-type exchange yields a non-monotonic dependence of $K_\mu(\mathbf{n})$ on exchange rate (Fig. 1H2), causes a fast decline in variance-kurtosis contributions (Fig. 1H1), and yields to relative contributions ($K_\mu(\mathbf{n})/K_t(\mathbf{n})$ and $K_v(\mathbf{n})/K_t(\mathbf{n})$) that are directionally independent (Fig. 1H3). These signatures are used in this study to separately determine the contribution of structural disorder and exchange in MRI experiments in healthy humans and multiple disease models, including stroke and three distinct cancer models.

Across all human and animal MRI experiments, our findings support a microscopic-kurtosis behavior indicative of structural disorder, rather than Kärger exchange. In healthy human white matter (Fig. 2), the directional profile of total-kurtosis ($K_t(\mathbf{n})$) was dominated by radial components (Fig. 2A5), consistent with previous reports (14, 45, 46). CTI decomposition showed that this radial dominance arose from both larger radial variance-kurtosis (Fig. 2A8) and larger radial microscopic-kurtosis (Fig. 2A11). These match the larger radial variance- and microscopic-kurtosis contributions predicted for impermeable structures with low structural caliber variation (c.f. $K_v^\perp$ and $K_\mu^\perp$ values in Fig. G1 and G2 for $SD(r)/r_0 < 0.2$). Supporting Material B shows that elevated $K_\mu^\perp$ values relative to $K_\mu^\parallel$ can be attributed to extracellular structural disorder.

Pathological models reinforced the dominance of structural disorder in microscopic kurtosis. In the stroke model (Fig. 3), regions affected by cytotoxic edema showed marked increases in $K_\mu^\parallel$, consistent with the expected rise in intra-axonal caliber variation caused by neurite beading, in agreement with histology. In medulloblastoma (Fig. 4), infiltrated cerebellar

regions showed the opposite behavior - lower directional microscopic-kurtosis and increased $\bar{K}_{ani}$, reflecting the highly uniform cellular morphology of proliferating tumor cells and compression of local cerebellar layers. The two glioma models (Fig. 5) further supported this interpretation even when differences between axial and radial kurtosis contributions were lower due to reduced directional coherence. Indeed, both CT2A and GL261 tumors exhibited low $\bar{K}_\mu$, consistent with limited intracellular caliber variation and no evidence for increased permeability. In CT2A tumors, which retained residual structural alignment (non-zero FA), $K_\mu^\perp$ significantly exceeded $K_\mu^\parallel$, matching histological signs of dense cellular packing. Beyond microscopic-kurtosis, CTI provided additional contrasts capable of differentiating tumor phenotypes, with $\bar{K}_{ani}$ clearly separating CT2A from GL261 according to their distinct morphologies, i.e. compact spindle-like cells in CT2A versus highly pleomorphic, disorganized features in GL261.

In general, our results have important implications for dMRI interpretation and modelling. First, the observation of non-vanishing microscopic-kurtosis across different brain regions and pathologies challenges the validity of the Standard Model for diffusion, even in white-matter, where its assumptions should hold best (7, 8). Second, expanded standard-models that incorporate Kärger-type exchange may also fall short from describing the microstructure: the directional signatures predicted by exchange were absent in all the datasets analyzed, from in vivo human data acquired on clinical scanners to ex vivo models of stroke and cancer. This indicates that structural disorder, not Kärger exchange, is the dominant source of microscopic-kurtosis in vivo, and that future modelling efforts should prioritize its proper representation. The Kärger model assumes Gaussian components with a single first order exchange rate, which is valid in barrier limited regimes; it remains unclear to which extent the barrier limited assumption is violated and which biological compartments are involved. Third, the results demonstrate that CTI and its new metrics reliably characterize diverse tissue types and disease states. By separating variance- and microscopic-kurtosis contributions and mapping their full directional structure, the expanded CTI framework offers a more specific and mechanistic view of microstructure without requiring new acquisitions or extra scan time relative to scalar CTI.

CTI vs. previous work using diffusion time-dependence. In line with this study, earlier work had already shown that the standard model of diffusion in tissues is incomplete. The observed time-dependence of the apparent diffusivity and of the total kurtosis directly challenged this model (53–59), which assumes that these quantities are time-invariant. As with the directional signatures shown here, structural disorder and Kärger-type exchange are expected to follow distinct time-dependent laws (35, 39). Several studies reported that the time-dependence of diffusivity and kurtosis matches the signatures expected from short-range structural disorder (35, 36, 39, 54, 60). These observations, however, did not exclude the simultaneous presence of Kärger exchange. Conversely, other approaches ignored structural disorder and assumed that the time-dependence predicted by the Kärger model could be used to quantify exchange from conventional (58, 61–63) or advanced diffusion encodings (64–66). Although these techniques are becoming popular, the specificity of their exchange estimates remains debatable (40, 67, 68). This is largely because diffusion-time studies require extensive sampling over wide time ranges, and because the informative regimes - such as the long-time power-law regimes used to infer disorder - are difficult to access in vivo. Moreover, the measured time-dependence often reflects mixed contributions from multiple compartments, making it difficult to isolate the source of the observed changes. CTI overcomes these

limitations by decoupling these interconnected effects: by separating the kurtosis into intra- and inter-compartmental components without relying on diffusion-time modulation, CTI provides an orthogonal and more specific way to characterize the distinct sources of kurtosis.

In addition to diffusion time dependence, recent theoretical work has proposed an alternative strategy using DDE with variable mixing times to disentangle exchange from "transient kurtosis," a term potentially relating to structural disorder effects (42). While this study successfully demonstrated via simulations that exchange can contribute to microscopic kurtosis, their heuristic approach to separating these sources relies on the validity of the Kärger model formulation. Our results, however, indicate that the directional signatures predicted by Kärger exchange are absent in the investigated biological tissues. Instead, the observed microscopic kurtosis exhibits strong directionality consistent with structural disorder.

Limitations. While our CTI framework provides novel insights into microstructural specificity, some limitations should be acknowledged. First, the simulations used simplified geometries. Our goal was to isolate two deviations from the standard model, structural disorder and exchange, and show how each produces distinct non-Gaussian signatures. Simple deviations from cylindrical geometries were used as this geometry forms the basis of the standard model under long diffusion-time assumptions. In practice, this assumption can break down as axonal caliber variation exhibits spatial periods comparable to the diffusion length, violating the idealized cylinder scenario. Although periodic caliber variations do not correspond to short-range disorder effects observed from diffusion time dependent studies (35, 36, 39, 54), we chose it as the minimal way to represent structural disorder effects (33, 48). Naturally, in MRI experiments, microscopic-kurtosis will capture not only more complex compartment caliber variations, but also disorder effects from the extra-cellular environments (54), axonal cross-sectional variation, undulation, neurites branching complexity, presence of spines and/or presence of organelles (35, 36, 39, 60, 68). Second, we modeled exchange using the Kärger formulation, as the standard choice in diffusion MRI. Although this ensures comparability with existing work, our results show the Kärger model's limitation to describe complex microstructure contributions to microscopic-kurtosis. Third, as with the standard axial and radial quantities of DTI and DKI, the independent information captured by the axial and radial microscopic- and variance-kurtosis tensors requires some degree of compartmental directional coherence. For biological tissues where directional coherence is absent, axial/radial quantities will converge toward their respective mean quantities. In this study, this scenario was observed in the GL261 tumors; for this highly disorganized tumor phenotype, the axial, radial, and mean kurtosis metrics presented identical maps. Finally, the signal-to-noise requirements of CTI remain a practical limitation (31, 32), particularly for clinical use. Reducing the complexity of directional sampling and improving preprocessing and reconstruction pipelines will be essential steps toward routine deployment.

Material and Methods

Simulations

To investigate the sensitivity of the expanded CTI framework to specific microstructural features, we conducted two sets of simulations:

1. Impermeable structures with variable caliber

Monte Carlo simulations were performed using the open-source DISIMPY package (69) on impermeable structures aligned to the z-axis (c.f. Fig. 1B2). The radius profile was defined as (33, 48):

$$r(z) = r_0 + \sqrt{2} \text{SD}(r) \sin\left(\frac{2\pi z}{l_p}\right) \quad (17)$$

where r_0 is the mean radius (set to $r_0 = 1 \mu\text{m}$), $\text{SD}(r)$ is the radius standard deviation (varied from 0 to a maximum of $0.42r_0$), and l_p is the caliber variation period (set to $l_p = 1.5 + 4r_0$ (70)). Random walks from inside the structures were produced for this geometry.

To consider diffusion contributions outside the structures, a periodic arrangement of structure replicas was generated around a central structure. To maximize packing, replicas at coordinates $(\pm s, \pm s)$ were phase-shifted by π relative to the central structure placed at $(0, 0)$. The spacing s was determined by a desired structure volume fraction of $f = 0.7$.

$$s = \sqrt{\frac{\pi}{2f} (\text{SD}(r)^2 + r_0^2)} \quad (18)$$

The meshes of structures were truncated at the periodic boundaries $(\pm s, \pm s)$. Random walk simulations were performed separately for intra- and extra-structural spaces with 10^5 walkers each. Trajectories were simulated over $\Delta = 10 \text{ ms}$ with bulk diffusivities set to $D_0 = 2.5 \mu\text{m}^2/\text{ms}$ (intra) and $0.6D_0 = 1.5 \mu\text{m}^2/\text{ms}$ (extra) and a time step of $0.1 \mu\text{s}$. Note that simulations are representative for any D_0, Δ, r_0 combination satisfying $\frac{D_0 \Delta}{r_0^2} = 25$, corresponding to a relative long diffusion time in respect to structure radius (71).

Diffusion and kurtosis tensors for each compartment (D_{ij}^c and W_{ijkl}^c) are calculated from walker displacements $\mathbf{R}$ using the tensors ground truth definitions (14):

$$D_{ij}^c = \frac{\langle R_i R_j \rangle}{2\Delta} \quad (19)$$

$$W_{ijkl}^c = 9 \frac{\langle R_i R_j R_k R_l \rangle - \langle R_i R_j \rangle \langle R_k R_l \rangle - \langle R_i R_k \rangle \langle R_j R_l \rangle - \langle R_i R_l \rangle \langle R_j R_k \rangle}{\langle \mathbf{R} \cdot \mathbf{R} \rangle^2} \quad (20)$$

To simulate orientation dispersion, replicas of the diffusion and kurtosis tensors D_{ij}^c and W_{ijkl}^c were produced with main axial directions sampled according to a Watson orientation distribution function (ODF) with concentration parameter $c = 5.24$ (FWHM $\approx 18^\circ$).

Total diffusion, kurtosis, and covariance tensors were then computed from D_{ij}^c and W_{ijkl}^c intra- and extra-structural replicas by:

$$D_{ij} = \langle D_{ij}^c \rangle \quad (21)$$

$$W_{ijkl} = \frac{\langle W_{ijkl}^c \bar{D}^2 \rangle}{\bar{D}^2} \quad (22)$$

$$C_{ijkl} = \langle D_{ij}^c D_{kl}^c \rangle - D_{ij} D_{kl} \quad (23)$$

These later tensors were used to derive the 3D profiles of variance- and microscopic kurtosis sources (equations 7-10) and respective derived quantities (equations 11-16) shown in Fig. 1.

2. Permeable structures with exchange

To simulate the effects of inter-compartmental water exchange on CTI-derived kurtosis components, an analytical approach was adopted based on the Kärger model. Specifically, as done in (41), we applied the second-order cumulant expansion of the Kärger model for DDE signals, which can be expressed by:

$$\log(E(\mathbf{q}_1, \mathbf{q}_2)) = -(q_{1i}q_{1j} + q_{2i}q_{2j})\Delta D_{ij} + \frac{1}{6}(q_{1i}q_{1j}q_{1k}q_{1l} + q_{2i}q_{2j}q_{2k}q_{2l})\Delta^2 \bar{D}^2 W_{ijkl}^0 h_{SDE} + q_{1i}q_{1j}q_{2k}q_{2l}\Delta^2 C_{ijkl}^0 h_{DDE} \quad (24)$$

where $\mathbf{q}_1$ and $\mathbf{q}_2$ are the q-vectors for the two diffusion-sensitizing gradients, W_{ijkl}^0 and C_{ijkl}^0 are the kurtosis and diffusion covariance tensors in the absence of exchange (i.e. exchange rate $k = 0 \text{ ms}^{-1}$), and h_{SDE} and h_{DDE} quantify how exchange modulates kurtosis under SDE-like and DDE-like acquisitions:

$$h_{SDE} = \frac{2}{(k\Delta)^2} (k\Delta - (1 - e^{-k\Delta})) \quad (25)$$

$$h_{DDE} = \frac{1}{(k\Delta)^2} e^{-k\tau_m} (1 - e^{-k\Delta})^2 \quad (26)$$

where Δ and τ_m are the diffusion and mixing times of the DDE.

By applying equations 9-16 to the exchange-corrected kurtosis and diffusion covariance tensors $W_{ijki} \equiv W_{ijkl}^0 h_{SDE}$ and $C_{ijki} \equiv C_{ijkl}^0 h_{DDE}$ (and noting that $W_{ijkl}^0 = \frac{3C_{ijkl}^0}{\bar{D}^2}$), the directional total-, variance- and microscopic-kurtosis quantities for the Kärger model are given by:

$$\left\{ \begin{array}{l} \bar{K}_t = h_{SDE} \bar{K}_0 \\ K_t^\perp = h_{SDE} K_0^\perp \\ K_t^\parallel = h_{SDE} K_0^\parallel \\ \bar{K}_v = h_{DDE} \bar{K}_0 \\ K_v^\perp = h_{DDE} K_0^\perp \\ K_v^\parallel = h_{DDE} K_0^\parallel \\ \bar{K}_\mu = (h_{SDE} - h_{DDE}) \bar{K}_0 \\ K_\mu^\perp = (h_{SDE} - h_{DDE}) K_0^\perp \\ K_\mu^\parallel = (h_{SDE} - h_{DDE}) K_0^\parallel \end{array} \right. \quad (27)$$

where $\bar{K}_0$, $K_0^\perp$, and $K_0^\parallel$ are the mean/radial/axial variance-kurtosis in the absence of exchange. From the equations in 27, one can note that the ratios between variance/microscopic contributions and the total kurtosis are directionally independent, i.e.:

$$\frac{\bar{K}_v}{\bar{K}_t} = \frac{K_v^\perp}{K_t^\perp} = \frac{K_v^\parallel}{K_t^\parallel} = \frac{h_{DDE}}{h_{SDE}}, \quad (28)$$

and

$$\frac{\bar{K}_\mu}{\bar{K}_t} = \frac{K_\mu^\perp}{K_t^\perp} = \frac{K_\mu^\parallel}{K_t^\parallel} = 1 - \frac{h_{DDE}}{h_{SDE}}. \quad (29)$$

For our simulations, variance- and microscopic kurtosis were computed from equations 25-29 by setting C_{ijkl}^0 to the diffusion covariance tensor obtained from the Monte Carlo simulations of impermeable cylinders with uniform radius ($r_0=1 \mu\text{m}$, $SD(r)=0 \mu\text{m}$), where disorder effects were shown negligible. Exchange rate was varied from $k = 0$ to $3/\Delta \text{ ms}^{-1}$, and $\tau_m = \Delta$ which is representative to the acquisition setting of our MRI experiments (vide infra).

Human MRI Experiments

All data acquisition procedures were approved by the Research Ethics Committee of the University of Trento, and written informed consent was obtained from all participants. As the expanded CTI framework developed does not impose additional acquisition requirements beyond those used for directionally averaged CTI metrics, the 3D profiles of $K_v(\mathbf{n})$ and $K_\mu(\mathbf{n})$ and respective directional quantities ($K_v^\perp$, $K_v^\parallel$, $K_\mu^\perp$, $K_\mu^\parallel$) were extracted from the data previously analyzed for CTI-derived mean kurtosis quantities ($\bar{K}_{ani}$, $\bar{K}_{iso}$, $\bar{K}_\mu$) (34).

Diffusion-weighted MRI data were acquired from ten healthy adult volunteers (mean age $\pm$ SD: 28.9 ± 6.0 years; 6 females) on a 3T clinical MRI system (MAGNETOM Prisma, Siemens Healthineers AG, Forchheim, Germany) equipped with a 64-channel head-neck receive coil. Data were collected using a prototype twice-refocused spine echo DDE sequence with the following (b_1 , b_2) pairing each with 60 directions: (1, 0) $\text{ms}/\mu\text{m}^2$; (2, 0) $\text{ms}/\mu\text{m}^2$; (1, 1) $\text{ms}/\mu\text{m}^2$ for ($\mathbf{q}_1 \parallel \mathbf{q}_2$); and (1, 1) $\text{ms}/\mu\text{m}^2$ for ($\mathbf{q}_1 \perp \mathbf{q}_2$). Each direction was acquired with reversed diffusion gradient polarity to suppress cross-term contributions from imaging gradients. To ensure accurate normalization, at least 9 $b = 0$ images were interleaved throughout the acquisition. Sequence timing parameters were: $\Delta = 31.8 \text{ ms}$, $\tau_m = 32.3 \text{ ms}$, $\delta = 15.8 \text{ ms}$, $\text{TE} = 127 \text{ ms}$, and $\text{TR} = 5500 \text{ ms}$ (subjects 1–2) or 5600 ms (subjects 3–10). Additional imaging parameters included matrix size = 84×84 , isotropic resolution = $2.5 \times 2.5 \times 2.5 \text{ mm}^3$, partial Fourier (PF) = 6/8, and GRAPPA/SMS acceleration factors of 2 and 4, respectively. Pre-processing steps were previously reported in detail (34). CTI fitting was performed using the constrained linear least-squares solution to the cumulant expansion (Eq. 24), as detailed in the Supplementary Materials Section A. To avoid that very low/large outlier kurtosis values substantially leverage statistical analysis, all kurtosis maps were clipped in a range between 0 and 3. For group-averaging, CTI maps were registered to a common template using symmetric diffeomorphic registration implemented in the Diffusion in Python (DIPY) software package (72). For ROI analysis, subject-wise and template-averaged CTI metrics were extracted from the 19 largest bilateral white matter regions of interest (ROIs) in the Johns Hopkins University (JHU) atlas (73), averaged across hemispheres. Statistical differences between radial and axial kurtosis quantities across the different ROIs is quantified using paired t-test - False Discovery Rate (FDR) was performed for multiple comparisons).

Animal Models and Preclinical MRI

1. Ethics

Mouse colonies were maintained in a certified animal facility in accordance with the European guidelines for laboratory animal use and care (Directive 2010/63/EU). All experimental procedures were approved by the local Animal Welfare and Ethics Body and the national authority, Direção-Geral de Alimentação e Veterinária (DGAV). Experimental protocols involving the SHH medulloblastoma model were specifically authorized under the license/protocol number 2020/008.

2. Ischemic Stroke Data

As with the human dataset, diffusion MRI data originally acquired for directionally averaged CTI metrics (33) were here reanalyzed to compute directional kurtosis source metrics in brains where ischemic stroke lesions were induced. This reuse of data aligned with ethical guidelines on animal use reduction.

These data were acquired ex vivo from ten adult male mice (C57BL/6J background, ~11 weeks old) in which focal ischemic lesions were induced using the photothrombotic stroke model (74). Each mouse was injected with meloxicam (5 mg/kg) 30 minutes prior to surgery and anesthetized with isoflurane (~2.5% in oxygen-enriched medical air composed of 70% nitrogen, 29% oxygen and the remaining 1% comprising mostly argon, carbon dioxide and helium). The skull was exposed by a median incision of the skin (dorsal aspect of the head), a photo-sensitive dye (Rose Bengal, Sigma Aldrich; 15 mg/ml in sterile saline) was administered via retro-orbital injection (10 μ l/g body weight) and light (3200 K, 10 W/cm²) was focused on the barrel cortex (S1bf; 1.67 mm posterior, 3 mm lateral to bregma) for 15 minutes. The incision was sutured and the animal was injected with meloxicam (5 mg/kg). Three hours post-induction, animals were perfused transcardially with 4% paraformaldehyde (PFA), and brains were post-fixed in 4% PFA for 24 hours, followed by PBS washing. Samples were then placed in 10-mm NMR tubes filled with Fluorinert (Sigma Aldrich, Lisbon, PT) for MRI scanning.

Ex vivo MRI acquisitions were performed on a 16.4T Aeon Ascend scanner (Bruker BioSpin MRI GmbH, Karlsruhe, Germany) using a Micro5 probe (3000 mT/m maximum gradient strength) and birdcage RF coil. Samples were maintained at 37 °C using the system's temperature control. A custom-developed DDE sequence (Paravision 6.0.1) was used with the following (b_1 , b_2) pairing each with 135 directions: (3, 0), (1.5, 0), (0.75, 0) ms/ μ m²; and (1.5, 1.5), (0.75, 0.75) ms/ μ m² for both ($\mathbf{q}_1 \parallel \mathbf{q}_2$) and ($\mathbf{q}_1 \perp \mathbf{q}_2$) settings. Sequence timing parameters were: Δ = 10 ms, τ_m = 10 ms, δ = 1.5 ms, TE = 50 ms, and TR = 4000 ms. Other imaging parameters: matrix size = 78 $\times$ 78, resolution=141 $\times$ 141 $\times$ 500 μ m³, no PF, number of segments = 2, number of averages = 8.

3. Model of Medulloblastoma

Sonic hedgehog medulloblastomas were induced in five genetically engineered mice (Math1-CreERT2/R26R-SmoM2, (50)) through the administration of 1mg/kg of tamoxifen at postnatal day 3. Six Math1-CreER mice that received 1mg/kg of tamoxifen at postnatal day 3 were used as littermate controls. Tamoxifen was diluted in a solution of 10% ethanol and 90% of sunflower oil.

Five weeks post-induction, animals were euthanized using transcardial perfusion with 4% paraformaldehyde (PFA). The intact heads were fixed for 24 hours in 4% PFA, washed in phosphate-buffered saline (PBS), and placed in a 20-mm NMR tube filled with Fluorinert (Sigma-Aldrich) for scanning.

MRI acquisitions were performed at room temperature using a 9.4T Bruker BioSpec scanner (Bruker BioSpin MRI GmbH, Karlsruhe, Germany) equipped with a 4-channel array surface array coil. DDE data were acquired with the following (b_1, b_2) pairing each with 135 directions: (2.45, 0.05), (1.25, 1.25), (0.625, 0.625) ms/ μm^2 for $(\mathbf{q}_1 \parallel \mathbf{q}_2)$; and (1.25, 1.25) ms/ μm^2 for $(\mathbf{q}_1 \perp \mathbf{q}_2)$ directions. Sequence timing parameters were: $\Delta = 10$ ms, $\tau_m = 12$ ms, $\delta = 4$ ms, TE = 49.2 ms, and TR = 3000 ms. Other imaging parameters: matrix size = 78×78 , resolution = $143 \times 143 \times 500 \mu\text{m}^3$, PF = 0.8, number of segments = 1, number of averages = 6.

4. Models of Glioma

Glioblastoma were induced in six adult mice (C57BL/6J background, ~11 weeks old) by intracranial stereotactic injection of GL261 (N=3) or CT2A cells (N=3) in the caudate nucleus (51). After 3 weeks post-inoculation, mouse brains were extracted following transcardial perfusion with 4% Paraformaldehyde (PFA) and placed in a 10mm NMR tube filled with Fluorinert (Sigma Aldrich, Lisbon, PT).

MRI acquisitions were performed on a pre-clinical 16.4 T Aeon Ascend scanner (Bruker BioSpin MRI GmbH, Karlsruhe, Germany), equipped with a cryogenic coil, at 37 °C using the probe's variable temperature capability. DDE data were acquired with the following (b_1, b_2) pairing each with 135 directions: (2.45, 0.15), (1.85, 0.15), (1.3, 1.3), (1, 1), (0.65, 0.65), (0.15, 0.15) ms/ μm^2 for $(\mathbf{q}_1 \parallel \mathbf{q}_2)$; and (1.3, 1.3), (1, 1) ms/ μm^2 for $(\mathbf{q}_1 \perp \mathbf{q}_2)$ directions. Sequence timing parameters were: $\Delta = 12$ ms, $\tau_m = 20$ ms, $\delta = 2$ ms, TE = 51 ms, and TR = 3000 ms. Other imaging parameters: matrix size = 74×74 , resolution = $150 \times 150 \times 300 \mu\text{m}^3$, PF = 0.8, number of segments = 1, number of averages = 10.

5. Preclinical data processing

To minimize the effects of thermal noise, all preclinical datasets were first denoised using the Threshold PCA (TPCA) algorithm (75). For the medulloblastoma datasets and its controls, TPCA was applied prior to magnitude reconstruction. This was performed for a more optimal denoising performance as this dataset was acquired with less DDE experiments and a lower field scanner. Medulloblastoma data was also smoothed with a 3D Gaussian kernel with FWHM=1.25 pixels. Note that smoothing was only used to enhance CTI visualization as it did not have any effect on statistical analysis.

For all other datasets, the algorithm was applied directly to magnitude-reconstructed images as raw complex data was not available, and smoothing was not applied. Data was then aligned using a sub-pixel algorithm (76), and image global signal intensity drifts were corrected based on the information provided by the interleaved low b-value acquisitions. CTI fitting was performed using the same constrained linear least-squares approach described in supporting material.

For statistical group comparisons, the following ROI strategies were employed:

- Stroke model: ROIs were manually defined in ischemic and contralateral WM, guided by both diffusion weighted images acquired for the higher b_1 employed and CTI FA maps, c.f. Supporting Material Fig. S2. Paired t-tests were used to assess averaged within-subject differences, and multiple comparisons were corrected using the false discovery rate (FDR).
- Medulloblastoma model: Due to diffuse tumor infiltration, whole cerebella of both tumor-bearing and control was segmented directly on the T2-weighted images acquired with the DDE sequence by setting $b_1 = b_2 = 0$. Averaged cerebella differences between tumor-bearing and control animals were assessed using two-sample t-tests with FDR correction.
- Glioma model: Averaged kurtosis values were computed over the entire segmented tumor mass for each animal. Group differences between CT2A and GL261 models were assessed via two-sample t-tests.

Note: To reduce the impact of outlier kurtosis values on group statistics, metric values outside the range [0, 3] were excluded from the stroke and medulloblastoma datasets. For the glioma data, where visually lower kurtosis values were observed, a broader acceptance interval of [-0.5, 3] was used.

6. Histopathological Analysis

For histological validation, additional mice were sacrificed, and their brains were harvested, fixed in formalin, embedded in paraffin, trimmed, and sectioned. Coronal sections were performed for animal models of stroke and gliomas, while sagittal sections were obtained for the animal model of medulloblastoma.

For the ischemic stroke model, coronal 50 μm sections were stained with Anti-GFP Alexa Fluor 488 antibody (Thermo Fisher Scientific) to amplify endogenous YFP fluorescence of transgenic Thy1-ChR2-YFP mice and facilitate the visualization of neuronal structures within the infarcted and contralateral areas. ROIs from the ipsi- and contralesional hemispheres were selected (barrel cortex and corpus callosum) and scanned using a Zeiss LSM 980 Confocal Microscope at 40x magnification.

For all three tumor models, hematoxylin and eosin (H&E) staining was used to assess cytoarchitecture and tumor cell morphology by a trained pathologist blinded to clinical outcome and experimental groups. Histological coronal 4 μm sections of glioma bearing mice were examined using an Axioscope 5 microscope (Zeiss) equipped with an AxioCam 208 color camera (Zeiss), while the whole 4 μm section of medulloblastoma bearing mice were acquired using a brightfield slide scanner (Ultra-Fast Scanner, Philips).

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Figure Legends

Figure 1 – Schematic of DDE acquisitions required for CTI and illustration of CTI-derived effects across different simulated microstructural scenarios. (A) In addition to acquisitions with at least two non-zero total b-values, CTI requires DDE signals obtained with: symmetric gradient intensities for both parallel (A1) and perpendicular (A2) directions, and asymmetric gradient intensities for parallel directions (A3). (B) Illustration of the three different geometries used for simulations: (B1) impermeable cylinders with uniform radius ($r_0=1\ \mu\text{m}$); (B2) impermeable structures with caliber variation (relative radius standard deviation $SD(r)/r_0=0.42$); and (B3) permeable cylinders modeled using the Kärger exchange model (exchange rate $k = 0.3/\Delta\ \text{ms}^{-1}$). Note that, to account for tissue dispersion, random walks for each scenario were convolved with a Watson fiber orientation distribution function (ODF). (C) Previous CTI's mean kurtosis quantities for the three simulated scenarios: mean total kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); and microscopic kurtosis ($\bar{K}_\mu$). (D) 3D directional profile of total-kurtosis $K_t(\mathbf{n})$ (information that could be resolved using the standard DKI approach), showing elevated values radially in all scenarios. (E) 3D directional profile of the variance-kurtosis $K_v(\mathbf{n})$, showing reduced contributions only in the presence of Kärger exchange. (F) 3D directional profile of the microscopic-kurtosis $K_\mu(\mathbf{n})$, showing residual values in uniform impermeable cylinders (F1), axial peaks in structures with caliber variation (F2), and maximum radial values in permeable cylinders (F3). (G) Directional kurtosis source sensitivity to cross-sectional variance: (G1-2) Radial/axial variance-kurtosis ($K_v^\perp$ and $K_v^\parallel$) and microscopic-kurtosis ($K_\mu^\perp$ and $K_\mu^\parallel$) as function of $SD(r)/r_0$ in impermeable structures. (H) Directional kurtosis source sensitivity to permeability: (H1-2) $K_v^\perp$, $K_v^\parallel$, $K_\mu^\perp$, $K_\mu^\parallel$ (and $K_t^\perp$ for reference) as function of $k\Delta$ in permeable cylinders; and (H3) ratios $K_v^\perp/K_t^\perp$, $K_v^\parallel/K_t^\parallel$, $K_\mu^\perp/K_t^\perp$, and $K_\mu^\parallel/K_t^\parallel$ as function of $k\Delta$. Clear signatures for Kärger exchange vs. structural disorder can be discerned.

Figure 2 – CTI results obtained from the human dataset. (A) CTI maps from the template averaged data, including: fractional anisotropy (FA), radial diffusivity ($D^\perp$), axial diffusivity ($D^\parallel$), mean total-kurtosis ($\bar{K}_t$), radial total-kurtosis ($K_t^\perp$), axial total-kurtosis ($K_t^\parallel$), mean variance-kurtosis ($\bar{K}_v$), radial variance-kurtosis ($K_v^\perp$), axial variance-kurtosis ($K_v^\parallel$), mean microscopic-kurtosis ($\bar{K}_\mu$), radial microscopic-kurtosis ($K_\mu^\perp$), axial microscopic-kurtosis ($K_\mu^\parallel$). (B) Kurtosis values for different white matter ROIs ordered to their mean values $K_\mu^\perp$: (B1) averaged radial and axial variance-kurtosis ($K_v^\perp$ and $K_v^\parallel$); (B2) averaged radial and axial microscopic-kurtosis ($K_\mu^\perp$ and $K_\mu^\parallel$); (B3) ratios between radial microscopic- and total-kurtosis ($K_\mu^\perp/K_t^\perp$) and between axial microscopic- and total-kurtosis ($K_\mu^\parallel/K_t^\parallel$). In panels B1–B3, bars represent values from the template-averaged data, while overlaid black dots represent values from individual subjects. Significant differences between radial and axial values are indicated by *, **, and *** for p-values < 0.05, 0.01, and 0.001, respectively (paired t-test, p-values adjusted for multiple comparisons using FDR correction). Note that in nearly all areas Kärger exchange signatures are not observed, while structural disorder signatures are. This supports a biophysical interpretation of structural disorder as the dominant source of microscopic kurtosis in the human brain.

Figure 3 – CTI and histological results in a mouse model of acute ischemic stroke. (A) CTI maps from a representative mouse 3 hours after photothrombotic stroke induction, showing typical diffusion abnormalities in the ischemic lesion. These include reduced fractional anisotropy (FA), radial diffusivity ($D^\perp$), and axial diffusivities ($D^\parallel$) along with elevated mean, radial and axial total-kurtosis ($\bar{K}_t$, $K_t^\perp$, $K_t^\parallel$). Marked increases are also observed in the newly derived variance-kurtosis ($\bar{K}_v$, $K_v^\perp$, $K_v^\parallel$) and microscopic-kurtosis ($\bar{K}_\mu$, $K_\mu^\perp$, $K_\mu^\parallel$) metrics within the affected region. (B) Quantitative analysis of averaged CTI metrics extracted from the delineated WM ROIs (c.f. Supporting Material Figure S.2, for more information about these ROI). (B1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_\mu$). (B2) Radial components: radial total-kurtosis ($K_t^\perp$); radial variance-kurtosis ($K_v^\perp$); radial microscopic-kurtosis ($K_\mu^\perp$). (B3) Axial components: axial total-kurtosis ($K_t^\parallel$); axial variance-kurtosis ($K_v^\parallel$); axial microscopic-kurtosis ($K_\mu^\parallel$). (B4) relative differences for all directional kurtosis quantities, calculated as $(M_s - M_c)/M_c$, where M_s and M_c represent average metric values in stroke and contralateral ROIs, respectively. The largest relative increase is observed in axial microscopic-kurtosis ($K_\mu^\parallel$). In panels B1–B4, bars represent group-averaged values across 9 mice; overlaid black dots show individual animal data. Significant differences between

stroke and contralateral ROIs (B1–B3) and between radial and axial values are indicated by *, **, and *** for $p < 0.05$, 0.01 , and 0.001 , respectively (paired t-test, FDR corrected). **(C–D)** Confocal histological analysis of white matter (corpus callosum, CC) and cortex (barrel cortex, BC, layers 2–3) in a Thy1-ChR2-YFP mouse ($40\times$ magnification): (C1) contralateral CC; (C2) Stroke-affected CC; (D1) contralateral BC; (D2) Stroke-affected BC. Anti-GFP Alexa Fluor 488 was used to amplify endogenous YFP fluorescence, enabling high-resolution neurite visualization. Note that widespread neurite beading is evident in stroke-affected regions, supporting the biophysical interpretation of microscopic kurtosis as emerging from structural disorder but not Kärger exchange.

Figure 4 – Histological and CTI results in a mouse model of medulloblastoma. (A–B) Haematoxylin-eosin-stained histological sections from the cerebellum of littermate controls (A) and Math1-CreERT2/R26-SmoM2 (B) mice five weeks upon tamoxifen administration: lower (A1/B1) and higher (A2/B2) magnification. Control mice show well-organized cerebellar folia, with intact layering and uniform cellularity (A). In tumor-bearing mice, the cerebellum is significantly expanded by a mass that overlies and distorts the parenchyma, with cerebellar coning (B1, arrow). High-power view (B2) reveals a densely cellular neoplasm infiltrating the molecular layer (arrows) and compressing the Purkinje cell layer, granule layer, and white matter. **(C–D)** Representative CTI-derived maps at the level of the cerebellar arbor vitae for control and tumor-bearing animals, showing: (C1/D1) fractional anisotropy (FA); (C2/D2) anisotropic kurtosis ($\bar{K}_{ani}$); (C3/D3) isotropic kurtosis ($\bar{K}_{iso}$); (C4/D4) mean microscopic-kurtosis ($\bar{K}_{\mu}$); (C5/D5) radial microscopic-kurtosis ($K_{\mu}^{\perp}$); and (C6/D6) axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). **(E)** Group-level quantification of CTI-derived kurtosis metrics across manually segmented cerebellums. (E1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_{\mu}$). (E2) Radial components: radial total-kurtosis ($K_t^{\perp}$); radial variance-kurtosis ($K_v^{\perp}$); radial microscopic-kurtosis ($K_{\mu}^{\perp}$). (E3) Axial components: axial total-kurtosis ($K_t^{\parallel}$); axial variance-kurtosis ($K_v^{\parallel}$); axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). In panels E1–E3, bars represent group-averaged values; overlaid black dots indicate individual animal values. Significant group differences are denoted by *, **, and *** for $p < 0.05$, 0.01 , and 0.001 , respectively (two-sample t-test, FDR corrected).

Figure 5 – Histological and CTI results in two glioma mouse models. (A–B) Hematoxylin-eosin-stained histological images acquired using bright-field microscopy of representative CT2A and GL261 tumors: lower (A1/B1) and higher (A2/B2) magnification (arrow points to tumor vessels, while cardinal points to multinucleated tumor cells). **(C–D)** Representative CTI-derived maps for CT2A and GL261 tumors, showing: (C1/D1) fractional anisotropy (FA); (C2/D2) anisotropic kurtosis ($\bar{K}_{ani}$); (C3/D3) isotropic kurtosis ($\bar{K}_{iso}$); (C4/D4) mean microscopic-kurtosis ($\bar{K}_{\mu}$); (C5/D5) radial microscopic-kurtosis ($K_{\mu}^{\perp}$); and (C6/D6) axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). **(E)** Group-level quantification of CTI-derived kurtosis metrics across manual segmented tumors. (E1) Mean kurtosis components: mean total-kurtosis ($\bar{K}_t$); anisotropic kurtosis ($\bar{K}_{ani}$); isotropic kurtosis ($\bar{K}_{iso}$); mean microscopic-kurtosis ($\bar{K}_{\mu}$). (E2) Radial components: radial total-kurtosis ($K_t^{\perp}$); radial variance-kurtosis ($K_v^{\perp}$); radial microscopic-kurtosis ($K_{\mu}^{\perp}$). (E3) Axial components: axial total-kurtosis ($K_t^{\parallel}$); axial variance-kurtosis ($K_v^{\parallel}$); axial microscopic-kurtosis ($K_{\mu}^{\parallel}$). In panels E1–E3, bars represent group-averaged values; overlaid black dots indicate individual animal values. Significant group differences are denoted by *, **, and *** for $p < 0.05$, 0.01 , and 0.001 , respectively (two-sample t-test, no multiple comparison is here performed to highlight $K_{\mu}^{\perp}$ trends).

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Supplementary Information

A. Constrained Correlation Tensor Imaging Fitting

To ensure physically plausible and robust estimation of diffusion, kurtosis, and covariance tensors, CTI fitting was performed using constrained linear least squares (CLLS), adapted from a previous approach developed for DKI fitting (46). The CLLS optimization is defined as:

$$\begin{aligned} & \text{Minimize } \|\mathbf{A}\mathbf{X} - \mathbf{L}\|^2 \\ & \text{subject to } \mathbf{C}\mathbf{X} \leq \mathbf{d} \end{aligned} \quad (30)$$

where $\mathbf{X}$ is a 43-element CTI parameter vector that includes: 1) the six independent elements of the diffusion-tensor; 2) the fifteen independent elements of the total-kurtosis tensor; 3) the twenty one independent elements of the covariance tensors; and 4) the predicted log signal at $b_t = 0$:

$$\mathbf{X} = [D_{xx}, \dots, D_{yz}, \bar{D}^2 W_{xxxx}, \dots, \bar{D}^2 W_{xyyz}, C_{xxxx}, \dots, C_{xyyz}, \log S_0]^T \quad (31)$$

$\mathbf{A}$ is a $N \times 43$ matrix that relates the DDE acquisition parameters (i.e. $\mathbf{q}_1$ and $\mathbf{q}_2$) to the signal, which is constructed according to Eq. 1 in the main text:

$$\mathbf{A} = \begin{bmatrix} -(q_{1x}^{(1)} q_{1x}^{(1)} + q_{2x}^{(1)} q_{2x}^{(1)})\Delta & \dots & \frac{1}{6}(q_{1x}^{(1)} q_{1x}^{(1)} q_{1x}^{(1)} q_{1x}^{(1)} + q_{2x}^{(1)} q_{2x}^{(1)} q_{2x}^{(1)} q_{2x}^{(1)})\Delta^2 & \dots & 1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ -(q_{1x}^{(N)} q_{1x}^{(N)} + q_{2x}^{(N)} q_{2x}^{(N)})\Delta & \dots & \frac{1}{6}(q_{1x}^{(N)} q_{1x}^{(N)} q_{1x}^{(N)} q_{1x}^{(N)} + q_{2x}^{(N)} q_{2x}^{(N)} q_{2x}^{(N)} q_{2x}^{(N)})\Delta^2 & \dots & 1 \end{bmatrix} \quad (32)$$

While the rows of $\mathbf{A}$ encode the N different DDE experiments performed, $\mathbf{L}$ contains the respective measured log signals:

$$\mathbf{L} = [\log S(\mathbf{q}_1^{(1)}, \mathbf{q}_2^{(1)}), \dots, \log S(\mathbf{q}_1^{(N)}, \mathbf{q}_2^{(N)})]^T \quad (33)$$

To enforce non-negativity on diffusivities, direction total-kurtosis, and the trace of the covariance tensor (directly related to $\bar{K}_{iso}$, c.f. Eq. 3 in the main text), the constraint matrix $\mathbf{C}$ is designed based on the following three portions:

$$\mathbf{C} = \begin{bmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \mathbf{C}_3 \end{bmatrix} \quad (34)$$

- **$\mathbf{C}_1$ (Diffusivity constraints):** the first N rows of matrix $\mathbf{C}$ ensure that apparent diffusivities remain positive. These rows contain non-zero entries only in columns one to six:

$$C_1(i, 1:6) = \frac{A(i, 1:6)}{b_t^{(i)}} \quad (35)$$

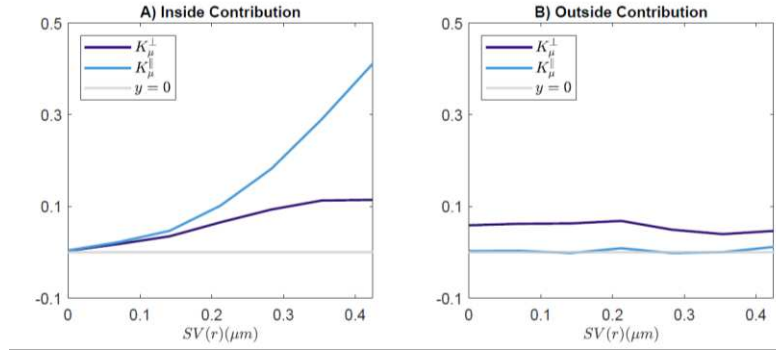
- **C_2 (Total kurtosis constraints):** the following N rows of matrix C enforce non-negativity of total kurtosis values. Non-zero entries appear only in columns seven to forty-two:

$$C_2(i, 7:42) = -\frac{A(i, 7:42)}{(b_t^{(i)})^2} \quad (36)$$

- **C_3 (Covariance tensor constraint):** a final single row that ensures the trace of the covariance tensor is non-negative. This is implemented by combining the relevant covariance tensor components: columns corresponding to C_{xxxx} , C_{yyyy} , C_{zzzz} are set to $-1/18$, columns for C_{xxyy} , C_{xxzz} , C_{yyzz} are set to $-1/9$; while all other column elements are set to zero.

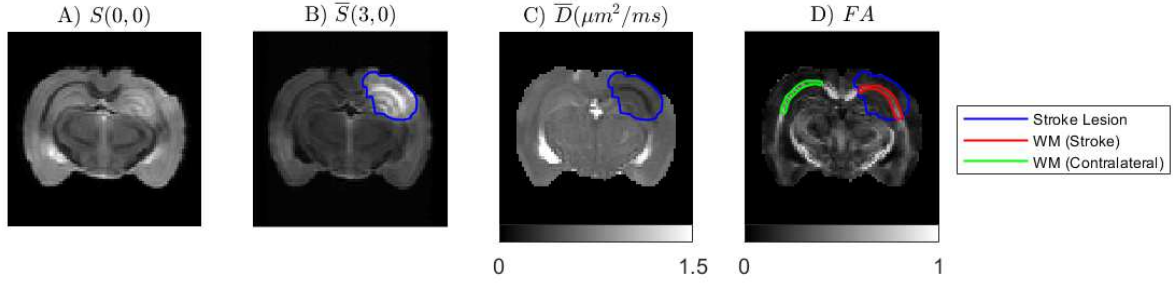
The inequality constraint $CX \leq d$ is enforced with d set to zero-elements vector, with number of elements matching the total number of rows in C (i.e. $2N+1$).

B. Supporting Simulations



Supporting Figure S1 – Simulated intra- and extra-compartment contributions to microscopic-kurtosis in impermeable structures with increasing caliber variation. (A) Axial and radial components of microscopic-kurtosis ($K_\mu^\perp$ and $K_\mu^\parallel$) computed exclusively from the intra-compartment kurtosis contributions. (B) Corresponding components computed from the extra-compartment kurtosis contributions. These contributions were obtained by selectively setting the extra- (panel A) or intra-compartment (panel B) terms of the W_{ijkl}^c tensor replicas to zero prior to averaging. The sum of values in panels A and B corresponds to the total $K_\mu^\perp$ and $K_\mu^\parallel$ reported in Fig. 1H. Notably, $K_\mu^\parallel$ primarily reflects intra-compartment caliber variation, while $K_\mu^\perp$ predominantly arises from extra-compartment non-Gaussian contributions due to structural packing.

C. Supporting Data Analysis



Supporting Figure S2 – Representative diffusion MRI maps used to define WM ROIs in stroked and contralateral hemispheres. **A)** Reference T2-weighted image acquired using the custom-developed DDE sequence with diffusion gradients (b_1/b_2) set to zero. **B)** Powder-averaged diffusion-weighted image, obtained by averaging all the images acquired with $(b_1/b_2) = (3,0)$ ms/ μm^2 across all gradient directions. This image provided enhanced delineation of the full extent of the stroke lesion and was used for initial segmentation of the ischemic region, including both grey and white matter (outlined in blue). **C)** Mean diffusivity $\bar{D}$ map computed from CTI model fitting. Reduced $\bar{D}$ values correspond well to the manually segmented stroke lesion (blue outline). **D)** FA map used to guide manual delineation of affected white matter within the global stroke lesion (red outline). The contralateral WM ROI was manually defined as a mirrored region of analogous size and location. This ROI definition procedure was repeated for all animals and across all imaging slices.