

Impact of within-voxel heterogeneity in fibre geometry on spherical deconvolution

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Abstract

Axons in white matter have been shown to have varying geometries within a bundle using ex vivo imaging techniques, but what does this mean for diffusion MRI (dMRI) based spherical deconvolution (SD)? SD attempts to estimate the fibre orientation distribution function (fODF) by assuming a single dMRI fibre response function (FRF) for all white matter populations and deconvolving this FRF from the dMRI signal at each voxel to estimate the fODF. Variable fibre geometry within a bundle however suggests the FRF might not be constant even within a single voxel. We test what impact realistic fibre geometry has on SD by simulating the dMRI signal in a range of realistic white matter numerical phantoms, including synthetic phantoms and real axons segmented from electron microscopy. We demonstrate that variable fibre geometry leads to a variable FRF across axons and that in general no single FRF is effective to recover the underlying fibre orientation distribution function (fODF). This finding suggests that assuming a single FRF can lead to misestimation of the fODF, causing further downstream errors in techniques such as tractography.

Highlights

- Variable fibre geometry within a voxel leads to variable fibre response functions within the voxel

- More complex fibre geometry leads to a wider range of fibre responses within a voxel
- No single choice of response function is typically effective at recovering the underlying fibre orientation distribution function using spherical deconvolution

Keywords

Fibre orientation

White matter

Diffusion MRI

Simulation

Abbreviations

CC: corpus callosum, ConFiG: contextual fibre growth, CSD: constrained spherical deconvolution, dMRI: diffusion MRI, EM: electron microscopy, fODF: fibre orientation distribution function, FRF: fibre response function, HCP: Human Connectome Project, NODDI: neurite orientation dispersion and density imaging, PDF: probability density function, SD: spherical deconvolution, SH: spherical harmonic, SNR: signal-to-noise ratio, WM: white matter

1 Introduction

Diffusion weighted magnetic resonance imaging (dMRI) has been widely used to probe the structure and organisation of brain tissue, with one particular area of focus being the estimation of the orientational distribution of neuronal fibres in a voxel. This fibre orientation distribution function (fODF) is particularly interesting since it is used in tractography techniques to probe the structural connectivity of the brain which is important in many clinical and basic neuroscience studies (Catani and Thiebaut de Schotten, 2013; Dell'Acqua and Tournier, 2019; Johansen-Berg and Behrens, 2006). Whilst tractography has found many uses, there remain a number of challenges to the technique, including typically generating a large number of false positive connections (Jbabdi and

Johansen-Berg, 2011; Maier-Hein et al., 2017; Schilling et al., 2019a; Thomas et al., 2014). One potential source of these issues could be due to difficulties in reliably estimating the fODF, where minor differences in fODF can lead to large differences in the tractograms created (Schilling et al., 2019a, 2019b). Accurate and reliable estimation of the fODF is therefore important to improve the accuracy of tractography techniques.

Many techniques have been developed for estimating the fODF, of which perhaps the most prominent are based on spherical deconvolution (SD). While there are a variety of spherical deconvolution methods, the central principle is the same - the diffusion weighted signal as a function of the azimuthal (ϕ) and elevation (θ) angles is modelled as a spherical convolution of the fODF, $F(\theta, \phi)$, with a kernel (called the fibre response function (FRF)), $R(\theta)$, the typical diffusion weighted signal from a single fibre population estimated a priori. By estimating an FRF from voxels where the signals are deemed typical of a single coherent fibre population, the fODF is determined by deconvolving this FRF from the signal. Implicit in this formulation is an assumption that one common FRF is shared across all fibre populations in the white matter (WM). Recently, some works have challenged this assumption, for instance (Schilling et al., 2019b) use known fODFs from histology to estimate the FRF in different WM regions, showing that the FRF does indeed vary across the WM and that this variation does affect the estimated fODF and tractography results.

However, the assumption behind the FRF not only requires it to be the same across WM voxels, but also to be identical across individual fibre populations within a voxel. In fact, recent works using electron microscopy (EM) to investigate WM axonal morphology show that axons within a voxel have different shapes (Abdollahzadeh et al., 2019; Andersson et al., 2020; Lee et al., 2019), with varying diameters along their length and non-straight trajectories. It is reasonable, therefore, to propose that this heterogeneity in fibre geometry will mean that different fibres will have different responses. This may lead to misestimation of the fODF when assuming a single FRF for all fibres, which has downstream consequences for techniques including tractography.

A related factor is that an assumption is made that there is no exchange between fibres or, equivalently, diffusion in different directions using this representation. In essence, this

means that the fibres are implicitly assumed to be perfectly straight and pointing a given direction since any deviation from straight (i.e. curved or undulating fibres) would introduce directions that are connected, violating the non-exchange assumption. Under some experimental conditions, such as those used in current clinical applications, these effects may not be negligible and may affect subsequent techniques such as tractography.

In this work we investigate what effect, if any, violation of these assumptions introduced by within-voxel heterogeneity in axonal morphologies has SD techniques. We use Contextual Fibre Growth (ConFiG) (Callaghan et al., 2020), our recently developed white matter numerical phantom generator capable of generating realistic WM morphology to investigate this in controllable environments, as well as real digital tissues reconstructed from EM (Lee et al., 2019) to test a limited sample of real tissue. Firstly, we investigate how microscopic variations in fibre geometry affect the diffusion within each fibre and whether the dMRI signal from each fibre is the same. We further evaluate what effect this has on fODF estimates by calculating them using FRFs representing the variable responses present in a voxel.

The rest of this paper is organised as follows: Section 2 describes the experiments performed to probe the assumptions outlined above, Section 3 presents the results and Sections 4&5 summarise the contributions and discuss future work.

2 Method

In order to test the impact of fibre geometry heterogeneity on the dMRI signal per-fibre and how any variability in response may affect fODF estimation, experiments were performed in a range of numerical phantoms generated using ConFiG and reconstructed from EM (Lee et al., 2019), using an acquisition typical of SD applications.

Two primary experiments were conducted:

1. **Per-fibre response heterogeneity** - To investigate the impact of fibre geometry heterogeneity on the dMRI signal per-fibre
2. **Impact on fODF estimation** - To investigate what impact variation in the FRF can have on fODF estimation

In this section, we describe the phantoms used in these experiments and the dMRI simulations that were performed to investigate the impact of microscopic structural variability.

2.1 Phantom Generation

In order to test SD techniques in realistic geometries, a set of digital-tissue phantoms were generated to represent a range of WM tissue configurations:

- A single bundle of fibres generated by ConFiG with varying amounts of orientation dispersion:
 - Watson distributed (Mardia and Jupp, 2008), $\kappa = 2$
 - Watson distributed, $\kappa = 6$
 - Watson distributed, $\kappa = 100$
- Crossing bundles of fibres generated by ConFiG
 - Two perpendicular bundles
 - Three perpendicular bundles
- Real fibres from mouse corpus callosum (CC) reconstructed from EM (Lee et al., 2019)

In the case of the single bundle phantoms, a low κ means high orientation dispersion, so phantoms with a lower κ were expected to have more complex morphology since higher OD means that they must grow around one another more to avoid intersections. A typical κ , estimated using NODDI (Zhang et al., 2012), for the corpus callosum of a healthy Human Connectome Project (HCP) (Sotiropoulos et al., 2013; Van Essen et al., 2012) subject is $\kappa \sim 6$ (Callaghan et al., 2020) Since the CC is expected to contain some of the most coherent fibre bundles in the brain, $\kappa \sim 6$ will be towards the higher end of OD *in vivo*. Crossing bundle phantoms were generated by using starting and target points arranged into two- or three-crossing bundles and grown using ConFiG to generate complex phantoms with interleaved fibres.

2.1.1 Real WM fibres from EM

To simulate diffusion in real axons, 3D meshes were generated from WM axon segmentations from EM of mouse corpus callosum presented by (Lee et al., 2019).

The axonal segmentations are provided in the NIfTI format, a volumetric format. In order to convert these into surface meshes for dMRI simulation, the `isosurface` function in MATLAB was used, however this produces meshes with some artifacts such as loose

surfaces inside the fibres. In order to account for this, a further mesh refinement procedure was developed using the shrinkwrap feature in Blender to create a smooth, closed surface mesh around each fibre.

2.1.2 Gold standard fODF extraction from microstructure

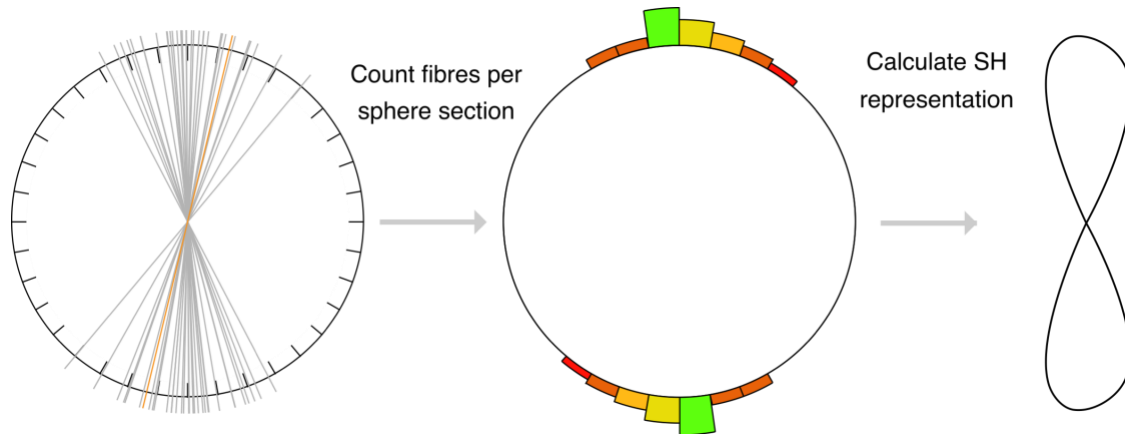


Figure 1 Gold standard fODF estimation from microstructure of WM numerical phantoms. Each fibre's main direction is projected onto a sphere and a spherical harmonic representation calculated.

In order to generate a ground truth to compare with fODFs estimated from the simulated dMRI signals, a gold standard fODF was estimated from the WM numerical phantom meshes. As an attempt to generate a microstructural fODF comparable to that estimated from the simulated dMRI based fODFs, the microstructural fODF was calculated using the assumption of one direction per fibre, namely the direction connecting the endpoints of the fibre that would subsequently be used to align each fibre to the z-axis (see Figure 2).

A triangulated unit sphere was used to store this fODF, with each triangle in the sphere storing the number of fibres whose direction went through that triangle scaled by the volume of each fibre, as illustrated in Figure 1. In order to compare this fODF to those calculated using SD from dMRI, the microstructural fODF was expanded in spherical harmonics (SHs). A spherical function $f(\theta, \phi)$, can be expressed in terms of spherical harmonics as:

$$f(\theta, \phi) = \sum_{l=0}^{l_{max}} \sum_{m=-l}^l c_l^m Y_l^m(\theta, \phi), \quad (1)$$

where

$$c_l^m = \int_0^{2\pi} \int_0^\pi f(\theta, \phi) Y_l^{m*}(\theta, \phi) \sin(\theta) d\theta d\phi. \quad (2)$$

Y_l^m are the so-called spherical harmonics of degree l and order m up to a maximum degree l_{max} and $*$ denotes complex conjugation. In our case, θ and ϕ are discrete samples in the centre of each triangle in our unit sphere meaning one approach to finding c_l^m is to turn the integral in into a summation. A more robust approach, however is a least squares approach (Alexander et al., 2002; Brechbühler et al., 1995) in which the spherical harmonics are re-indexed to have single index $j(l, m) = l^2 + l + m$. The discrete fODF values stored in each triangle are turned into a vector of length n_{tri} , $[f] = \{f(\theta_i, \phi_i), i = 1, \dots, n_{tri}\}$ and an $n_{tri} \times j(l_{max}, l_{max})$ matrix, X , constructed with elements $X_{i,j(l,m)} = Y_l^m(\theta_i, \phi_i)$. Essentially, X maps the SH coefficients for each l, m into amplitudes along each θ_i, ϕ_i . The $j(l_{max}, l_{max})$ vector of SH coefficients, $[c]$ can then be found as

$$[c] = (X^{*T}X)^{-1}X^{*T}[f]. \quad (3)$$

The number of coefficients in $[c]$ can be reduced since the fODF is real-valued and antipodally symmetric (since the dMRI process is antipodally symmetric). Being real valued means that the SH coefficients exhibit conjugate symmetry (that is, $c_l^m = (-1)^m c_l^{-m*}$) and the antipodal symmetry means that all odd m terms are 0 (Alexander et al., 2002; Tournier et al., 2004). In the end, this means that $[c]$ has $(l_{max} + 1)(l_{max} + 2)/2$ elements.

Each fODF was normalised such that

$$\int_0^{2\pi} \int_0^{\pi} f(\theta, \phi) \sin(\theta) d\theta d\phi = 1, \quad (4)$$

to ensure that the fODF is a probability density function (PDF), describing the probability of fibre pointing in a given unit of solid angle.

2.2 Experiments

2.2.1 Per-fibre response heterogeneity (Experiment 1)

We test to what extent variable fibre geometry results in variable fibre responses by simulating the intracellular dMRI signal from the phantoms described in Section 2.1 following the procedure outlined in Figure 2.

For testing individual fibre response functions, only the intracellular signal was needed since the ideal FRF comes solely from the intracellular space. In this case, each fibre was rotated to be aligned with the z -axis and then extended with a reflected copy as in (H. Lee et al., 2020). The rotation matrix used to align the fibre with z was stored so that signals could be rotated back into the dispersed directions to generate an overall voxel signal. In the case of EM fibres, only axons that were longer than $18 \mu\text{m}$ before extension were used in simulation to remove very short fibres that had been segmented at the edge of the volume.

Diffusion MRI signals were simulated from ConFiG phantoms using the Camino dMRI simulator (Cook et al., 2006; Hall and Alexander, 2009) to perform the experiments described below. For all experiments a bulk diffusivity $D = 2.0 \mu\text{m}^2/\text{ms}$ was used in agreement with similar Monte Carlo experiments as in with standard Camino periodic boundaries (Panagiotaki et al., 2010).

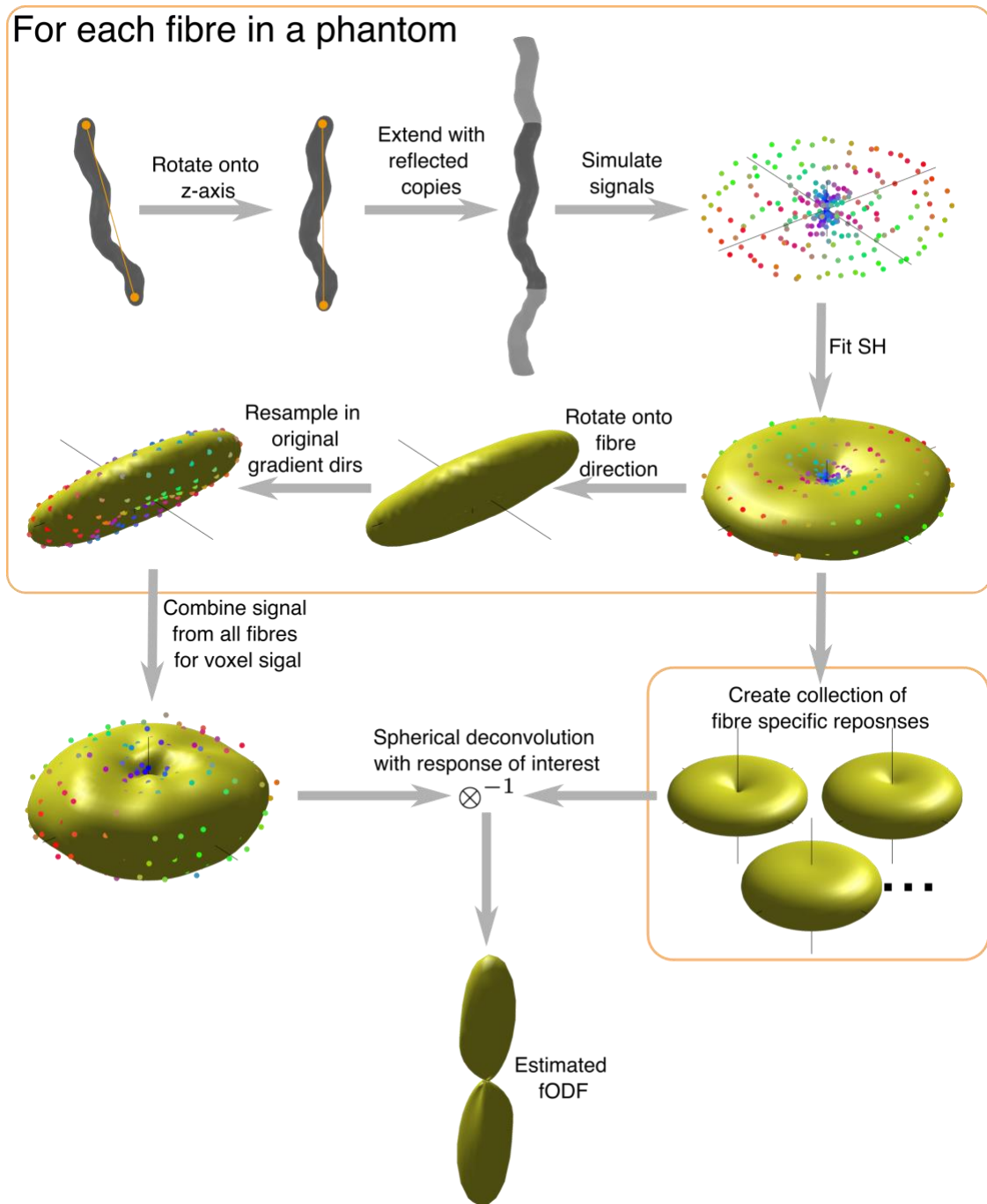


Figure 2 Calculation of per-fibre response and fODF from a phantom. Each fibre is treated individually, being rotated onto the z-axis and extended with reflected copies as in (Callaghan et al., 2020; H. Lee et al., 2020) to avoid artefacts from the closed ends. The dMRI signal is then simulated, decomposed into SH, rotated back onto the original fibre direction and resampled in the original directions. Each fibre signal is then combined to give total voxel signal and deconvolved with a given FRF to estimate the fODF.

For each fibre 10,000 spins were initialised uniformly within the intra-axonal space and the simulations were performed using 5000 timesteps. Each phantom had ~ 300 fibres giving $\sim 3 \times 10^6$ spins in total per phantom. These settings were confirmed to be adequate by comparing to a set of test simulations performed using 10^5 spins per fibre and 10^4 timesteps. The measurement parameters were $\Delta = 28\text{ms}$, $\delta = 24\text{ms}$, $b = 1000, 2000, 3000 \text{ s/mm}^2$ and 256 equidistributed gradient directions (Saff and Kuijlaars, 1997) at each shell. This gives a diffusion time $d_t = 20 \text{ ms}$, chosen so that the diffusion length scale ($\sqrt{2Dd_t} \approx 8\mu\text{m}$ at $D = 2.0 \mu\text{m}^2/\text{ms}$) is small relative to the axon length ($\geq 18\mu\text{m}$). Additionally, these settings give $G = 60 \text{ mT/m}$ at $b = 3000 \text{ s/mm}^2$, a feasible gradient strength on a high-end clinical system. Rician noise was optionally added at 30 SNR.

To compare to the collection-of-straight-fibres assumption implicit in spherical deconvolution techniques, an infinite cylinder representing each fibre was generated using the endpoints of each fibre to give the direction and the mean radius of the fibre as the cylinder radius. For simulation, each cylinder was aligned with the z-axis similarly to the ConFiG fibres so that everything was in the same space to compare the signals. The same measurement scheme was simulated in each cylinder in order to compare to the ConFiG fibres.

Since the individual axons have been aligned with the z-axis, the signals from each fibre can be directly compared with one another as the gradient directions are aligned with respect to each fibre. To demonstrate the variability in dMRI response, each fibre's response was calculated and the median, 10th and 90th percentile responses found (in terms of mean squared difference between fibre and cylinder responses). Additionally, to relate the type and size of morphological variation to the signal changes, the microscopic orientation dispersion (Brabec et al., 2019) (μOD), a measure of undulation, and the coefficient of variation of diameter (CV), a measure of beading (H. Lee et al., 2020), was calculated for each fibre.

Throughout this work, SH representations of signals are used. The MATLAB implementation of constrained spherical deconvolution (CSD) (Tournier et al., 2007)

available from (<https://github.com/jdtournier/csd>) is used to calculate SH decompositions of signals. This is the same technique as used in popular dMRI tractography tool MRtrix3 (Tournier et al., 2019) and follows the procedure outlined in Section 2.1.2 for SH decomposition of the dMRI signal, where θ , ϕ are the gradient directions.

2.2.2 Impact on fODF estimation (Experiment 2)

To investigate the impact on the fODF of assuming a single fibre response per voxel, we compared fODF estimates from CSD using three different FRFs per phantom derived from Experiment 1: the median response (representing a typical response) and the 10th percentile and 90th percentile FRF (representing extrema of responses). To generate an overall voxel signal for deconvolution with each FRF, the volume-weighted mean signal calculated for each phantom, as shown in bottom half of Figure 2 by rotating each fibre's signal onto the original fibre direction and weighting by fibre volume. Additionally, the fODF estimated using CSD is not a true PDF since it does not integrate to one, so throughout this work the fODF from CSD is normalised as outlined in Section 2.1.2.

To help illustrate the differences in the fODF, the main peak direction and dispersion angle (calculated as the angle away from main direction to cover 75th percentile of main peak) were calculated for the single bundle and EM fibre cases.

3 Results

3.1 Per-fibre response heterogeneity

Variations in intra-voxel fibre geometries are present in real fibres and ConFiG phantoms as demonstrated in Figure 3 which shows each digital phantom alongside the fibres which give the median, 10th and 90th percentile response.

Under the experimental conditions investigated, this morphological variation in the fibres causes the dMRI signal response per-fibre to vary as can be seen in the middle of Figure 3, which shows the median, 10th and 90th percentile signals across all fibres in each phantom at $b=2000s/mm^2$ and 30 SNR. The variation in the response function depends on the

complexity of the fibre arrangement, with the most complex three-crossing bundle arrangement leading to the largest variation in response functions.

This variation in the FRF is seen across each b -value from 1000 to 3000s/mm² as demonstrated in Figure 4 for the $\kappa = 2$, three-crossing and EM fibre phantoms, chosen since these display the most variation for each phantom category. Here $SNR = \infty$ to isolate the effect of b from noise.

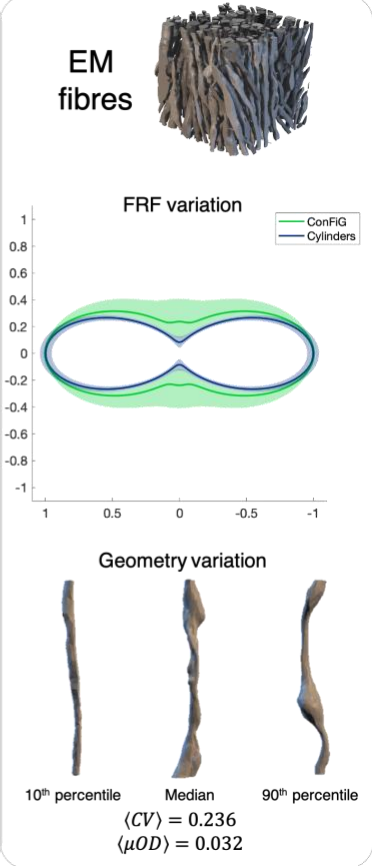
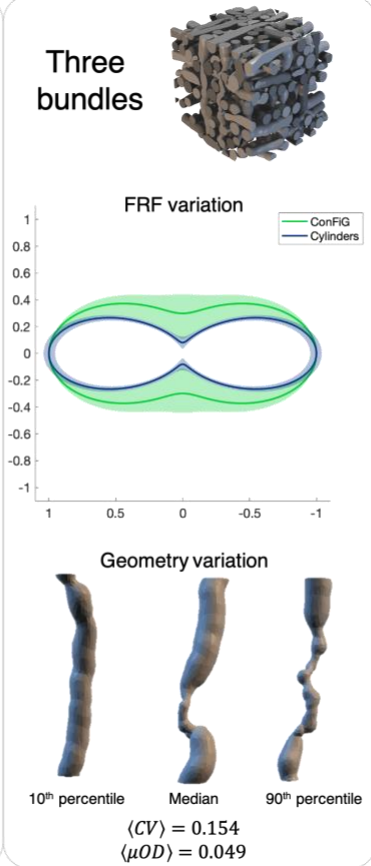
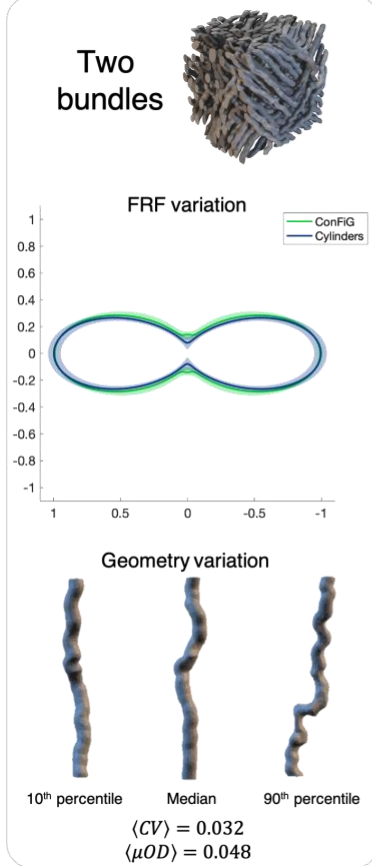
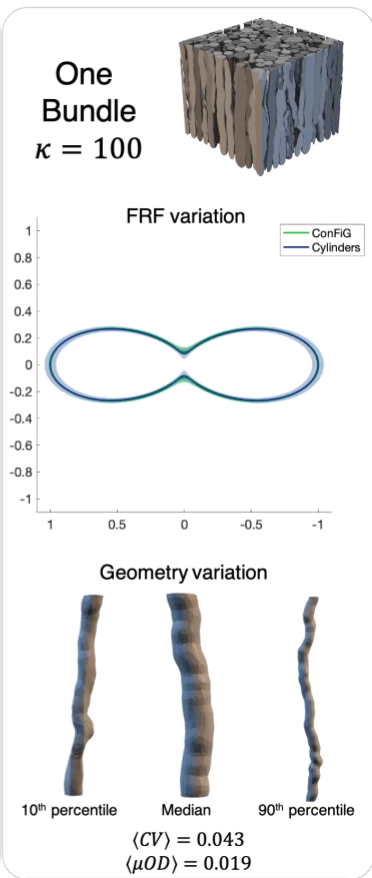
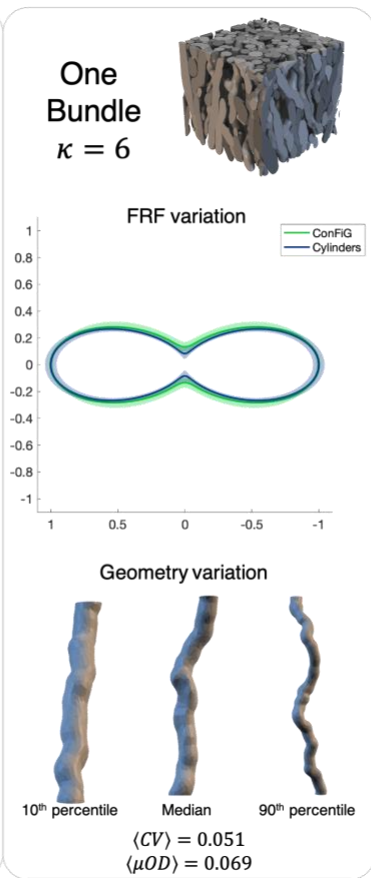
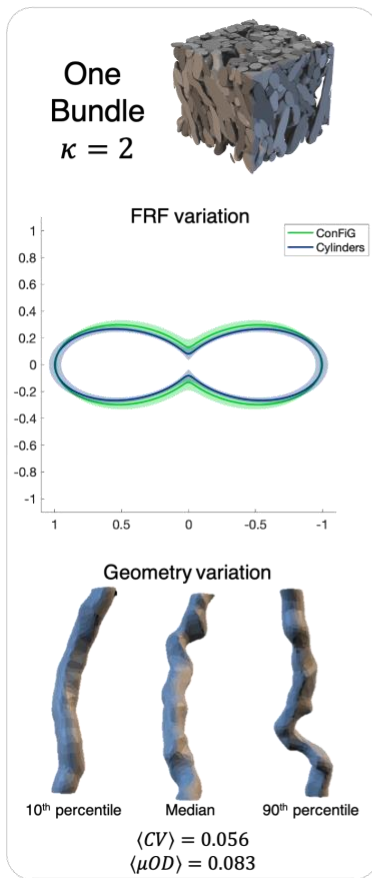


Figure 3 Variability in fibre responses within a voxel at $b=2000$ s/mm² at SNR=30 along with geometrical variation in fibres responsible for median, 10th and 90th percentile response. Notably, the 10th percentile fibres tend to be more stick-like while 90th percentile have more beading. Solid green line in FRF figures represents median response, shaded green area represents 10th and 90th percentile response. Same values for representative cylinders are in blue to demonstrate the variability due to noise.

3.2 Impact on fODF estimation

The variation in the FRF for each fibre leads to a variation in the estimated fODF as seen in Figure 5-Figure 7. Again, the magnitude of differences in fODF tends to depend on the complexity of the fibre arrangements since the more complex arrangements have more variation in the FRF. The resultant fODF is dependent on the b -value as demonstrated by Figure 6. While the direction of peaks in the fODF does not differ greatly at different b -values, the overall shape can change. Generally, the fODF calculated from SD picks out the correct main peak direction that is seen in the gold standard fODF from the microstructure, with differences in the overall peak amplitude and shape. A notable exception to this is the $\kappa = 2$ and EM fibre phantoms which at low b -value identifies two peaks with some FRFs which are not present in the gold standard fODFs.

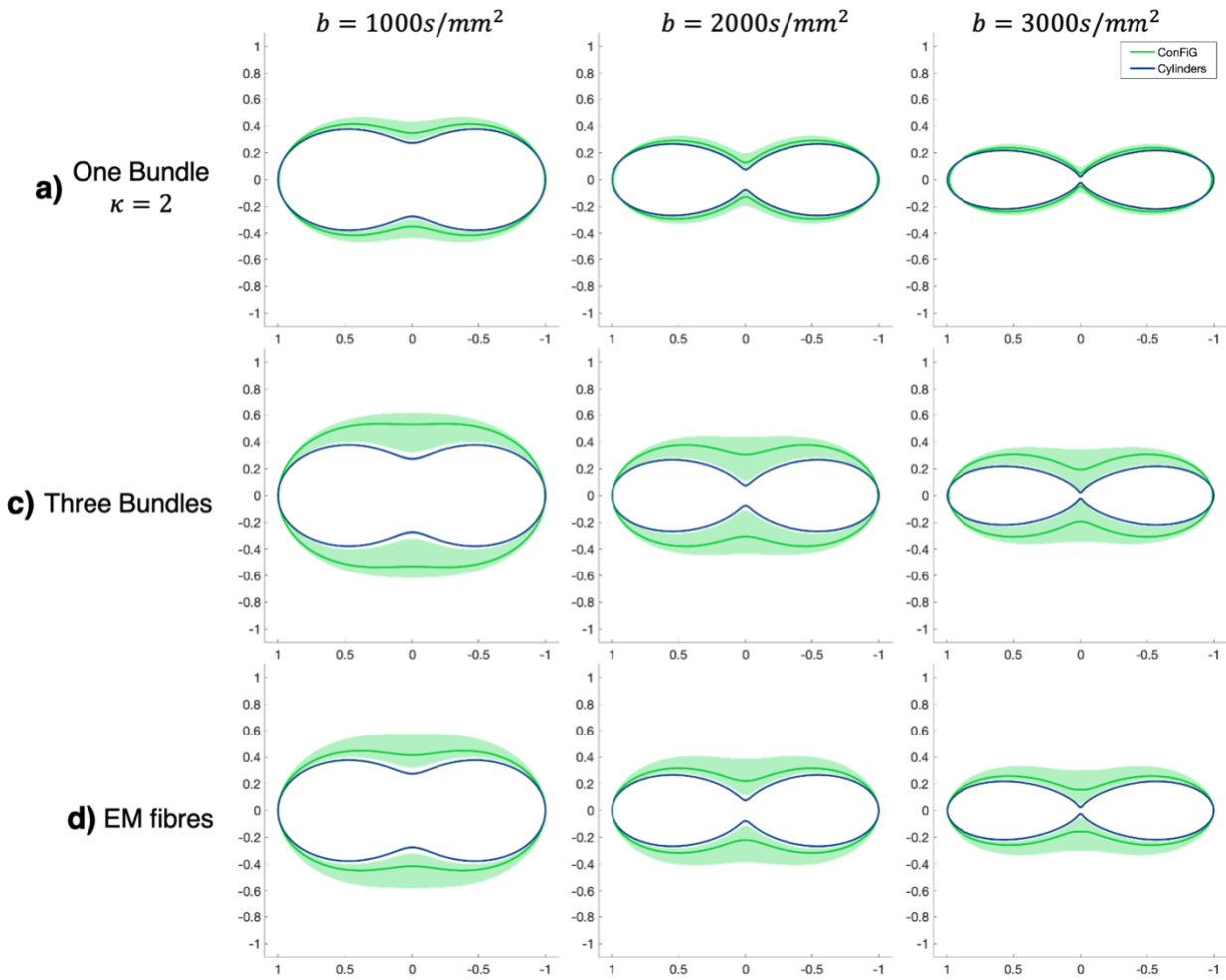


Figure 4 Per-fibre response function at $b = 1000, 2000, 3000 \text{ s/mm}^2$ (left-to-right) for (a) the $\kappa = 2$ phantom, (b) the three-crossing phantom and (c) the EM fibres.

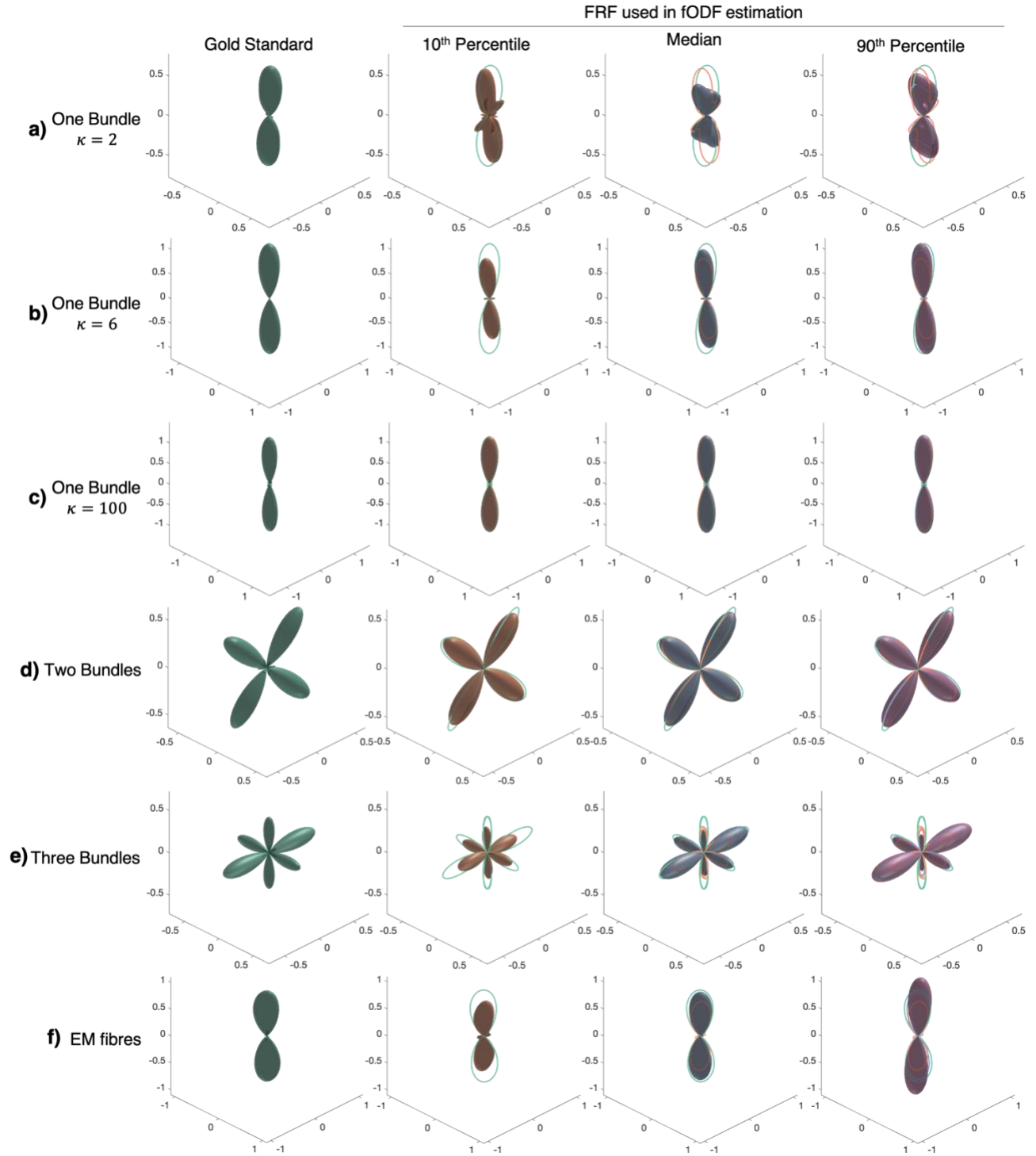


Figure 5 Variations in the FOD estimated using the 10th percentile, median and 90th percentile signal for the FRF for a range of phantoms. Left-to-right: the gold standard FOD from microstructure, FOD using 10th percentile, FOD using median, FOD using 90th percentile. 3D glyphs represent the FOD for each column and lines show previous FODs

overlaid.

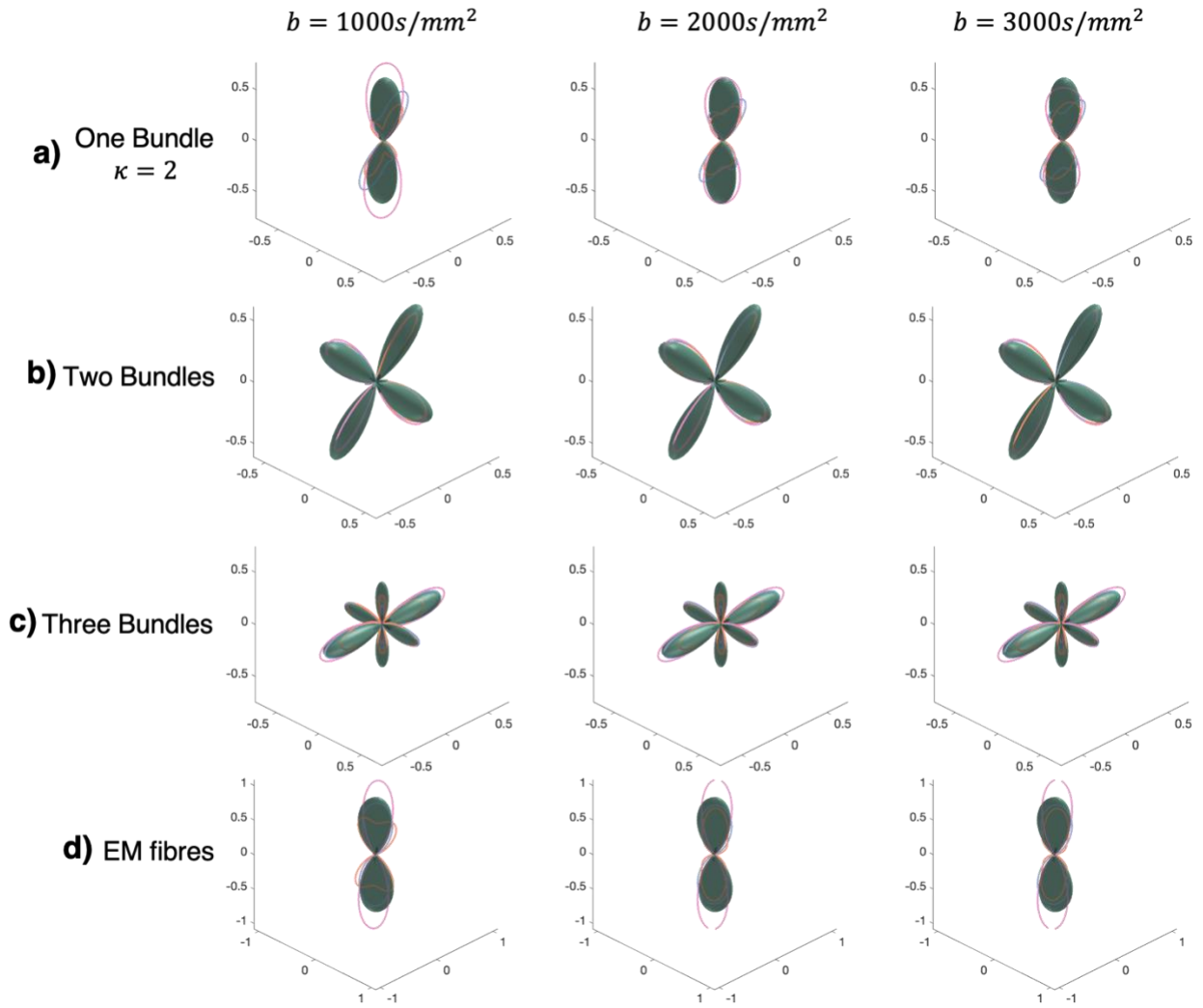


Figure 6 Variation in FOD estimated using 10th percentile, median and 90th percentile FRF at $b = 1000, 2000, 3000 \text{ s/mm}^2$ (left-to-right) for (a) the $\kappa = 2$ phantom, (b) the two-crossing bundles phantoms (c) the three-crossing bundles phantom and (d) the EM fibres. Green 3D surface is gold standard FOD from microstructure and lines outline the FOD using the 10th percentile, median and 190th percentile signals.

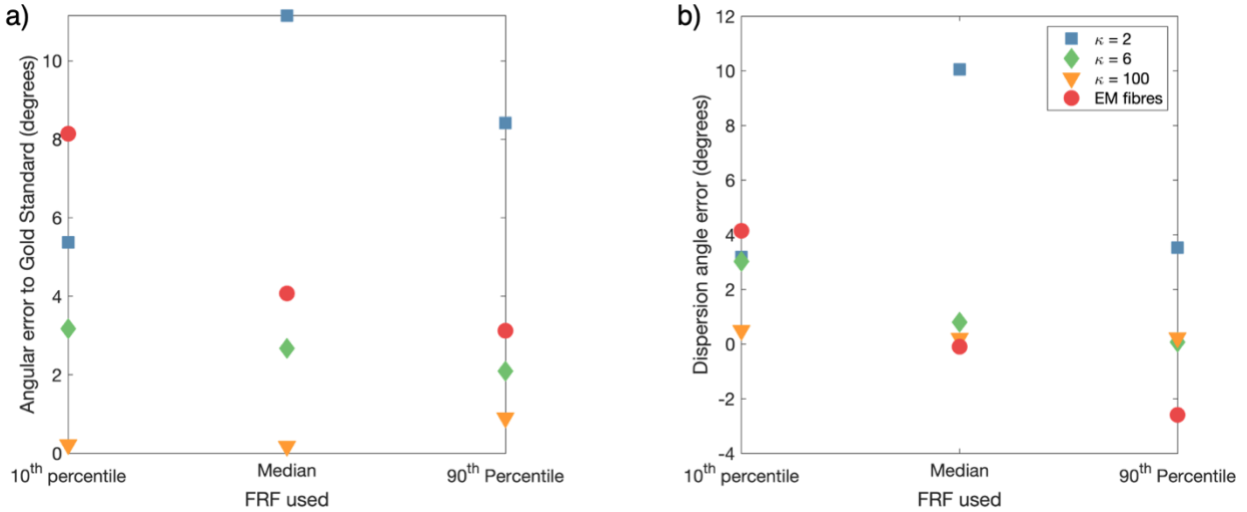


Figure 7 Variation in fODF properties when estimated using different FRFs. a) the angular error between the main peak in gold standard fODF and that of the fODFs estimated using CSD and b) the error in dispersion angle of the main peak (angle from main peak direction to cover 75th percentile) compared to the gold standard for each fODF.

4 Discussion

The microscopic variations in fibre morphology challenge the assumption in SD techniques that there exists a unique and shared FRF even within a single voxel. Here we have used dMRI simulations to demonstrate that variations in individual axonal morphology do indeed lead to different response functions per-fibre which in turn can have a knock-on effect on SD techniques to estimate the fODF.

As demonstrated in Figure 3Figure 4, the response function can vary substantially across different fibres, particularly in complex fibre arrangements such as the three-crossing bundles scenario. Indeed, the variation in responses in fibres reconstructed from real EM images of WM is large, similar to that of the ConFiG three-crossing simulations, and much larger than ConFiG phantoms containing a single fibre bundle. This suggests that there is in fact more variation in real axons than in the axons generated by ConFiG, even in simple arrangements of a single bundle of fibres. This is something that may be used to inform future versions of ConFiG to generate more realistic phantoms.

In the main, the largest variation in the per-fibre response, as well as the largest difference between cylinders and realistic axons happens in the axial direction which is to be

expected. Even with diameter variations and undulation, the radial diffusion is still strongly restricted under the assumption of no axonal permeability, while recent studies have shown that real axonal morphology causes time-dependent deviations from Gaussian diffusivity along the axial direction (H. Lee et al., 2020). Phantoms which show large amounts of beading (high CV in EM fibres and three-crossing bundles) show the largest variability in response, while μOD affects the response less, suggesting that fibre beading drives fibre response variability more than undulation.

Variations in the FRF have an impact on the estimated fODF as demonstrated in Figure 5- Figure 7. In the simplest fibre arrangements with the lowest dispersion (Figure 5b&c), the FRF variation is relatively small and so the fODF variation is small and the fODF peak directions match the gold standard fODF well, however in the $\kappa = 2$ case, the fODF varies greatly when using different FRFs (Figure 5a, Figure 6a and Figure 7).

Interestingly, when using the 10th percentile and median FRF, the fODF seems to pick out two-crossing bundles which are not present in the gold standard fODF, while the 90th percentile FRF picks up one dispersed bundle much closer to the gold standard fODF. On top of this, the relative amplitudes of the fODF change with b -value as seen in Figure 6a in which the median and the 10th percentile fODF changes from two distinct peaks at $b=1000s/mm^2$ to one wider merged peak at $b=3000s/mm^2$. These kinds of effects could have significant impacts on tractography techniques which use the fODF to trace fibre bundles through the brain.

In the case of crossing fibres, the fODF varies depending on the FRF used too. In the case of two-crossing bundles of fibres (Figure 5d), each bundle responds in a similar way, with peak directions and amplitudes largely unaffected by the FRF used and matching the gold standard fODF well. In the three perpendicular bundle case, however, peak amplitudes are affected by the FRF used as seen in Figure 5e and Figure 6c, in which different FRFs create fODFs with different relative peak amplitudes. The 90th percentile FRF selects the largest peak very strongly, creating smaller perpendicular peaks while the 10th percentile (and, in fact gold standard) FRF pick out more even lobes. This is significant because as shown by

(Schilling et al., 2019b), even changes in fODF peak amplitudes without peak direction changes can have an impact on tractography results.

In the real EM fibres, the large differences in FRF lead to large differences in the fODF as seen in Figure 5f and Figure 6d. Similarly to the $\kappa = 2$ case, the 10th percentile FRF seems to pick out two-crossing peaks, while the others pick out a single peak, closer to the gold standard fODF. This effect is dependent on b -value, with the two peaks at $b = 1000 \text{ s/mm}^2$ merging into one at $b=3000 \text{ s/mm}^2$. One possible explanation for this is that the Tikhonov regularisation in CSD (Tournier et al., 2007) encourages directions in which there is little diffusion to zero and it may be over-regularising in the $\kappa = 2$ and EM fibres cases.

The main takeaway from these investigations is that in general, even for a single voxel, there is often not a single FRF that can properly explain the data - at least under the experimental conditions investigated. Within-voxel heterogeneity in fibre geometry leads to heterogeneity in the per-fibre response to the extent that using a single FRF in CSD cannot always accurately recover the underlying fODF. Some techniques account for some FRF variation voxel-to-voxel (Christiaens et al., 2017; Kaden et al., 2016b, 2016a), however the investigations presented here suggest that the FRF may vary even within a voxel.

Additionally, these simulations lend further support to challenge the assumption that FRF is constant across the brain as differences in the median FRF per phantom demonstrate that the overall FRF from different fibre arrangements will be different as a result of the different axonal morphology in each environment. Further, as demonstrated by the fODF experiments, small changes in the FRF can lead to significant changes in the estimated fODF, meaning that using the wrong FRF in different brain regions could have large impacts on fODF-based techniques such as tractography.

4.1 Limitations and future work

In this work we investigated how complex fibre morphology affects SD techniques using a dMRI scheme chosen to use clinically feasible gradient strength and duration, though it may be possible that other measurement schemes exhibit greater or lesser sensitivity to these effects. For instance (Yeh et al., 2010) have shown that the gradient pulse duration can impact fibre orientation estimation. Another factor to investigate is the impact of the

diffusion time on the observed effects, since longer diffusion times can ‘smooth out’ these microscopic morphological variations as spins are able to diffuse further. At present, the diffusion time we can simulate is limited by the size of our phantoms, however work is ongoing to produce larger phantoms with ConFiG to allow us to study these effects at longer diffusion time.

Throughout this work we have referred to the fODF generated from the phantom microstructure as the ‘gold standard’ fODF in inverted commas. This is deliberate since it is not straightforward to define an fODF from microstructure that exactly corresponds to that from dMRI, in part due to the assumptions made in modelling the fODF from dMRI, which have been discussed here. Efforts have been made to make the two fODFs comparable in this work by defining the gold standard fODF using a single direction per fibre and normalising all fODFs to one.

The variability that is demonstrated in the per-fibre response in this experiment is suggested to arise due to the complexity of fibre morphology introduced due to complex fibre arrangements. This seems to be the case, as shown in Figure 3, though the exact nature of the link is not known for certain since there are many sources of morphological complexity (undulation, beading, non-circular cross-sections, etc.) which could all contribute to these variations. Future work will aim to isolate each of these effects to probe which morphological features have the largest impact on the FRF/fODF. Should these effects be understood, it may be possible to estimate them from the dMRI signal to improve the accuracy of SD techniques.

Another important consideration is that in this work we use CSD as in MRtrix3, however there are a wide range of SD techniques for fODF estimation, each with slightly different derivations and assumptions. While this will affect the fODFs presented in this work, the per-fibre signals and compartmental signals presented do not rely on any SD model, so the FRF variations will impact any models which use the FRF.

It is worth pointing out that here we merely demonstrate that the response varies on a per-fibre basis, meaning that the concept of a fibre response function needs to be treated carefully. Most SD techniques estimate their FRF from averaging across a number of voxels,

meaning that the FRF is an average single bundle response (including many fibres, extracellular space, other cells etc.) rather than purely a *fibre* response function. The variability in the per-fibre response may contribute less to the variable overall FRF seen across the brain in previous studies (Christiaens et al., 2020; Schilling et al., 2019b) than other factors such as the extracellular space but it should be considered.

It is also worth noting that this effect will impact other dMRI modelling techniques which model the signal as a combination of a diffusion response with an orientation distribution. For instance, NODDI models the intracellular signal with a Watson distribution as the orientation component and diffusion in sticks as the MR response, assuming that all the fibres can be treated as sticks. As shown here, microscopic variations in fibre morphology mean that the signals from each fibre are not identical and this could affect results of NODDI and other similar models (Alexander et al., 2017). Similarly, previous studies have looked into the effect of morphological features such as undulation on axon diameter estimation (Brabec et al., 2019; H.-H. Lee et al., 2020; Nilsson et al., 2012). Future work will aim to shed more light on these effects and investigate whether it is possible for it to be accounted for in our models.

5 Conclusion

The complex axonal morphology introduced by axons packing together in complex arrangements leads to differences in the dMRI response across different fibres. These variations in per-fibre response functions lead to differences in the fODF estimated using CSD, suggesting that the assumption of a single FRF across all fibres and all voxels may need to be considered carefully. Indeed, where there are large variations in FRF, no single choice of FRF is able to accurately estimate the fODF.

All of this means that the interpretation of the FRF and fODF in SD needs to be carefully considered and future models may seek to disentangle some of these effects for more accurate FRF and fODF estimation.

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Author Credit

Ross Callaghan: Conceptualisation, Methodology, Software, Investigation, Visualisation, Writing – Original Draft. **Daniel C. Alexander:** Conceptualisation, Writing – Review & Editing, Supervision, Resources, Funding acquisition. **Marco Palombo:** Conceptualisation, Methodology, Writing – Review & Editing, Supervision. **Hui Zhang:** Conceptualisation, Methodology, Writing – Review & Editing, Supervision, Resources, Funding acquisition.

Data and Code Availability

ConFiG code and simulated data will be made available at <https://rcallagh.github.io>. EM data is available at <https://www.cai2r.net/resources/software/intra-axonal-space-segmented-3d-scanning-electron-microscopy-mouse-brain-genu>.

Conflicts of Interest

The authors confirm that there are no conflicts commercial or financial conflicts of interest affecting this work.

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