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Does powder averaging remove dispersion bias in diffusion MRI diameter estimates within real 3D axonal architectures?

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Abstract

Noninvasive estimation of axon diameter with diffusion MRI holds the potential to investigate the dynamic properties of the brain network and pathology of neurodegenerative diseases. Recent studies use powder averaging to account for complex white matter architectures, but these have not been validated for real axonal geometries from regions that contain fibre crossings. Here, we present 120 – 304 μm long segmented axons from X-ray nano-holotomography volumes of a splenium and crossing fibre region of a vervet monkey brain. We show that the axons in the complex crossing fibre region, which contains callosal, association, and corticospinal connections, are larger and exhibit a wider distribution than those of the splenium region. To accurately estimate the axon diameter in these regions, therefore, sensitivity to a wide range of diameters is required. We demonstrate how the q -value, b -value, signal-to-noise ratio and the assumed intra-axonal parallel diffusivity influence the range of measurable diameters with

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Abbreviations: ADD, axon diameter distribution; EAS, extra-axonal space; IAS, intra-axonal space; MRI, magnetic resonance imaging; OD, orientation dispersion; PA, powder average; PGSE, pulsed gradient spin echo; PL, power law; SMT, spherical mean technique; SNR, signal-to-noise ratio; WM, white matter; XNH, X-ray nano-holotomography.

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powder average approaches. Furthermore, we show how Gaussian distributed noise results in a wider range of measurable diameter at high b -values than Rician distributed noise, even at high signal-to-noise ratios of 100. The number of gradient directions is also shown to impose a lower bound on measurable diameter. Our results indicate that axon diameter estimation can be performed with only few b -shells, and that additional shells do not improve the accuracy of the estimate. For strong gradients available on human Connectom and preclinical scanners, Monte Carlo simulations of diffusion confirm that powder averaging techniques succeed in providing accurate estimates of axon diameter across a range of sequence parameters and diffusion times, even in complex white matter architectures. At relatively low b -values, the diameter estimate becomes sensitive to axonal microdispersion and the intra-axonal parallel diffusivity shows time dependency at both in vivo and ex vivo intrinsic diffusivities.

Keywords: Fibre architecture, axon diameter, diffusion MRI, powder average, microstructure, white matter

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1. Introduction

Action potentials propagate along axons and enable communication across different parts of the central nervous system. The axonal morphologies are crucial for the signal conduction process [1, 2, 3] and determine the conduction velocity with which signals are propagated. By electrophysiological modelling of the axon, Drakesmith et al. showed that axon diameter is the most important determinant of conduction velocity in myelinated axons [4]. Axon diameter is also a potential biomarker of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis [5] and Multiple Sclerosis [6], and has been suggested to correlate with clinical scores of cognitive impairment in Multiple Sclerosis patients [7]. Thus, axon diameter sheds light on brain health, as well as the structural and functional properties of the brain network.

Diffusion Magnetic Resonance Imaging (MRI) non-invasively probes the microstructural brain tissue environment by measuring the diffusion of water

15 molecules across millisecond time scales. By fitting biophysical models that de-
 scribe the underlying tissue microstructure to the diffusion MRI signal, axon
 diameter can be estimated [8, 9]. The AxCaliber method [10, 11] uses pulsed
 gradient spin echo (PGSE) measurements with numerous combinations of gra-
 dient strengths and diffusion times to output the axon diameter distribution
 20 (ADD), as demonstrated in vivo in the rat [10] and human [12] brains. AxCal-
 iber requires prior knowledge of the axon orientation since it relies on measure-
 ments being made perpendicular to the axons. The ActiveAx approach has been
 demonstrated in vivo in humans and ex vivo in primates [13, 14] and outputs
 a mean axon diameter index. Contrary to AxCaliber, ActiveAx is invariant to
 25 the orientation of the main fibre direction and implements an optimised acqui-
 sition consisting of three b -value shells, the minimum number of shells required
 to fit the three parameters in the signal model used by ActiveAx. These are
 sampled in ~ 90 unique directions distributed uniformly on the unit sphere.
 These two methods have in common that they do not account for non-parallel
 30 axons, i.e. orientation dispersion (OD), or multiple bundles of crossing axons,
 factors which bias the axon diameter measurement. Zhang et al. extended the
 ActiveAx approach in two ways. They relaxed the assumption of a single main
 fibre direction to enable axon diameter estimation in regions of the ex vivo mon-
 key brain in which there were crossing axon bundles [15], but the OD within
 35 those bundles was not taken into account. Later, Zhang et al. modelled the
 OD as a Watson distribution to fit axon diameter in the in vivo human brain
 [16], but the method assumed a single main bundle direction. As such, diffusion
 MRI-based axon diameter studies have mostly targeted the corpus callosum, an
 organised white matter (WM) region that consists of aligned interhemispheric
 40 axonal connections. The corpus callosum has also been the subject of light
 and electron microscopy studies on axon diameter, and these have been used
 as validation for the diffusion MRI-based axon diameter metrics [10, 17, 18].
 However, recent 3D imaging studies in the monkey and mouse corpus callosum
 demonstrate the complex morphologies, OD and trajectory variations of axons
 45 [19, 20, 21], and show how even in the highly organised corpus callosum these

will bias axon diameter measurements [19, 20, 22, 23]. Diffusion MRI-based estimates of axon diameter should thus take into account three different classes of orientation effects: 1) the macroscopic fibre architecture, describing the relative orientations of different fibre bundles e.g. in crossing fibre regions; 2) the OD, describing the average dispersion exhibited by axons within each bundle; and 3) the microdispersion, describing the changes in trajectory and curvature along individual axons on the length scale of the measured diffusion.

The effects of the macroscopic fibre architecture and OD can be removed by powder averaging (PA). The PA involves calculating the arithmetic mean of the diffusion MRI signal in isotropically distributed directions on the unit sphere. Each diffusing spin can be described as probing a micro-domain, a microscopic region of the tissue environment within a voxel. The PA signal thus represents the spherical mean of the set of micro-domains, regardless of their individual orientation or organisation within the voxel. Several studies have used the PA to disentangle the effects of fibre architecture and OD from diffusion metrics [24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 22], and it has recently been implemented to estimate axon diameter in the entire brain WM [34, 35]. To obtain estimates of axon diameter index in the in vivo human brain, Fan et al. [34] fitted a multi-compartment spherical mean technique (SMT) model to the PA signal. The signal was sampled in up to 64 uniformly distributed directions for two diffusion times. In total, 16 unique b -values up to $b \approx 20$ ms μm^{-2} were acquired, enabled by the high in vivo gradient strengths of up to 300 mT/m of the Connectom scanner [36, 37, 38]. Veraart et al. [35], on the other hand, modelled only the intra-axonal space (IAS) by fitting a power law (PL) to the PA signal at high b -values that suppress the signal from the extra-axonal space (EAS) [39, 40]. This used $b \geq 20$ ms μm^{-2} for ex vivo experiments and $b \geq 6$ ms μm^{-2} for in vivo experiments. The signal was measured along 60 uniformly distributed gradient directions at a single diffusion time for up to 18 b -values. Axon diameter was calculated for the ex vivo rat brain and, also using a Connectom scanner, for the in vivo human brain.

Although the PA techniques remove fibre architecture and OD effects, they

rely on the assumption that the micro-domain probed by diffusing spins is cylindrical. With increasing diffusion times, the spins diffuse further and increasingly probe the microdispersion of the axons, violating the assumption of a cylindrical micro-domain and making the axon diameter estimate time-dependent. The effects of diameter and trajectory variations on estimated axon diameter have been described [22, 41, 42], but the diffusion times and b -values for which the PA-based axon diameter estimate becomes sensitive to the microdispersion in axons is unknown. The signal-to-noise ratio (SNR) of the signal [43] and the gradient strength of the applied magnetic field [14] affect the sensitivity profile of the acquisition, placing limits on the upper and lower bounds of measurable axon diameter. How different sequence parameters, the number of gradient directions or the SNRs affect these bounds has not been investigated for PA-based AD estimates.

Validating the estimated axon diameters from the PA methods for different sequence parameters and in real axons is therefore important. The PL implementation from Veraart et al. has been evaluated within axon segments of length $\sim 20 \mu\text{m}$ from electron microscopy of the mouse corpus callosum using Monte Carlo simulations of diffusion [22]. Given that non-axonal structures in the EAS can impact the trajectories of axons for up to $20 \mu\text{m}$ [19], longer axonal segments may to a greater extent represent the characteristics of the IAS. Notably, although the PA is expected to factor out the effects of fibre crossings and OD, it has only been validated on segments of axons from the corpus callosum in which the fibre architecture is simple and does not contain substantial crossings.

In this study, we adopt a simulation-based *modus operandi* to validate PA-estimates of AD in real axons between 120 and $304 \mu\text{m}$ in length, segmented from large field-of-view X-ray nano-holotomography (XNH) volumes of two inherently different WM architectures of the vervet monkey brain. The two regions are: a) the ordered splenium corpus callosum (Fig. 1C) and b) a heterogeneous crossing fibre region (Fig. 1B). Throughout, we restrict the analysis to the IAS to analyse how accurately it is represented by the PA model at different b -values,

and the results are relevant to both multi-compartment and single-compartment models that include the IAS. Firstly, we explore how the SNR, noise distribution, gradient strength (hence, also b -value) and number of unique gradient directions determine the sensitivity profile of the PA acquisition to different diameters in cylinders. For the analysis on cylinders, a preclinical setup is assumed as the segmented axons originate from monkey tissue, and animal scanners typically allow for the implementation of a broader sequence parameter space than clinical scanners. This includes the strong gradients required for sensitivity to typical axon diameters [14, 44, 43]. Secondly, we demonstrate the impact (or lack thereof) on estimated axon diameter of using inaccurate assumptions of the parallel diffusivity for different b -values. Lastly, we validate the axon diameter estimates from the SMT and PL at different diffusion times, gradient strengths (typical of pre-clinical and human Connectom scanners), diffusivities (both in vivo and ex vivo) and, pertinently, within the complex segmented axons of the primate brain where axon diameters are similar to those of the human brain [45].

2. Theory

To model the IAS, the SMT and PL approaches both assume that the microdomains probed by spins within the IAS are cylindrical. Here, we present an outline of the origins of the SMT expression for cylinders, based on the theory presented elsewhere [46, 47]. It is this expression that is used to represent the IAS in the SMT-based approach used in Fan et al. [34]. From the SMT expression, the assumption of high b -values entails that the SMT can be formulated as a PL, as that in Veraart et al. [35].

2.1. Modelling the powder averaged signal in a cylinder

In a cylinder, the apparent diffusion coefficient at any angle α to its axis is [48]:

$$D(\alpha) = \cos^2(\alpha)D_{\parallel} + \sin^2(\alpha)D_{\perp} \quad (1)$$

Figure 1: Can axon diameter be accurately estimated in different WM regions using PA approaches, despite complex axonal morphologies? Validation of PA-estimates of axon diameter was carried out in two different fibre architectures of the vervet monkey brain. A) Axons were segmented from XNH volumes of a heterogeneous crossing fibre region in the anterior semiovale (blue cross) and the splenium (yellow cross). B) 59 segmented crossing fibre axons and C) 58 segmented splenium axons from [19]. The axon diameters are indicated by the respective colorbars and the black circles represent uniformly distributed directions on the unit sphere. D) A magnified view of one of the axons from B, showing its trajectory and diameter variations.

where $D_{\parallel}$ is the diffusivity parallel to the cylinder axis and $D_{\perp}$ is that perpendicular to it. Powder averaging of the diffusion MRI signal involves
 135 integrating it over the inclination angles α relative to the cylinder axis to give the powder averaged signal, $\bar{S}_{\text{SMT}}$ [46] :

$$\begin{aligned}\bar{S}_{\text{SMT}}(b) &= \int_0^{\pi} \frac{\sin(\alpha)}{2} e^{-b(\cos^2(\alpha)D_{\parallel} + \sin^2(\alpha)D_{\perp})} d\alpha \\ &= \left(e^{-bD_{\perp}} \cdot \sqrt{\frac{\pi}{4b \cdot (D_{\parallel} - D_{\perp})}} \cdot \text{erf}\left(\sqrt{b \cdot (D_{\parallel} - D_{\perp})}\right) \right)\end{aligned}\quad (2)$$

where the $\sin(\alpha)/2$ term ensures that the weighting of the angles is uniform, $\text{erf}(x)$ is the error function of x , and b is the diffusion weighting. Eq. 2 is what is here referred to as the "SMT implementation" and is the analytical description
 140 of the spherically averaged signal within a cylinder. Aside from the b -value, the signal depends on two variables: $D_{\parallel}$ and $D_{\perp}$. Only a finite number of directions, N , can be used in practice. As such, N is one of the variables that determines the accuracy of the measurement.

In cylinders, where $D_{\parallel} > D_{\perp}$ it can be assumed that $b \cdot (D_{\parallel} - D_{\perp}) \gg 1$ for
 145 high b -values,. In these conditions, $\text{erf}(x) = 1$ and Eq. 2 can be rearranged to take the form of a PL [49, 35]:

$$\bar{S}_{\text{PL}}(b) = \beta e^{-bD_{\perp}} b^{-0.5} \quad (3)$$

where $\beta = \sqrt{\frac{\pi}{4(D_{\parallel} - D_{\perp})}}$. Eq. 3 is what is here referred to as the "PL implementation" and is an alternative representation of the SMT at high b -values.

150 In practise, whether using single- or multi-compartment models of the WM, the fraction of the total signal that the IAS represents, f_a , is unknown. It thus needs to be included as a multiplicative constant in Eqs. 2 or 3. This introduces an additional third variable into the SMT implementation in Eq. 2, such that the signal depends on f_a , $D_{\parallel}$ and $D_{\perp}$. In the case of the PL, f_a can simply be
 155 incorporated into the existing constant β such that $\beta = f_a \cdot \sqrt{\frac{\pi}{4(D_{\parallel} - D_{\perp})}}$. This removes the need to fit a third parameter to the PA signal.

2.2. Converting $D_{\perp}$ into a diameter

By fitting Eqs. 2 or 3 to the PA signal, the apparent diffusion coefficient perpendicular to the cylindrical micro-domains, $D_{\perp}$, is obtained. The cylinder diameter can be calculated from $D_{\perp}$ using the Gaussian phase distribution approximation in van Gelderen et al. [48]:

$$-bD_{\perp} = \ln S_{\perp} = \ln S_0 - 2\gamma^2 G^2 \sum_{m=1}^{\infty} \frac{1}{D_0^2 \alpha_m^6 (R^2 \alpha_m^2 - 1)} \cdot [2D_0 \alpha_m^2 \delta - 2 + 2e^{-D_0 \alpha_m^2 \delta} + 2e^{-D_0 \alpha_m^2 \Delta} - e^{-D_0 \alpha_m^2 (\Delta - \delta)} - e^{-D_0 \alpha_m^2 (\Delta + \delta)}] \quad (4)$$

where $S_{\perp}$ is the diffusion weighted signal perpendicular to the cylinder, S_0 is the signal with no diffusion weighting, γ is the gyromagnetic ratio, G is the strength of the gradient pulse, D_0 is the intrinsic diffusivity, δ is the duration of the gradient pulse, Δ is the separation of the gradient pulses, R is the cylinder radius and α_m is the m th root of $J_1'(\alpha_m R) = 0$ where J_1' is the derivative of the first order Bessel function of the first kind. It is assumed that the cylinder is impermeable. In this study, Eq. 4 is calculated up to $m = 6$.

If $\delta \gg R^2/D_0$, as can be assumed to be the case for most axons [35], the cylinders are said to fall within the wide pulse or Neuman limit [50] and Eq. 4 simplifies to:

$$\ln S_{\perp} = -\frac{7}{48} \frac{\delta \gamma^2 G^2 R^4}{D_0}. \quad (5)$$

However, this was not always the case for the XNH-segmented axons in this study. Fig. 2 plots R^2/D_0 against diameter for the ex-vivo and in-vivo intrinsic diffusivities adopted in later simulations. The ADDs from the splenium and crossing fibre regions are shown (see Section 3.1.2 for how the diameters were analysed), along with the ADD along a single example axon from the crossing fibre region and the pulse widths used in later simulations.

Figure 2: **The Neuman (wide pulse) limit does not apply to large axons from the splenium and crossing fibre region.** R^2/D_0 is plotted against diameter for an in vivo (blue) and ex vivo (red) intrinsic diffusivity. Two pulse widths used in later simulations are marked by the dashed (7 ms) and dotted (13 ms) lines. The pooled ADDs of all splenium axons (yellow) and all crossing fibre axons (blue), and the ADD along a single example axon (grey) from the crossing fibre region, are shown. The Neuman limit requires that the pulse width exceeds R^2/D_0 , which is only true for diameters to the left of the intersections between the lines representing R^2/D_0 and the pulse widths. As such, some segments along a single axon may fall into the wide pulse limit and others not.

For a pulse width of 13 ms (dotted line) and an ex-vivo diffusivity, the single example axon (grey histogram) contains some segments that fall into the Neuman limit, and others that do not. Strictly speaking, the same is true for the pulse width of 7 ms and both the ex-vivo and in-vivo intrinsic diffusivities. The only situation in which the wide pulse limit would apply to all segments of the example axon is for the combination of the in-vivo intrinsic diffusivity and pulse width of 13 ms.

This is also the case for other segmented axons. Consequently, we use the full Gaussian phase approximation expression of van Gelderen et al. in Eq. 4 to calculate diameter. Importantly, knowledge or an assumption of D_0 is required in order to estimate a diameter from $D_{\perp}$. However, since $R \sim (D_0)^{\frac{1}{4}}$ in Eq. 5,

R is relatively insensitive to small inaccuracies of D_0 .

190 3. Materials and Methods

3.1. Simulations

The simulations in this study are divided into two categories: simulations of the diffusion MRI signal from cylinders of different diameters and simulations in the IAS of segmented axons from XNH volumes of the vervet monkey brain presented in [19]. By simulating the signal from cylinders, the impact of scanning parameters, SNR and model assumptions on estimated diameter could be isolated. By simulating diffusion within segmented axons from the splenium and crossing fiber regions, the impact on the estimated axon diameter of real fiber architectures, OD and microdispersion was investigated.

200 3.1.1. Simulating the diffusion MRI signal from cylinders

The signals arising from cylinders of different diameter, aligned with the z -axis, were generated analytically. For given PGSE parameters δ , Δ and G and radius R , Eq. 4 was used to calculate $D_{\perp}$ for each cylinder. From this, the apparent diffusion coefficient and signal in any normalised gradient direction $\hat{e}_G = [e_x, e_y, e_z]$ could be calculated using Eq. 1.

3.1.2. Simulating diffusion within the real IAS from XNH volumes of the monkey brain

We used segmented axons from the brain of a 32-month old female vervet monkey, imaged with 3D synchrotron XNH acquired at the European Synchrotron Research Facility, beamline ID16A. The axons originated from two different brain regions: the splenium of the corpus callosum and a "crossing fiber region", located in a position of the anterior centrum semiovale where the diffusion MRI data indicated the crossing of the corticospinal tract, interhemispheric callosal fibers and association fibers [19]. A description of both XNH volumes, as well as the segmentation and analysis of splenium axons is given in Andersson et al [19]. In short, the XNH volume of the splenium had an isotropic

voxel size of 75 nm and cylindrical field of view of diameter and length 153.6 μm . From the splenium, 54 axons of minimum length 120 μm were segmented at the native 75 nm image resolution. The XNH volume of the crossing fiber region had an isotropic voxel size of 100 nm and cylindrical field of view of diameter and length 204.8 μm . The larger diameters of axons in this region entailed that the segmentation of 58 axons of minimum length 120 μm could be manually performed in ITK-SNAP [51] (RRID:SCR_002010) at a downsampled isotropic voxel size of 200 nm. This was less time consuming than segmenting the axons at the smallest 100 nm voxel size. Smaller axons were present in both XNH volumes, but could not be segmented due to their small diameters in comparison to the voxel size and low SNR [19]. After segmentation, the equivalent diameters [19, 20, 21] of the axons were quantified in the plane perpendicular to their local trajectory. Thick axonal segments contain more water molecules and thus contribute more to the MRI signal than narrower segments [13]. As a measure of the ground-truth morphology of the intra-axonal space of each axon, we calculate the the volume-weighted axon diameter, d , of each axon as $d = \sum_{i=1}^N 2R_i \cdot \left(\frac{\pi R_i^2}{\sum_{i=1}^N \pi R_i^2} \right)$ where R_i is the i th measured radius of N equidistant measurement points along the axonal trajectories. The volume-weighted diameters of the splenium and crossing fibre populations were similarly calculated.

The axon segmentations were converted to triangulated surface meshes using the `isosurface()` function in Matlab R2020a. They were then decimated and smoothed (using the Corrective Smooth Modifier) in Blender (RRID:SCR_008606) to reduce the triangle density and thus the computational demand of the simulations. The Monte Carlo Diffusion and Collision framework [52] was used to simulate diffusion within each mesh, with the assumption that the axonal membranes were impermeable. The simulations used an intrinsic ex vivo diffusivity of $D_0 = 0.6 \mu\text{m}^2\text{ms}^{-1}$, $2 \cdot 10^5$ uniformly distributed spins per axon and $1 \cdot 10^{-5}$ seconds per time step, as in Andersson et al [19] where the parameters were chosen based on a bootstrap-analysis (Rafael-Patino et al. 2020) of the convergence of the simulation for the splenium axons. Simulations were also performed using an in vivo diffusivity of $D_0 = 2 \mu\text{m}^2\text{ms}^{-1}$ to mimic diffusion within the

living human brain, but with $3.4 \cdot 10^{-6}$ seconds per time step to ensure the same step length at the higher diffusivity. Initialisation of the spins was performed
 250 at a minimum distance from the ends of the axons (minimum $20 \mu\text{m}$ for ex vivo simulations and $30 \mu\text{m}$ for in vivo diffusivities), to prevent their escape from the IAS with regards to the diffusivity and the maximum diffusion times used (~ 40 ms for ex vivo simulations and ~ 30 ms for in vivo simulations).

The axons were simulated separately and, to evaluate the diameter estimate
 255 based on the PA signal of the populations of splenium or crossing fibre axons, the individual signals from the axons were weighted by the respective axonal volumes and summed.

3.1.3. Diffusion MRI scanning parameters

For all experiments, the PGSE waveform was used. Throughout the investigation,
 260 different sequence parameters were varied to isolate the effects of different variables on the estimated diameter.

In the simulations on cylinders, a gradient duration of $\delta = 7.1$ ms was used, similar to in [34, 35]. The gradient separation was kept at $\Delta = 20$ ms, and the effective diffusion time, t_d , was given by $t_d = \Delta - \delta/3$. The simulations on
 265 cylinders used only ex vivo diffusivities, and b -values were referred to as "high" if they surpassed $\gtrsim 20 \text{ ms } \mu\text{m}^{-2}$, the value at which the EAS was said to be suppressed in [35]. Most simulations in cylinders used high b -values in the range of $b = [19.25, 63.62] \text{ ms } \mu\text{m}^{-2}$ to allow a direct comparison between the SMT and PL implementations, the latter of which required the use of high b -values.
 270 Different b -values were obtained by varying the gradient strength, G , given that $b = q^2 t_d$ where q is the diffusion encoding $q = \gamma \delta G [\text{m}^{-1}]$.

In the simulations of the real IAS, the pulse width, δ , was set to either 7 ms or 13 ms. Both G and Δ were varied to assess the effects of different diffusion times and b -values. In these simulations, the SMT and PL were fitted to several
 275 sets of b -values, ranging between $b = [0.44, 65] \text{ ms } \mu\text{m}^{-2}$ for ex vivo diffusivities and $b = [0.44, 27.94] \text{ ms } \mu\text{m}^{-2}$ for in vivo diffusivities.

For the most part, fits of the SMT and PL to the PA signal from several

b -values used three shells, similar to ActiveAx [13, 14], since three was the minimum number of shells needed to calculate f_a , $D_{\parallel}$ and $D_{\perp}$ in the SMT implementation. Uniformly distributed directions on the unit sphere were generated according to the electrostatic repulsion method [53, 54].

Table 1 provides an overview over the main simulation datasets in terms of substrate, sequence parameters, experimental parameters and the parameter(s) that was/were investigated in each.

3.1.4. Distinguishing the signal from noise

The effect of noise on the diameter estimation was studied by adding Rician noise of variable SNR to the noise-free, normalised signals. The total variance of the noise was defined as $1/\text{SNR}^2$. Rician distributed noise was simulated calculating the magnitude of complex Gaussian noise in which the real and imaginary components each had a standard deviation of $1/\text{SNR}$ [55].

As a measure of sensitivity, we considered whether or not the normalised signal along a single gradient direction could be distinguished from either 0 (the noise floor) or 1 (the maximum). For this, we used the sensitivity criterion of Nilsson et al. [43]:

$$\bar{\sigma} = \frac{z_{\alpha}}{\text{SNR}\sqrt{n}} \quad (6)$$

where $\bar{\sigma}$ is the smallest robustly measurable difference in attenuation, n is the number of repeated measurements and z_{α} is the z -threshold for the significance level α . As in [43], we chose $\alpha = 0.05$, giving $z_{\alpha} = 1.64$.

With this definition, the signal can be distinguished from the noise floor and from 1 if it is in the range $[\bar{\sigma}, 1 - \bar{\sigma}]$. This implies that if a signal is measured perpendicular to the axis of a cylinder and is within this range, the cylinder has a measurable diameter i.e. the signal is distinguishable from the noise floor and that produced by a cylinder of zero diameter (a stick). Similarly, the cylinders for which the perpendicular signal is equal to $\bar{\sigma}$ or $1 - \bar{\sigma}$ respectively have the maximum and minimum measurable diameters given the SNR and n . These

305 diameters can be calculated using the relationship between the perpendicularly measured signal and cylinder radius in Eq. 4.

The definitions of the bounds differ for the PA signal. The PA of a cylinder with zero diameter, S_{stick} , is given by:

$$S_{\text{stick}} = \sqrt{\frac{\pi}{4b \cdot D_{\parallel}}} \cdot \text{erf}(\sqrt{bD_{\parallel}}) \quad (7)$$

As such, a cylinder has a measurable diameter if its PA signal is distinguish-
 310 able from 0 and from the PA of a stick i.e. the signal should be in the range $[\bar{\sigma}, S_{\text{stick}} - \bar{\sigma}]$ to be measurable. For the PA, n in Eq. 6 is set to the number of unique gradient directions. Given these bounds on the PA signal, the perpendicular diffusivities of the cylinders with the smallest and largest measurable diameters could be calculated by fitting Eq. 2 to the PA signal. Lastly, the
 315 perpendicular diffusivities could be converted into diameters using Eq. 4, producing estimates of the upper and lower bounds of measurable diameter from the PA signal.

Expressions for the minimum measurable diameter for the case of parallel cylinders and for fully dispersed cylinders (similar to taking the PA) are de-
 320 rived in [43]. However, we take the above approach instead of directly using the derived expressions in [43] as it is general for all sequence parameters and enables the definition of a maximum measurable diameter. Importantly, Eq. 6 assumes that the noise follows a normal distribution. Also, the calculation of the bounds of measurable diameter are for single b -values only, meaning that
 325 the theoretical upper and lower bounds of measurable diameter in multi-shell acquisitions cannot be calculated.

3.2. Fitting the Spherical Mean Technique and Power Law to the PA signal

3.2.1. The Spherical Mean Technique implementation

The signal fraction of the IAS, f_a , was incorporated into the SMT formulation in Eq. 2 such that:

$$\overline{S_{\text{SMT}}} = f_a \left(e^{-bD_{\perp}} \cdot \sqrt{\frac{\pi}{4b \cdot (D_{\parallel} - D_{\perp})}} \cdot \text{erf} \left(\sqrt{b \cdot (D_{\parallel} - D_{\perp})} \right) \right) \quad (8)$$

The SMT was fitted to the PA signals with the non-linear least squares solver `lsqnonlin()` in Matlab R2020a. Once $D_{\perp}$ had been fitted, the diameter was calculated using Eq. 4. We implemented three different variations of the SMT fit:

- (i) **SMT-1**: a single-shell fit to obtain $D_{\perp}$ in the range $[0, D_{\parallel}]$. Assumes known f_a and ex vivo $D_{\parallel} = 0.6 \mu\text{m}^2\text{ms}^{-1}$.
- (ii) **SMT-2**: a multi-shell fit to obtain $D_{\perp}$ in the range $[0, D_{\parallel}]$ and f_a in the range $[0, 1]$. Assumes known ex vivo $D_{\parallel} = 0.6 \mu\text{m}^2\text{ms}^{-1}$, or in vivo $D_{\parallel} = 2 \mu\text{m}^2\text{ms}^{-1}$.
- (iii) **SMT-3**: a multi-shell fit to obtain $D_{\perp}$ in the range $[0, D_0 \cdot 1.5]$, f_a in the range $[0, 1]$ and $D_{\parallel}$ in the range $[D_0/2, D_0 \cdot 1.5]$ where D_0 was the known intrinsic diffusivity of the simulations. For ex vivo and in vivo simulations, $D_0 = 0.6 \mu\text{m}^2\text{ms}^{-1}$ and $D_0 = 2 \mu\text{m}^2\text{ms}^{-1}$ were used respectively.

SMT-1 was used to assess the effect of the number of directions and SNR on the estimated diameter in the best case scenario in which f_a and $D_{\parallel}$ are known. Moving to a more realistic scenario, SMT-2 was used to assess the accuracy of SMT-based diameter estimation at different, unknown values of f_a . SMT-2 was also used to investigate the consequences of enforcing an incorrect value of $D_{\parallel}$ at different b -values and SNRs. Lastly, SMT-3 placed no assumptions on any of the variables, similarly to the PL implementation. SMT-3 was used to investigate the ability of the SMT to estimate diameter in the segmented axons for in vivo and ex vivo D_0 , assuming no prior knowledge of diffusivities (other than their upper and lower bounds). SMT-3 also outputted estimates of axonal $D_{\parallel}$.

355 3.2.2. The Power Law implementation

To assess the diameter estimates using the PL formulation, the expression in Eq. 3 was fitted to the PA signal from cylinders of different diameters, providing estimates of $D_{\perp}$ and β . This allowed for a comparison of the PL-derived diameter, d_{PL} , with those from SMT-2 and SMT-3. To fit the PL to the
 360 PA signal, the non-linear least squares solver `lsqnonlin()` in Matlab R2020a was used. As for the SMT fits, the diameter was calculated from $D_{\perp}$ using Eq. 4.

3.3. Data availability and ethics

The data that support the findings of this study will be made openly available
 365 on the website of the Danish Research Centre for Magnetic Resonance upon publication. This includes the Matlab code used to fit SMT-1, SMT-2 and SMT-3, as well as the XNH volume of the crossing fiber region and the associated segmentations. The XNH volume of the splenium region and associated axon segmentations are available from ref. [56].

370 The vervet monkey from which the tissue originated was cared for on the island of St Kitts and treated in line with a protocol approved by The Caribbean Primate Center of St. Kitts. The tissue was obtained from the Montreal Brain Bank and has also been studied in [13, 57, 14, 19].

4. Results

375 The Results section is organised as follows. First, we verify that it would be theoretically feasible to calculate the diameters of the XNH-segmented axons by analysing how SNR and gradient strength influence the upper and lower bounds of measurable diameter. We proceed to verify the multi-shell SMT-2 and PL descriptions for different numbers of shells, and investigate the effects of both
 380 Rican and Gaussian distributed noise on the diameter estimates. The SMT-2 and PL formulations implement different assumptions and degrees of a priori knowledge. We therefore evaluate whether the assumption of a known parallel

diffusivity is valid, using both low and high b -values. Then, we present segmentations of axons from the splenium and a crossing fiber region of the monkey
 385 brain. In these, we investigate the ability of the SMT-2, SMT-3 and PL implementations to estimate the volume-weighted diameters within real axonal geometries for in/ex vivo diffusivities and different b -values, given by combinations of different gradient strengths and diffusion times between $\sim 10 - 40$ ms.

390 4.1. Angular sensitivity of the diffusion MRI signal in cylinders

The sensitivity profile of a PA acquisition to diameter can be determined through the calculation of the upper and lower bounds of measurable diameter, as described in Section 3.1.4. This profile is shown in Fig. 3B for a range of SNRs and q -values. The mechanisms behind the widening/narrowing of the sensitivity
 395 profile in panel B can be explained by considering the sensitivity of the PGSE acquisition to different cylinder diameters and inclination angles α relative to the cylinder axis. As shown in Fig. 3C, the range of α for which the signal could be distinguished from the noise floor or from 1 also varied with the SNR and the diffusion encoding q (and hence the gradient strength). Additionally,
 400 the angular sensitivity profile of a given acquisition varied depending on the cylinder diameter. With increasing SNR (Fig. 3C, top row), the sensitivity to both the high and low α increased. At SNR = 100, an increasing q increased the sensitivity to high α i.e. perpendicular to the cylinder, but the additional attenuation of the signal caused by the higher diffusion weighting decreased the
 405 sensitivity to small α .

4.2. Number of gradient directions

The number of gradient directions determines how well the PA signal represents the analytical PA of a cylinder. Although it would be ideal to use very many directions, a compromise that factors in limited scan time must be made.
 410 To investigate how the angular resolution affects the diameter estimate, the signals from cylinders of different diameters with different numbers of gradient

Figure 3: **The range of diameters that are measurable with the PA vary with SNR and the diffusion encoding, q .** A) The angle α is defined as the inclination from the cylinder axis. The PGSE parameters in the table were used for the sensitivity analysis. B) The sensitivity profile to measured diameter using the PA varies with the SNR, as shown using $q = 1.1397 \cdot 10^6 \text{ m}^{-1}$. For SNR = 100, the range of measurable diameters varies with the q -value (G is varied to obtain different q , but δ and Δ are as in the table). The contours of the shaded areas represent the upper and lower bounds of measurable diameter for the given SNR or q -value. The calculation of the bounds assumes 30 gradient directions, Gaussian distributed noise, $f_a = 1$ and an ex vivo $D_0 = 0.6 \mu\text{m}^2\text{ms}^{-1}$. C) Variation with SNR and q -value of the angular angular range, in terms of α , to which the measurement is sensitive in cylinders of diameter [1, 5, 10] μm for the PGSE parameters in B. This sensitivity criterion is as in Eq. 6.

directions (6, 15, 30 and 512) and levels of Rician noise were generated, emulating the noise distribution present in magnitude diffusion MRI images. The sequence parameters were the same as in Fig. 3 and SMT-1 was fitted to the PA signal, giving a diameter estimate $d_{\text{SMT-1}}$, as shown in Fig. S1. The diameter from the signal measured in the one direction perpendicular to the cylinders (using Eq. 4), d_{VG} , was also calculated. The theoretical upper and lower bounds

of $d_{\text{SMT-1}}$ and d_{VG} were calculated as outlined in Section 3.1.4.

We found that the number of gradient directions imposed an additional
 420 lower bound of measurable diameter separate from that incurred by finite SNR
 in Fig. 3. Furthermore, if the directions were too few, the PA signal was not
 representative of the cylinder diameter i.e. it did not match the theoretical PA
 (that assumes an infinite number of directions) in Eq. 8. One implication of this
 was also that there was a dependence of $d_{\text{SMT-1}}$ on the orientation of the cylinder
 425 for fewer gradient directions than 30, as discussed in Supplementary Information
 S1 and shown in Fig. S2. Consequently, the predictions of the upper and lower
 bounds were only accurate starting from 30 directions using the given sequence
 parameters. At SNR = 20, there was a general underestimation of diameter
 regardless of the number of gradient directions, as a result of the Rician bias
 430 [55]. This disappeared when Gaussian distributed noise was used, as shown in
 Fig. S3.

4.3. Estimating diameter and intra-axonal signal fraction from multiple high *b*-value shells

The impact of different high *b*-values on diameter estimation was studied by
 435 fitting SMT-2 and the PL to the PA signals from cylinders with ground truth
 values of $f_a = 0.8$, an approximation of the expected axonal signal fraction. The
b-values were chosen to cover a similar range to those in the ex vivo acquisition
 in [35], and all were $\gtrsim 20 \text{ ms } \mu\text{m}^{-2}$ to simulate suppression of the EAS. For
 one set of ten *b*-values, Fig. 4 shows the estimated parameters from the SMT-1,
 440 SMT-2 and PL fits at SNR = 100 with Rician and Gaussian distributed noise.

For Rician noise in Fig. 4A, $d_{\text{SMT-2}}$ and d_{PL} dropped to 0 at small and large
 diameters, in contrast to the single shell SMT-1 which plateaued at large di-
 ameters. At large diameters, the underestimation of diameter was accompanied
 by an underestimation of estimated f_a for the SMT-2 approach. A reduction
 445 in β was seen for the PL approach. The use of data with Gaussian distributed
 noise in Fig. 4B produced $d_{\text{SMT-2}}$, f_a and β estimates that were accurate at
 smaller and larger diameters than with Rician noise, albeit with a high variance

Figure 4: Multi-shell fit of the SMT-1, SMT-2 and PL implementations to the PA signal of cylinders of diameter d at SNR = 100 with A) Rician distributed noise and B) Gaussian distributed noise. The PA signals were generated using $D_{||} = 0.6 \mu\text{m}^2\text{ms}^{-1}$, $f_a = 0.8$, 30 isotropically distributed directions and PGSE parameters $\delta = 7.1 \text{ ms}$, $\Delta = 20 \text{ ms}$ and ten b -values in the range $[19.25, 63.62] \text{ ms } \mu\text{m}^{-2}$, obtained by linearly spaced gradients in the interval $G = [550, 1000] \text{ mT/m}$. All datapoints reflect the mean of $N = 50$ measurements, and the error bars reflect their standard deviation.

at large diameters. Consequently, at diameters $\gtrsim 8 \mu\text{m}$, the estimates of f_a and β could not be robustly estimated, even with the mean of $n = 50$ repeats. One contributor to the high variance was that the SMT-2 and PL fits failed for many large diameters, defaulting to 0.

To explore how the b -value range and number of shells affected the diameter estimates, the experiments in Fig. 4 were repeated for A) three shells that spanned the same b -value range with $b = [19.25, 35.79, 63.62] \text{ ms } \mu\text{m}^{-2}$, B) three closely spaced b -values at the lower end of the range $b = [19.25, 22.90, 26.88] \text{ ms } \mu\text{m}^{-2}$ and C) three closely spaced b -values at the higher end of the range, $b = [51.54, 57.42, 63.62] \text{ ms } \mu\text{m}^{-2}$. The results are shown in Fig. 5. Other than

a small decrease in variance (likely due to the increased number of sampling points), there seemed to be no clear advantage to sampling more shells that
 460 cover the same range of b -values, as clear from the comparison of the results using ten shells in Fig. 4A and using three shells in Fig. 5A. Secondly, fitting to the three b -values on the lower end of the range in Fig. 5B resulted in a wider range of measurable diameters than fitting to three significantly higher b -values in Fig. 5C. The equivalent experiments using Gaussian noise are shown in Fig.
 465 S4. Here, the use of Gaussian noise widened the range of measurable diameters and provided more accurate estimates of f_a .

How different noise levels influenced the diameter estimates from the SMT-2 and PL fits was examined by repeating the experiment in Fig. 5A for SNR = [20, ∞], as shown in Fig. S5. For then noise-free case, we also examined
 470 the effect on d_{PL} of calculating the diameter assuming the Neuman limit as in Eq. 5 as opposed to the full Gaussian phase approximation of Van Gelderen et al. [48]. At SNR = ∞ , d_{SMT-2} , d_{PL} (Eq. 4), f_a and β were accurately estimated up to $\sim 11 \mu\text{m}$, aside from at very small diameters. The assumption of the Neuman limit to calculate d_{PL} , however, resulted in an underestimation
 475 of diameter. As expected, reducing the SNR to 20 in Fig. S5B-C increased the variance of the estimates and decreased the range of measurable diameters. This effect was particularly prominent for Rician distributed noise, but also for Gaussian distributed noise where 50 repetitions were not sufficient to robustly estimate the diameter, f_a or β for diameters $\geq 5 \mu\text{m}$. Since f_a multiplies the
 480 signal attenuation, a change of its value directly determined a change in the SNR and the range of measurable diameters (Fig. S6).

4.4. The dependence of estimated diameter on $D_{\parallel}$

Whether or not SMT-2 can be fitted to a multi-shell acquisition depends on how robust the estimation of diameter, d_{SMT-2} , is to inaccuracies in the assumed
 485 prior $D_{\parallel}$ for the given diffusion time. For cylinders, the estimated d_{SMT-2} and d_{VG} for different assumed values of $D_{\parallel}$ are shown in Fig. 6, with ground truth $D_{\parallel} = 0.6 \mu\text{m}^2\text{ms}^{-1}$. For the diameter estimation, it was assumed that $D_0 = D_{\parallel}$

Figure 5: **The choice of b -values affects the range of measurable diameters.** Fit of SMT-1, SMT-2 and PL to the PA signal from cylinders of diameter d at SNR = 100 (Rician noise) and $f_a = 0.8$. The PA signals were generated using A) three shells with $b = [19.25, 35.79, 63.62]$ ms μm^{-2} B) three shells with $b = [19.25, 22.90, 26.88]$ ms μm^{-2} and C) three shells with $b = [51.54, 57.42, 63.62]$ ms μm^{-2} . The signal was generated using $D_{\parallel} = 0.6 \mu\text{m}^2 \text{ms}^{-1}$, 30 directions, PGSE parameters $\delta = 7.1$ ms, $\Delta = 20$ ms and varying G . $n = 50$ repeats of each acquisition were performed for each diameter.

for all $D_{\parallel}$. The range $D_{\parallel} \pm 15\%$ is marked.

Using an incorrect value of $D_{\parallel}$ to within $\pm 15\%$ of the ground truth did not noticeably change $d_{\text{SMT-2}}$ for cylinders with $d > 2 \mu\text{m}$ at high b -values and infinite SNR in Fig. 6A. For diameters $> 2 \mu\text{m}$, there was thus little dependence of $d_{\text{SMT-2}}$ on the value of $D_{\parallel}$. For cylinders with $d = 2 \mu\text{m}$, the use of a larger-than ground truth $D_{\parallel}$ caused SMT-2 to fail and diameters of $1 \mu\text{m}$ could not be

resolved, regardless of the assumed value of $D_{\parallel}$. The value of d_{VG} showed some
 495 dependence, albeit small, on the assumed value of D_0 for diameters $> 1 \mu\text{m}$. The
 inclusion of Rician noise in Fig. 6B caused an increased dependency of $d_{\text{SMT-2}}$
 on the assumed value of $D_{\parallel}$, apparent from the increased slope of the plots. As
 expected from Fig. 4, the Rician noise caused a general underestimation of all
 diameters, particularly in the largest diameters for which the signal attenuation
 500 was strong and the Rician noise floor constituted a larger portion of the signal
 (e.g. $7 \mu\text{m}$ in Fig. 6B). For the lower b -values in Figs. 6C-D, there was a much
 clearer dependence of $d_{\text{SMT-2}}$ on $D_{\parallel}$. The trends were almost identical for the
 noise-free/noisy conditions.

Figure 6: **The sensitivity of $d_{\text{SMT-2}}$ and d_{VG} to assumed value of $D_{\parallel}$ depends on b -value regime.** Estimated $d_{\text{SMT-2}}$ and d_{VG} in cylinders vs. assumed value of $D_{\parallel}$ for high diffusion weighting $b = [19.25, 35.79, 63.62] \text{ ms } \mu\text{m}^{-2}$ for A) $\text{SNR} = \infty$ and B) $\text{SNR} = 100$ (Rician), and lower diffusion weighting $b = [0.64, 2.54, 5.73] \text{ ms } \mu\text{m}^{-2}$ for C) $\text{SNR} = \infty$ and D) $\text{SNR} = 100$ (Rician). The signal was generated using 30 isotropically distributed directions, $f_a = 1$ and PGSE parameters $\delta = 7.1 \text{ ms}$ and $\Delta = 20 \text{ ms}$. The applied gradient strengths, G , are indicated in the figure. The true $D_{\parallel} = 0.6 \mu\text{m}^2 \text{ms}^{-1}$, is marked by the black striped line, and $D_{\parallel} \pm 15\%$ is represented by the green shaded area. The datapoints represent the mean of $n = 50$ repeats.

4.5. Diameter estimation in segmented axons from XNH volumes of the vervet monkey WM

We found that the fiber architecture, axonal OD and axonal microdispersion differed considerably between the splenium and crossing fiber regions of the vervet monkey brain. The axons segmented from the splenium region, shown in Fig. 7A, were significantly smaller (mean = $2.8 \mu\text{m}$, SD = $0.53 \mu\text{m}$) and exhibited a narrower ADD than those from the crossing fiber region (mean AD = $4.8 \mu\text{m}$, SD = $1.28 \mu\text{m}$) in Fig. 7B. In comparison to axons from the organised corpus callosum environment, the axons from the crossing fiber region were very heterogeneous in terms of length, diameter, shape and OD. The thinnest, thickest and longest axons are shown in Fig. 7D-F.

To measure the diffusivity along the axons without correcting for microdispersion by using the PA, the main directions of the axons were first calculated via a principal component analysis of their trajectories. Intra-axonal diffusion of spins was simulated for up to 100 ms and the displacements of the spins were recorded in the respective main direction of each axon. From the mean-squared-displacements of the spins, the diffusion coefficient in the main direction, here denoted as the z-direction (D_z), was approximated and its variation with diffusion time, t_d , is shown in Fig. 7G-H for the splenium and crossing fiber axons respectively. The values of D_z in the splenium axons were higher than those in the crossing fiber region.

For evaluation of the SMT and PL implementations in the realistic IAS and under different conditions, we simulated four acquisitions within the axons from the splenium and crossing fiber regions. To isolate the effects of the real axonal geometries on the estimated diameter, no noise was added. The four acquisitions consisted of three shells sampled in 30 gradient directions each (due to the similar lower bounds obtained using 30 and 512 directions in Fig. S1). The acquisitions used either a higher or a lower gradient set, and either a short ($t_d = 12.7 \text{ ms}$) or a long ($t_d = 37.7 \text{ ms}$) diffusion time. The intrinsic diffusivity D_0 was set to an ex vivo diffusivity of $0.6 \mu\text{m}^2\text{ms}^{-1}$. SMT-2, SMT-3 and the PL were fitted to the PA signals to obtain estimates of $d_{\text{SMT-3}}$, $D_{\parallel}$ from

Figure 7: **Properties of real axons from different WM fiber architectures in the vervet monkey brain.** 3D reconstructions of A) 54 splenium axons (segmented at 75 nm isotropic resolution) and B) 58 crossing fiber axons (segmented at 200 nm isotropic resolution) in their respective XNH volumes. C) Combined 3D ADDs over all measured diameters in the splenium (yellow) and crossing fiber region (blue). The striped lines mark the means, $\bar{d}$, or volume-weighted means $\bar{d}_w$, of the distributions. The D) thinnest, E) thickest and F) longest axons from the crossing fiber region demonstrate the significant variability of axonal morphology that can exist on the MRI subvoxel scale. G) and H) show the variation of the diffusion coefficient, D_z , in the respective main direction of each axon with diffusion time t_d (data points every 1 ms per axon), using $D_0 = 0.6 \mu\text{m}^2\text{ms}^{-1}$. Different colours represent different axons.

535 the SMT-3 fit, $d_{\text{SMT-2}}$ and d_{PL} , as shown in Fig. 8.

At the higher b -values in Fig. 8A and C, $d_{\text{SMT-3}}$, $d_{\text{SMT-2}}$ and D_{PL} mostly provided accurate approximations of both the individual axon diameters and the volume-weighted mean diameter of the splenium/crossing fiber axon populations

Figure 8: **Diameter estimation in real axonal architectures for different sequence parameters.** SMT-2, SMT-3 and PL were fitted to the PA signals of axons from the splemium (yellow) and crossing fibre (blue) regions with ground truth volume-weighted diameters, d . The simulated sequences used short ($\Delta = 15$ ms) and long ($\Delta = 40$ ms) gradient sets with higher ($G = [500, 600, 700]$ mT/m) or lower ($G = [100, 200, 300]$ mT/m) strengths, as indicated. This gave three-shell acquisitions with A) $b = [11.11, 15.99, 21.77]$ ms μm^{-2} , B) $b = [0.44, 1.78, 4.00]$ ms μm^{-2} , C) $b = [33.03, 47.56, 64.73]$ ms μm^{-2} and D) $b = [1.32, 5.28, 11.89]$ ms μm^{-2} . The signals were generated with Monte Carlo simulations using ex vivo $D_0 = 0.6 \mu\text{m}^2\text{ms}^{-1}$ and were sampled in 30 gradient directions. For all acquisitions, $\delta = 7$ ms and SNR = ∞ (barring the intrinsic noise associated with Monte Carlo simulations). Square marker: volume-weighted axon diameter of splemium axon population, cross marker: volume-weighted axon diameter of crossing fiber population.

at short and long diffusion times. There was, however an underestimation of the
 540 largest crossing fibre axons, potentially due to the inherent noise of the Monte Carlo simulation. The $D_{||}$ estimates from the SMT-3 were scattered over the range of possible values and further tests found that the $D_{||}$ estimates were

sensitive to the initial value in the SMT-3 optimisation (the default initial value for $D_{\parallel}$ was 0). As shown in Fig. S7, a finite SNR of 100 perpetuated the underestimation of the largest diameters for the high b -value acquisition in Fig. 8C, in line with the underestimation of large diameters in Fig. 4.

For the lower b and the short diffusion time, as in Fig. 8B, $d_{\text{SMT-3}}$ slightly overestimated the diameters of all axons. There was a very subtle positive trend between d and $D_{\parallel}$, the values of which were similar to or higher than D_z in Fig. 7G-H. $d_{\text{SMT-2}}$ underestimated the diameters of the smaller splenium axons, but overestimated those from the crossing fiber region. The PL fit failed for all axons. Interestingly, at the longer diffusion time in Fig. 8D, there was a further overestimation of $d_{\text{SMT-3}}$ for the crossing fiber axons and a small shift in $D_{\parallel}$ towards lower values. In $d_{\text{SMT-2}}$, the under- and overestimation of the splenium and crossing fiber axons respectively were both enhanced. At this longer diffusion time, d_{PL} was non-zero for some of the larger crossing fiber axons, but was significantly underestimated.

To evaluate axon diameter estimation with in vivo diffusivities and gradient strengths offered by human Connectom MRI scanners, the simulations were repeated for $G = [100, 200, 300]$ mT/m and two different diffusion times, using an intrinsic diffusivity of $D_0 = 2 \mu\text{m}^2\text{ms}^{-1}$. The results are shown in Fig. 9. Strikingly, the estimated diameter of the splenium axon population was only non-zero for one metric and under one condition: it was accurate for $d_{\text{SMT-3}}$ at the shortest investigated effective diffusion time $t_d = 12.7$ ms in Fig. 9A. At the longer diffusion time, $d_{\text{SMT-3}}$ provided accurate estimates of a subset of the individual splenium axons, but the mean of the population could not be fitted. The individual and population mean diameters of the splenium axons could not be robustly estimated using the SMT-2 or PL approaches for either of the diffusion times. The diameters of the crossing fiber axons were generally overestimated by SMT-3 at all both times. Contrary to the splenium axons, SMT-2 could fit the diameters of all but the smallest crossing fiber axons. The PL implementation could fit very few axons at short diffusion times (Fig. 9A), but its performance improved with increasing diffusion time (Fig. 9B). As in

the simulations with ex vivo diffusivities, the values of $D_{\parallel}$ decreased with increasing diffusion time.

Limited slew rates entail that short pulse widths of ~ 7 ms are not always achievable on human scanners. For their experimental in vivo scans, Fan et al. applied a pulse width of 8 ms [34] and Veraart et al. a pulse width of 13 ms [35]. To evaluate the effects of a longer pulse width, therefore, the simulations in Fig. 9 were repeated for a longer $\delta = 13$ ms. The results are shown in Fig. S8; the $d_{\text{SMT-3}}$ estimates were similar to in Fig. 9, but the accuracy of $d_{\text{SMT-2}}$ and d_{PL} improved for both the splenium and crossing fibre axons.

Figure 9: **Diameter estimation in real axons for an in vivo intrinsic diffusivity**, $D_0 = 2 \mu\text{m}^2\text{ms}^{-1}$. SMT-2, SMT-3 and PL were fitted to the PA signals of axons from the splenium (yellow) and crossing fibre (blue) regions with ground truth volume-weighted diameters, d . This was done for two different diffusion times A) $\Delta = 15$ ms and $b = [0.44, 1.78, 4.00]$ ms μm^{-2} and B) $\Delta = 30$ ms and $b = [0.97, 3.88, 8.73]$ ms μm^{-2} . Each acquisition consisted of three shells with 30 gradient directions, $G = [100, 200, 300]$ mT/m, $\delta = 7$ ms and SNR = ∞ . Square marker: volume-weighted axon diameter of splenium axon population, cross marker: volume-weighted axon diameter of crossing fiber population.

5. Discussion

By segmenting axons from synchrotron X-ray nano-holotomography volumes of a splenium and crossing fiber region of the vervet monkey brain, we explored

the impact of real axonal morphologies on axon diameter estimation with powder average diffusion MRI approaches. Simulations of diffusion within the segmented axons, using both in vivo and ex vivo diffusivities, show that accurate measures of volume-weighted mean axon diameter can be obtained. In the real axonal substrates, we also find that the diameter estimates exhibit a time dependence, provided that the q -values of the applied sequence are low enough to preserve the diffusion MRI signal arising from large displacements. Given the broad distribution of axon diameters found in the crossing fiber region, it is imperative that the diffusion MRI acquisition has sensitivity to both small (including those too small to be segmented from the XNH volumes) and very large diameters. Similar to the sensitivity profiles of acquisitions based on a single measurement perpendicular to the cylinder/axon [14, 9], we show here how powder average-based methods have sensitivity profiles to diameter that are determined by the whole experimental setup. This includes the gradient strength [14, 44, 58], b -value, the number of gradient directions and the choice of signal model. Ultimately, we find that the effective SNR of the measurement is the key limiting variable of measurable diameter, and we show the importance of removing Rician bias from the diffusion MRI signal.

5.1. Sequence parameter considerations

5.1.1. The effect of q -value and the number of gradient directions on the sensitivity profile to diameter

We show that sensitivity profiles to different diameters of PA-based methods (Fig. 3B) are influenced by sensitivity of the acquisition to different angles within the cylindrical micro-domains (Fig. 3C). The q -value and the diffusion time of the acquisition act as spatial filters, restricting the maximum detectable displacements of the spins. A higher q -value increases the sensitivity of the acquisition to smaller length scales in directions perpendicular to the cylinder. However, we show that if the q -value is high enough to cause attenuation of the signal from an ensemble of spins before it has diffused for the entire diffusion time, the acquisition loses sensitivity in the axial direction. For a given diffusion

time and SNR, we show that an increase in q narrows the angular sensitivity profile. Although an increased gradient strength will move the lower bound to smaller diameters [14, 44], the upper bound will also be reduced, narrowing the range of measurable diameters. This is true also for methods that estimate axon diameter from measurements perpendicular to axons [11, 10, 13, 14, 16, 15], but the increased attenuation of the PA due to its averaging across many directions and length scales typically incurs a higher location of the lower bound, as shown in the comparison between $d_{\text{SMT-1}}$ and d_{VG} in Fig. S1. The sensitivity analyses in Fig. 3 are valid for an intra-axonal volume fraction of 1 and an ex-vivo diffusivity. A lower and more realistic volume fraction would reduce the effective SNR of the measurements and also act to narrow the sensitivity profiles shown in Fig. 3B. A higher, in vivo diffusivity would have the same effect; the lower bound of measurable diameter would increase [43] and the increased diffusivity would increase the attenuation for large diameters, lowering the upper bound. Although the sensitivity profiles in Fig. 3 can be used to predict whether the PA signal will have sufficient sensitivity to the underlying ADD in a voxel for given sequence parameters and experimental conditions, thus guiding the sequence design, the theoretical upper and lower bounds of measurable diameter have not been formulated for multi-shell fits. The locations of the bounds are therefore only applicable to single-shell data (e.g. in Figs. S1 and S3), which is typically not the case in experiments, making simulations of the signal important for predicting the sensitivity of a multi-shell acquisition to diameter.

We found that the number of uniformly distributed gradient directions influenced the lower bound of measurable diameter, with smaller diameters demanding measurements with higher angular resolution. This is in line with the findings of Li et al. [59] who show that at $\text{SNR} = \infty$, the number of directions determines how accurately the measured PA signal reflects the ground truth signal. The angular resolution thus places a lower bound on the measurable diameter, separate to that incurred by the sequence parameters and finite SNR. We demonstrate that with Rician noise, increasing the angular resolution cannot decrease the SNR-incurred lower bound. For Gaussian noise, however, the

higher number of sampling points given by the higher angular resolution may increase the effective SNR and provide access to smaller diameters. In practice, it has been shown that it is better to increase the number of directions than to perform many repeats of the same shell [60]. Furthermore, an increased angular resolution increases the robustness of the axon diameter estimate to different underlying fiber configurations and OD, as seen in Fig. S2. This agrees with other studies that find that more gradient directions lower the variance in parameter estimates from the PA signal [33, 61]. Our results also indicate that an increased angular resolution does not yield noticeably increased rotational invariance after a certain number of directions, similar to the findings of [62] where little improvement in the rotational invariance of the fractional anisotropy measurement was found beyond 20 directions at $b = 1 \text{ ms } \mu\text{m}^{-2}$.

All simulations on cylinders in this investigation assume a single cylinder direction. In practise, this is not realistic even in the corpus callosum [19, 20]. For models that assume a single fiber direction [11, 10, 13, 14], this is a limitation. PA methods, on the other hand, may benefit from fiber dispersion as it has been shown that the less anisotropy there is on the voxel scale, the fewer directions are required to obtain rotational invariance of the PA [59, 62, 61]. It is thus possible that axon diameter estimates in ordered WM regions such as the corpus callosum require the use of more gradient directions than in heterogeneous crossing fiber regions.

5.1.2. Is it necessary to fit the intra-axonal parallel diffusivity?

We show here that the need to fit $D_{\parallel}$ depends on the sequence parameters used. For b -values high enough to suppress the EAS, the signals at low α (roughly parallel to the cylinder axis) are heavily attenuated and contribute little to the overall PA. The dependency of the estimated diameter on $D_{\parallel}$ was therefore low and the assumption of erroneous $D_{\parallel}$ and D_0 to within $\pm 15\%$ of the true value did not have a significant impact on the estimated diameter (Fig. 6A-B). At lower b , as in Fig. 6C-D, on the other hand, the contribution of the low α signals to the overall PA was more significant and the dependency

on $D_{\parallel}$ could not be ignored. Some axon diameter estimation methods, such as ActiveAx [13] and the SMT implementation by Fan et al. [34] assume a value of $D_{\parallel}$. Other methods e.g. AxCaliber [11, 10] and the PL implementation [35] (either directly or indirectly) fit it. For PA methods, this entails that estimates of $D_{\parallel}$ obtained by simply measuring the signal parallel to axons in the corpus callosum at low b are likely to be sufficient priors and SMT-2 can be used to calculate the diameter for ex vivo intrinsic diffusivities and $b \geq 20 \text{ ms } \mu\text{m}^{-2}$. For acquisitions that use b -values low enough for the EAS signal to persist, $D_{\parallel}$ may need to be fitted (in addition to the signal from other tissue compartments), especially considering the time-dependence of the intra-axonal axial diffusivity [63, 64, 19, 65].

5.1.3. *Choice, but not number, of b -values affects the sensitivity profile to diameter*

Fitting the SMT and PL implementations to the signal from different sets of b -values (all $b \gtrsim 20 \text{ ms } \mu\text{m}^{-2}$ to simulate suppression of the EAS signal [35]) showed that there may be little advantage to increasing the q -value (and thus the b -value) of an acquisition. This supports the trend in Fig. 3B, in which an increase in q -value after a certain point does little to lower the lower bound, but narrows the range of measurable diameter. Furthermore, the finding that there appears to be little advantage to densely sampling many b -values lends support to the optimisation framework of Alexander et al. [66] in which the optimal acquisition design for axon diameter estimation used few shells (3 to 6), and to the approach of ActiveAx [13]. Our results thus indicate that it may not be necessary to perform a dense sampling of b -values as in the SMT implementation of Fan et al. [34], AxCaliber [11] and the PL implementation [35]. A similar suggestion was recently made by Veraart et al. [67].

The drops to zero at small and large diameters for multi-shell fits in the presence of Rician distributed noise in Fig. 4 introduce a problematic degeneracy: axons with diameters above the upper bound of measurable diameter may instead appear as smaller axons. We show here the existence of axons up

to 8 μm in mean diameter in the crossing fiber region. In the Rician regime and high b -values, as in Fig. 4, these are underestimated by both the SMT and PL approaches. Therefore, to work in the Rician regime one must ensure that the upper bound is sufficiently high to prevent the largest axons in the tissue from being underestimated. We show here that the use of Gaussian distributed noise ensures improved sensitivity to larger diameters for both the SMT and PL approaches. Sensitivity to larger axons may also be gained by using longer diffusion times [14], and as we show in Fig. 3B by increasing the effective SNR or reducing the gradient strength, although the latter may cause a preservation of the EAS signal which must be taken into account in the signal model. Similar to the results of Fan et al. [34], we observed a decreasing estimated IAS signal fraction (and the PL parameter, β) with increasing cylinder diameter. This was not the case at infinite SNR or with Gaussian noise, indicating that it may not be an issue at lower b -values, where the signal attenuation is lower and effective SNR is higher.

5.1.4. The importance of the SNR and noise type

For a single b -value, we show here how an increase in SNR (assuming Gaussian distributed noise) widened the sensitivity profile of the acquisition (Fig. 3B), as expected from the sensitivity criteria presented in [43]. Similarly, for fits of diameter to multiple high b -value signal sets, we found that Gaussian distributed noise resulted in a wider range of measurable diameters (Fig. 4) and provided more accurate estimates of the intra-axonal signal fraction. As such, Gaussian distributed noise prevented the systematic underestimation of diameter that is seen with Rician noise. This agrees with the findings of Fan et al. [34], in which it is also argued that the use of real-valued diffusion MRI data with Gaussian noise is more independent of the underlying fiber orientation distributions. The advantages of using Gaussian distributed noise stem from the fact that powder averaging of signals with a noise distribution centred around the mean avoids bias in the estimated average of the signal. In light of this, recovery of the real-valued diffusion MRI data with Gaussian distributed noise

by performing a phase correction [68] is likely an important step in improving both the range and accuracy of measurable axon diameters.

5.2. Microdispersion affects PA-based axon diameter estimates in segmented axons from the vervet monkey brain

At high b -values and ex vivo intrinsic diffusivities (Figs. 8A and C), we show that wide range of axon diameters from the splenium and crossing fiber WM regions could be accurately estimated using the SMT-3, SMT-2 and PL implementations, regardless of diffusion time. Furthermore, the scattered and inaccurate estimates of $D_{\parallel}$ from SMT-3 (some even surpassing the intrinsic diffusivity in Fig. 8A), yet the accurate d_{SMT-3} estimates of axon diameter, support our finding that the diameter is not very sensitive to inaccuracies in $D_{\parallel}$ at high b -values (Figs. 6A and B).

At the comparatively lower gradient strengths and b -values ($b \lesssim 12 \text{ ms } \mu\text{m}^{-2}$) in Figs. 8B and D, the effects of axonal microdispersion manifested as a slight overestimation of the crossing fiber diameters that increased with increasing diffusion time. Fitting $D_{\parallel}$ was necessary to obtain accurate axon diameter estimates for the smaller splenium axons. Despite the extremely tortuous trajectories of the crossing fiber axons, their fitted $D_{\parallel}$ were generally higher than those of the smaller splenium axons. This contrasts with the trend in D_z , the diffusivity measured in the single main direction of each axon, that was markedly lower in the crossing fiber region than in the splenium (Figs. 7G and H). Although $D_{\parallel}$ and D_z cannot be directly compared since the former is calculated from the diffusion MRI signal and the latter from the mean-squared-displacements of diffusing spins (akin to measuring the signal with a pulse width approaching zero), the trend flip highlights the importance of factoring out microdispersion to obtain unbiased estimates of intra-axonal diffusivity. The smaller $D_{\parallel}$ for the thinner splenium axons could potentially be due to spins in smaller axons probing the curvature of the IAS to a greater extent. The curvature could be caused by microdispersion and diameter variations [19, 22, 64, 69]. Lastly, we observed a decrease in $D_{\parallel}$ at longer diffusion times. This time dependence could

be indicative of the axonal diameter variations [19, 64] and the microdispersion of the axons, and thus also an indication of the density of cells or other extra-axonal structures in the WM [19]. The time dependence of both $D_{\parallel}$ and the
 770 diameter could then potentially act as biomarkers of situations where the WM cell density is expected to change, such as in pathology or inflammation.

The simulations within the realistic IAS at in vivo diffusivities (Fig. 9, Fig. S8) highlight the importance of mapping the sensitivity profile of the whole acquisition setup including the model and acquisition parameters. For
 775 two different pulse widths, and even with gradient strengths accessible to human diffusion MRI experiments only via Connectom scanners, the mean diameter of the splenium population could be accurately estimated only by SMT-3 at the shortest diffusion time, i.e. provided that $D_{\parallel}$ was fitted. The fits of SMT-2 and the PL did, however, improve for the longer pulse width as the higher
 780 resulting b -values implied that the sensitivity of d_{SMT-2} to the incorrect fixed value of $D_{\parallel} = D_0$ was reduced and the assumptions of the PL were valid. The overestimation of the diameters of the crossing fiber axons at all diffusion times could be explained by the high in vivo intrinsic diffusivity entailing that spins probe larger distances, and thus more microdispersion, than at ex vivo intrinsic
 785 diffusivities. It is possible that the lack of an observable time-dependence in the crossing fiber diameters could be a result of the spins already having probed the representative microdispersion after the short diffusion time in Fig. 9A, diffusing further than in the ex vivo case as a result of the higher intrinsic diffusivity.

790 5.3. Considerations

This investigation restricted the analysis of PA-based axon diameter estimates to the IAS, for different sequence parameters and SNRs. At high b -values, the observation of a signal decay proportional to $b^{-0.5}$ indicates that the signal mostly arises from thin, cylindrical structures [39, 49, 40], and that the EAS is
 795 suppressed. However, in the splenium XNH volume, we observed cell clusters and vacuoles that together constituted 6.1% of the total volume fraction [19].

Recent studies show that the cell somas could contribute to the PA signal at short diffusion times [70], complicating the SMT and PL fit to the PA signals. The presence of any restricted or hindered compartment from which the signal remains at high b -values will complicate the fits, unless it is explicitly modelled. These compartments could include e.g. irregularities in the axonal myelin or cellular processes. The observed dot compartment in ex vivo tissue [13, 35], representing fully restricted and immobile water, would cause a systematic underestimation of PA-based axon diameter measurements unless it independently measured and included in the model [35]. Alternatively, it may be possible to analyse the spherical-variance approach to separate the signal contribution of the dot compartment from the axonal signal [71]. For in vivo experiments, the contribution of the dot compartment to the signal has been shown to be negligible [72, 40, 73].

Furthermore, use of the single-compartment PL implementation as in Veraart et al. [35] requires high b -values both for the suppression of the EAS and to fulfil the assumptions of the PL model. In Fan et al. [38] and Veraart et al. [35], the use of Connectom scanners for the in vivo applications enabled high gradient strengths, and thus high q - and b -values. On regular clinical scanners with limited gradient strengths, high b -values could be achieved with longer diffusion times, but this would be at the cost of a reduced sensitivity to small diameters, bias due to axonal microdispersion [22] as seen in Fig. 9, and a long echo time that would reduce the SNR of the acquisition. Longer effective diffusion times could alternatively be obtained via stimulated echo acquisitions [74, 75], but also at the cost of lower SNR.

At b -values for which the condition $b(D_{\parallel} - D_{\perp}) \gg 1$ is not valid, the PL can no longer be applied and only the SMT implementation provides an adequate fit to the PA signal. Given that the EAS is not suppressed at such b -values, the signal contributions from other compartments must be modelled, as in Fan et al. [34]. The accuracy of the axon diameter estimate thus depends not only on how accurately the geometry of other compartments are modelled, but also the compartmental T_2 relaxation times [76] and potential exchange rates.

However, the sensitivity to microdispersion and $D_{\parallel}$ at these lower b -values, and the time-dependence of $d_{\text{SMT-3}}$ and $D_{\parallel}$ could provide valuable insight into axonal morphology [43, 41, 20, 22, 64, 77].

One key challenge to axon diameter estimation with diffusion MRI is that real WM voxels contain a full ADD, whereas we have segmented only its upper tail. The smallest axons, that constitute the majority of axons in the monkey corpus callosum [78, 17, 79, 45], were not possible to segment from the XNH volumes. Given the weighting of the diffusion MRI-based diameter estimate by the tail of the ADD [80, 35], it is interesting to study the properties of large axons, as done here. Simulating diffusion within these axons enabled an investigation and validation of the ability of PA approaches to factor out OD effects for complex axonal architectures and different sequence parameters, under achievable experimental conditions. Their diameters entailed that many of the XNH axons were within the sensitivity profile of the acquisitions simulated in Figs. 8 and 9. However, if a significant fraction of axons within the same voxel are below the lower bound of measurable diameter, the diameter estimate may drop to zero as seen for the diameter estimate based on the PA signal from the population of splenium axons in Fig. 9B. Therefore, quantifying and simulating the full ADD within an MR voxel, including the smaller axons, will be important to assessing the feasibility of an axon diameter from the signal produced by the full ADD from a voxel. Recently, smaller axons have been segmented from 3D electron microscopy volumes of the mouse corpus callosum with fields of view similar to those of our XNH volumes [81]. Simulating diffusion within these could provide insight into how well, and with which sequence parameters, the weighted mean diameter of the full ADD can be characterised from the diffusion signal.

Defining a ground truth diameter against which to compare the fitted diameters from the diffusion MRI signal is not entirely straightforward. Here, we used the volume-weighted diameter as a measure of the average intra-axonal diameter experienced by the water molecules, independent of the applied PGSE sequence parameters and intrinsic diffusivity. For axons with diameter varia-

tions, there exist formulations for the calculation of the effective radius that is
 860 retrieved from the diffusion MRI signal [22]. These are sequence- and diffusivity-
 dependent and rely on axons being classified as falling into either the Neuman
 (wide pulse) limit ($\delta \gg R^2/D_0$) or the narrow pulse limit ($\delta \ll R^2/D_0$) some-
 thing we show is not trivial for all axons (Fig. 2). Nonetheless, as shown in
 Figure S9, the volume-weighted diameters of all the axons segmented in this
 865 study are very similar to the diameters calculated assuming both the narrow
 pulse limit and wide pulse limit.

Similarly, it is important to consider whether or not the assumption of the
 Neuman limit can be made for the conversion of $D_{\perp}$ into a diameter estimate,
 regardless of whether the full SMT or PL approach is taken. Fig. 2 shows
 870 examples of cases for which the wide pulse assumption cannot be made for large
 axons. Using the assumption of the Neuman limit in Eq. 5 for these cases,
 instead of the full Gaussian phase approximation expression of van Gelderen et
 al. in Eq. 4, causes a significant underestimation of diameter, as shown in Fig.
 S5A.

875 Lastly, the SMT and PL implementations (Eqs. 3 and 8) and the van
 Gelderen expression for the restricted signal within an impermeable cylinder
 (Eq. 4) all assume that the Gaussian phase approximation holds. The Gaus-
 sian phase approximation may break down for strong gradients, biasing the
 diameter estimation [22]. In this investigation, the analytical generation of sig-
 880 nals using Eq. 4 cannot, by definition, represent such non-Gaussian effects, and
 it is an assumption of the parameter analysis that they are negligible. On the
 other hand, the Monte Carlo simulations of diffusion within the XNH-segmented
 axons would be able to represent non-Gaussian effects in the signal, and the
 success of the diameter estimation in the splenium and crossing fibre axons in
 885 Figs. 8 and 9 suggests that the Gaussian phase approximation is an adequate
 assumption for the applied sequence parameters.

6. Conclusion

We demonstrate that powder averaging techniques can succeed in providing accurate estimates of axon diameter, even in axons from a complex crossing fiber region, by simulating diffusion within axons segmented from X-ray nano-holotomography volumes of the vervet monkey brain. This is done for strong gradients that are typical of pre-clinical or human Connectom scanners, and for ex vivo and in vivo diffusivities. We show that in order for the diameter estimation to succeed, the acquisition must have a broad sensitivity profile to accommodate the many different axon diameters present within a voxel, as presented here. Furthermore, we show how the signal model, gradient strength, diffusion time and number of gradient directions, as well as the SNR and type of noise distribution, influence the lower and upper bounds of measurable diameter. Finally, by simulating different diffusion times and b -values, we show that the acquisition can become sensitive to axonal microdispersion, which could be an interesting biomarker of WM health and pathology. We foresee that this characterisation of the limits and potential of PA-based approaches to axon diameter estimation will contribute to the development of new methods and models to study the WM microstructure with diffusion MRI.

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8. Declaration of competing interest

Declarations of interest: none.

Table 1: Simulations on cylinders and segmented XNH axons were performed to investigate the effects of different sequence and experimental parameters on the estimated cylinder/axon diameter from the PA signal. For each simulation dataset, the sequence details, fitting method and the investigated parameters are listed.

Substrate	D_0 ($\mu\text{m}^2 \text{ms}^{-1}$)	$C2$ (mT ms^{-1})	δ (ms)	Δ (ms)	b ($\text{ms } \mu\text{m}^{-2}$)	Gradients directions	Noise type	SNR	Investigated parameter(s)	Fit	Figure
		600	7.1	20	22.90	4, 15, 35, 512	Helian Gaussian	20, 100 ∞	Gradients directions Noise type	SMT-1	31
Cylinders (ex-vivo)	0.6	[550, 600, 650, 700, 750, 800, 850, 900, 950, 1000]	7.1	20	[19.25, 22.90, 26.58, 31.18, 35.79, 40.72, 45.97, 51.54, 57.42, 63.62]	30	Helian Gaussian	100	SNR	SMT-1	4
									Noise type	SMT-2	
									Shell number	PL	
									Shell choice	SMT-2	
									(Noise type)	PL	
	1.0	[550, 750, 1000]	7.1	20	[19.25, 22.90, 26.58, 31.18, 35.79, 40.72, 45.97, 51.54, 57.42, 63.62]	30	Helian (Gaussian)	100	Shell number	SMT-1	5
									Shell choice	SMT-2	
									(Noise type)	PL	
									SNR	SMT-2	
									∞	PL	
	2.0	[550, 750, 1000]	7.1	20	[19.25, 22.90, 26.58, 31.18, 35.79, 40.72, 45.97, 51.54, 57.42, 63.62]	30	Helian Gaussian	20	SNR	SMT-1	56
									∞	PL	
									Values of varying f_{ax}	SMT-2	
									100	PL	
									∞	SMT-2	
XNH axons	0.6 (ex vivo)	[500, 600, 700]	7	15, 40	[11.11, 15.99, 21.77, 33.03, 47.56, 64.73]	30	Helian	(100) ∞	Diffusion time	SMT-2	8
									b-value	SMT-3	
									(SNR)	PL	
									∞	SMT-2	
									∞	PL	
	2 (in vivo)	[100, 200, 300]	7	15, 30	[0.44, 1.78, 4.90, 13.32, 5.28, 11.69]	30	-	∞	In vivo D_0	SMT-2	9
									b-value	SMT-3	
									(SNR)	PL	
									∞	SMT-2	
									∞	PL	
axons	2 (in vivo)	[100, 200, 300]	13	15, 30	[1.25, 5.14, 11.61, 3.10, 12.42, 27.94]	30	-	∞	Pulse width	SMT-2	58
									∞	PL	

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10. Tables

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