Assessment of the structural complexity of diffusion MRI voxels using 3D electron microscopy in the rat brain

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A B S T R A C T

Validation and interpretation of diffusion magnetic resonance imaging (dMRI) requires detailed understanding of the actual microstructure restricting the diffusion of water molecules. In this study, we used serial block-face scanning electron microscopy (SBEM), a three-dimensional electron microscopy (3D-EM) technique, to image seven white and grey matter volumes in the rat brain. SBEM shows excellent contrast of cellular membranes, which are the major components restricting the diffusion of water in tissue. Additionally, we performed 3D structure tensor (3D-ST) analysis on the SBEM volumes and parameterised the resulting orientation distributions using Watson and angular central Gaussian (ACG) probability distributions as well as spherical harmonic (SH) decomposition. We analysed how these parameterisations described the underlying orientation distributions and compared their orientation and dispersion with corresponding parameters from two dMRI methods, neurite orientation dispersion and density imaging (NODDI) and constrained spherical deconvolution (CSD). Watson and ACG parameterisations and SH decomposition captured well the 3D-ST orientation distributions, but ACG and SH better represented the distributions due to its ability to model asymmetric dispersion. The dMRI parameters corresponded well with the 3D-ST parameters in the white matter volumes, but the correspondence was less evident in the more complex grey matter. SBEM imaging and 3D-ST analysis also revealed that the orientation distributions were often not axially symmetric, a property neatly captured by the ACG distribution. Overall, the ability of SBEM to image diffusion barriers in intricate detail, combined with 3D-ST analysis and parameterisation, provides a step forward toward interpreting and validating the dMRI signals in complex brain tissue microstructure.

Introduction

Interpretation and validation of diffusion magnetic resonance imaging (dMRI) relies on a deep understanding of the tissue characteristics at the level of the MRI voxels. Without ground truth information, we can only speculate how the microenvironment and structural barriers of healthy and diseased tissue modulate the diffusion of water molecules responsible for the dMRI signal (Basser and Pierpaoli, 1996; Le Bihan, 2003). The content of MRI voxels from images with approximately 100-μm isotropic resolution in small animal settings or with greater than 1-mm isotropic resolution in human studies is highly complex and heterogeneous. One single voxel in the brain, depending on its volume, can contain several from tens to thousands of neurons, glial cells, neurites, and vessels. Also, the proportions of these cellular components vary, change their morphology, and degrade or proliferate under pathological conditions. Voxels from different brain areas vary in tissue composition, organization, and complexity. White matter areas are homogeneous and mainly exhibit highly organized myelinated axons with micrometre in diameter, with a small contribution of glial cells and vasculization. On the other hand, grey matter areas show a heterogeneous, randomly organized and more complex microenvironment with cell bodies at the level of tens of micrometres in diameter with an important contribution of neurites, neuropil, and vasculization.

Recent advances in dMRI have introduced new methodologies that provide information beyond the conventional diffusion tensor model (Aganj et al., 2010; Jansons and Alexander, 2003; Özarslan et al., 2006; Scherrrer et al., 2016; Topgaard, 2017; Tournier et al., 2004, 2007; Tuch, 2004; Wedeen et al., 2005; Zhang et al., 2012). These

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; ACG, angular central Gaussian; BIC, Bayesian information criterion; CSD, constrained spherical deconvolution; dMRI, diffusion magnetic resonance imaging; DTI, diffusion tensor imaging; EM, electron microscopy; FOD, fibre orientation distribution; MRI, magnetic resonance imaging; NODDI, neurite orientation dispersion and density imaging; SBEM, serial block-face scanning electron microscopy; SH, spherical harmonics; ST, structure tensor; VOI, volume of interest.

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advanced dMRI techniques can resolve multiple fibre configurations and recover complex information from the dMRI voxel, which is averaged out in the diffusion tensor imaging (DTI) voxel. Multi-fibre dMRI models produce complex fibre orientation distributions (FOD) with the potential to extract novel information related to the tissue microstructure. Among the multi-fibre reconstructions, constrained spherical deconvolution (CSD) deconvolves the diffusion signal into a response function that represents the signal of coherently oriented fibre populations within a voxel (Tournier et al., 2007; Dell’Acqua and Tournier, 2019). Because of its capability to resolve crossing fibres, CSD is extensively used in tractography applications (Farquharson et al., 2013; Hyde et al., 2019). Another model, neurite orientation dispersion and density imaging (NODDI), is a multi-compartment model that accounts for differences in water diffusion of the intracellular, extracellular, and isotropic diffusion compartments of the tissue, and provides geometric models of the tissue microstructure, such as neurites modelled as sticks (Zhang et al., 2012). NODDI has been applied to estimate neurite density or increased cell density during inflammation (Collorone et al., 2019; Yi et al., 2019; Wang et al., 2019).

Structure tensor (ST) has been used for the validation of complex FODs extracted from dMRI reconstructions to extract analogous information from the tissue (Rezakhanli et al., 2012; Krause et al., 2010; Schmitt et al., 2004; Schmitt and Birkoholder, 2011; Budde and Frank, 2012; Schilling et al., 2016; Schilling et al., 2018; Khan et al., 2015). ST estimates the predominant directions of the intensity gradient in a specified neighbourhood of an image point and the degree of coherency between those directions (Bigan and Granlund, 1987). As a result, ST analysis applied to histological images can determine the orientation and dispersion from cellular components stained in the preparation. Previous studies applied ST analysis to evaluate primary fibre orientations on two-dimensional (2D) histological photomicrographs for the validation of DTI (Budde and Frank, 2012; Budde and Annese, 2013; Seehaus et al., 2015; Wang et al., 2017). Khan and collaborators extended 2D-ST to three-dimensional (3D) ST using stacks of confocal microscope images of the hippocampal proper obtained from an adult threus macaque brain in parallel with DTI measurements of the same sample (Khan et al., 2015). Schilling and collaborators also applied 3D-ST analysis using stacks of confocal microscope images of squirrel monkey brain to study the fibre distribution and compare them with the corresponding dMRI-estimated FODs (Schilling et al., 2016; Schilling et al., 2018).

Conventional and advanced histological approaches, including light microscopy and electron microscopy (EM) can provide 2D and 3D tissue information to validate dMRI (Breu et al., 2019; Budde and Annese, 2013; Budde et al., 2011; Jelescu and Budde, 2017; Schilling et al., 2019; Salo et al., 2017, 2018; Rojas-Vite et al., 2019). In recent years, novel EM imaging modalities have begun to offer ultra-high image resolution and large tissue volumes in 3D (Helmstaedter et al., 2008). The tissue preparation for EM with heavy metals reveals the membranes of all the cellular components, which are the real barriers for water diffusion. In our previous study (Salo et al., 2018), we introduced 3D-EM for the validation of DTI at the voxel level. We utilised serial block-face scanning electron microscopy (SBEM), which produces 3D datasets in high-resolution up to 10 nm at the mesoscopic scale (in the order of a few hundred micrometres) (Denk and Horstmann, 2004). Altogether, these properties indicate 3D-EM as a method of choice for the validation of dMRI.

In the present study, we exploited the ability of SBEM to image the membranes of all the cellular components of the rat brain. After imaging the brain using high-angular resolution diffusion imaging acquisition, we selected several white and grey matter areas with a different cellular composition, organisation, and orientation to challenge ST and dMRI analyses. We implemented 3D-ST on the SBEM datasets using three models, Watson and angular central Gaussian (ACG), with single and multiple fittings, and spherical harmonic (SH) decomposition. We compared SBEM-FODs with dMRI-FODs extracted from two widely used dMRI reconstructions, CSD and NODDI. We tested the angular correspondence between SBEM- and dMRI-FOD primary and/or secondary population distributions, and explored how the dispersion values could potentially distinguish between different tissue microenvironments with both 3D-ST and dMRI analyses.

Materials and methods

Animal information

One adult male Sprague-Dawley rat (10-weeks old, approximately 300 g, Harlan Netherlands B.V., Horst, Netherlands) was used in the study. The animal included in this study served as a control in our previous study, and underwent a sham operation, a craniotomy between bregma and lambda on the left convexity of the skull (see Salo et al., 2018 for detailed information). The brain tissue and the dura of this animal was intact after the sham operation. The tissue samples processed for EM were collected from the right hemisphere, contralateral to the sham operation five months after the procedure. The animal was housed in a room (22 ± 1 °C, 50–60% humidity) with a 12-h light/dark cycle and allowed free access to food and water. All animal procedures were approved by the Animal Care and Use Committee of the Provincial Government of Southern Finland and performed according to the guidelines set by the European Community Council Directives 86/609/EEC.

Tissue processing

Five months after the sham operation, the rat was transcardially perfused with 0.9% NaCl (30 ml/min) for 2 min followed by 4% paraformaldehyde (30 ml/min) at 4 °C for 25 min. The brain was removed from the skull and post-fixed in 4% paraformaldehyde/1% glutaraldehyde overnight at 4 °C, and then placed in 0.9% NaCl for at least 12 h to remove excess paraformaldehyde.

Ex vivo dMRI acquisition

The rat brain was scanned ex vivo in a vertical 9.4 T/89 mm magnet (Oxford Instruments PLC, Abingdon, UK) interfaced with a DirectDrive console (Varian Inc., Palo Alto, CA, USA) using a quadrature volume RF-coil (Ø = 20 mm; Rapid Biomedical GmbH, Rimpar, Germany) as both the transmitter and receiver. During imaging, the brains were immersed in perfluoropolyether (Solexis Galden®, Solvay, Houston, TX, USA) to avoid signals from the surrounding area.

The data were acquired using a 3D segmented spin-echo EPI sequence (TR = 1000 ms, TE = 35 ms, data matrix 128 × 96 × 96, FOV 19.2 × 14.4 × 14.4 mm³, resolution 0.150 × 0.150 × 0.150 mm³) with four segments. The acquisition was performed as a single scan and comprised a total of 129 volumes, with 42 uniformly distributed directions repeated 3 times with different diffusion weightings (b-values = 2000, 3000, and 4000 s/mm², Δ = 6 ms, Δ = 11.5 ms) and three images without diffusion weighting. The total scan time was 14 h, 24 min, and 27 s.

Data analysis for dMRI

The multi-shell dMRI data were fitted using two dMRI reconstruction methods that were essentially different: CSD as a multiple fibre orientation model, and NODDI as a multi-compartment model. The dMRI data were first converted to NIfTI format followed by correction for motion and eddy currents using FSL (FSL 5.0, http://fsl.fmrib.ox.ac.uk/fsl/fswiki) eddy (Andersson and Sotiropoulos, 2016). In CSD, the tissue response function was estimated for three tissue types – white matter, grey matter, and cerebrospinal fluid – using the dholander algorithm with the number of spherical harmonics determined automatically (DHollander et al., 2016). After that, a multi-shell multi-tissue CSD was computed using the msmt_csd algorithm (Jeurissen et al., 2014). The number and orientations of the
separate populations were determined by the MRtrix3 peak-finding algorithm (Jeurissen et al., 2013). We extracted distribution orientations and dispersions using fixed representation. The angular dispersion was computed using CSD spherical harmonic coefficients and Eqs. (4) and (5) (see Data analyses for SBEM datasets below for detail). In NODDI, the Watson distribution was fitted using an ex-vivo-based four-compartment model WatsonSHStickTortIsoVisoDot_B0 (Zhang et al., 2012). We acquired Watson concentration parameter κ, mean orientation μ, and orientation dispersion index (ODI) values from the model fit. We generated a FOD-directionally encoded-coloured apparent fibre density map using MRtrix3 (https://www.mrtrix.org) to visualize the location of the VOIs in the right hemisphere of this brain: cingulum/corpus callosum, external capsule/Layer VI of the somatosensory cortex, dentate gyrus (granule cell layer and molecular layers), and ventroposterior thalamic nucleus/internal capsule (Fig. 1A).

Tissue preparation for SBEM

EM studies are challenging because of the labour-intensive sample preparation, which limits the number of samples that can be analysed. For this proof-of-concept study, we prepared a total of four samples, from the right hemisphere of the brain, where we did not expect to see any tissue alterations caused by the sham operation.

After the ex vivo dMRI, the brain was placed in 0.9% NaCl for at least 4 h to remove excess perfluoropolyether and then sectioned into 1-mm thick coronal sections with a vibrating blade microtome (VT1000s, Leica Instruments, Germany). A section –3.80 mm from bregma was selected and further dissected into smaller samples containing one or two areas of interest. We collected four tissue samples from the right hemisphere and preparations were done by R.A. and T.M. from Germany).

After this sectioning, each sample was imaged using SBEM (Deerinck et al., 2010). The protocol was described in detail in our previous work (Salo et al., 2018). Briefly, the samples were incubated with potassium ferrocyanide and aqueous osmium tetroxide followed by thiocarbohydrazide solution. After a second osmication in osmium tetroxide, the samples were treated with uranyl acetate and stained with en bloc Walton’s lead aspartate. Then, after dehydration, samples were embedded in Durecupan ACM resin (Electron Microscopy Sciences, Hatfield, PA, USA).

Before mounting the specimens, the excess resin in the hardened tissue blocks was trimmed. Then, 5 semi-thin 500-nm sections were selected from the face of the block (Fig. 1A–D). The first section was stained with toluidine blue. The fifth section was used for co-registration and selection of the area of interest for imaging. After selecting the area, the blocks were further trimmed in a pyramidal shape with a base of 1 × 1 mm² and a top or face of approximately 600 × 600 μm². The blocks were then mounted on aluminium specimen pins using conduc-
tive silver epoxy (CircuitWorks CW2400), and painted with silver paint (Ted Pella, Redding, CA, USA) to electrically ground the exposed block edges to the aluminium pin. Finally, the entire surface of the specimen was sputtered with a thin layer of platinum coating to improve conductivity and reduce charging during the sectioning process.

**SBEM data acquisition**

The blocks were imaged on an SEM microscope (Quanta 250 Field Emission Gun; FEI Co., Hillsboro, OR, USA) equipped with 3View system (Gatan Inc., Pleasanton, CA, USA) using a backscattered electron detector (Gatan Inc., Pleasanton, CA, USA). The top of the mounted block or face was the x-y plane, and the z-direction was the cutting direction. When the mounted block was positioned in the microscope, we obtained an overview image of the volume-of-interest (VOI) before scanning.

For imaging, we used a 2.5-kV beam voltage and a pressure between 2.28 × 10^{-5} to 0.15 Torr. The obtained datasets were acquired at a 16-bit colour depth with a magnification between 457× to 537× and a resolution of 50.0 × 50.0 × 50.0 nm³, covering an area of 100.6 ± 2.3 µm in the x-direction, 202.6 ± 2.4 µm in the y-direction, and 62.5 ± 3.1 µm in the z-direction (2048 × 4096 × 1192-1306 voxels³). After imaging, Microscopy Image Browser (MIB; http://mib.helsinki.fi; Belevich et al., 2016) was used to process (calibrate, normalise contrast, align, and convert to an 8-bit colour depth) the SBEM image stacks. Potential image distortions or artefacts were removed using upright-speeded up robust features (U-SURF; Bay et al., 2006). U-SURF found local features in each image that was then affinely aligned with an adjacent image using those features with an m-estimator sample consensus (MSAC) algorithm (Torrid and Murray, 1997). Finally, any mean drifts of stretch, shear, or translation were removed from the images by applying a 40-image running average window.

**Co-registration of dMRI and SBEM data**

MRI and SBEM datasets were co-registered to re-position the SBEM datasets into the dMRI reference frame and to select dMRI voxels that spatially corresponded with the SBEM datasets, as described previously in Salo et al. (2018). Briefly, we used the fractional anisotropy map to co-register the dMRI images into the photomicrograph of a 1-mm thick section of the whole brain using Amira software (version 5.6.0; Thermo Fisher Scientific, USA), using translations, rotations, and voxel size scaling. This co-registration provides an estimate of the 3D rotations between the fractional anisotropy map and whole brain section. The second step was to co-register the SBEM datasets to the 1-mm thick section. The fifth semi-thin section was first co-registered to the dissected tissue sample using landmarks, such as tissue borders. Next, the SBEM overview was co-registered to the fifth semi-thin section using local landmarks, such as vessels and cell bodies. Combining these steps provides the rotation of SBEM datasets into the rotational frame of reference of the 1-mm thick section. The selected voxels on the dMRI map were confirmed by testing the signal of neighbouring voxels and by an anatomy and MRI expert (A.S.).
Selection of volume-of-interest (VOI) in SBEM datasets

From each tissue sample (Fig. 2A–D), we imaged one dataset, except in the sample from the thalamus, from which we acquired two; one from the internal capsule and another from the ventroposterior thalamic nucleus (see Fig. 2B). In total, we acquired five datasets containing one or two VOIs from different brain areas (red outlines in Fig. 1), resulting in seven VOIs for analysis, four from the white matter and three from the grey matter. The corpus callosum and cingulum were imaged in the same dataset, which contained approximately two-thirds of the corpus callosum and one-third of the cingulum (Fig. 1B). The VOIs analysed for the corpus callosum and cingulum were 120.0 × 102.2 × 65.3 μm³ (2400 × 2044 × 1306 voxel³) and 47.3 × 102.2 × 65.3 μm³ (947 × 2044 × 1306 voxel³) respectively. The internal capsule occupied approximately two-thirds of the dataset and the VOI analysed was 87.4 × 119.4 × 59.9 μm³ (1748 × 2388 × 1198 voxel³) in size (Fig. 1C). The dataset for the ventroposterior thalamic nucleus exclusively included this area in the images. The VOI was 86.4 × 194.3 × 59.8 μm³ (1728 × 3885 × 1169 voxel³) (Fig. 1D), only slightly cropped from the full dataset to avoid heterogeneous borders due to misalignments. Similarly, the dentate gyrus dataset only included this area and the VOI was 98.6 × 189.4 × 61.3 μm³ (1971 × 3788 × 1225 voxel³) (Fig. 1F). We also separately analysed the molecular layer (98.6 × 104.5 × 61.3 μm³) and the granule cell layer (98.6 × 60.0 × 61.3 μm³; VOIs shown in Fig. 5). The datasets for the somatosensory cortex contained one-third the dataset of cortical layer VI (75.0 × 102.4 × 66.4 μm³; 1500 × 2048 × 1327 voxel³) and one-fourth that of the external capsule (54.8 × 102.4 × 66.4 μm³; 1096 × 2048 × 1327 voxel³) (Fig. 1E). In the VOIs described above, we avoided including interfaces, such as between the cingulum and corpus callosum (Fig. 1B), internal capsule and ventroposterior thalamic nucleus (Fig. 1C), and somatosensory cortex and external capsule (Fig. 1E).

Data analyses for SBEM datasets

The SBEM VOIs were analysed by ST analysis (Bignon and Granlund, 1987; Budde and Frank, 2012). We implemented three models, Watson, ACG, and SH, to compare with NODDI and CSD. Image intensity gradients were produced by convolving the image voxels with the first derivative of the 3D Gaussian function (size 11 × 11 × 11 voxels or 0.55 × 0.55 × 0.55 μm³; standard deviation 2.34 voxels or 0.12 μm) in the three orthogonal directions. A dyadic tensor was formed from the partial derivatives, and the ST was summed element-wise from within a 19 × 19 × 19 voxel-sized window to form a well-defined (semi-positive definite, rank-two) ST. The ST was eigen-decomposed, and the eigenvector corresponding to the smallest eigenvalue was collected. Combining these component vectors formed our estimate of the distribution of water diffusion within the VOI. For parameterisation of the distributions, we fitted two antipodally symmetric probability distributions to the data: 1) 3D Watson distribution

\[ W(x; \mu, \kappa) = Ce^{(\mu^T x)^2}, \]

where \( \mu \) is the mean direction, \( \kappa \) is the concentration of the distribution, and \( x \) are points on the unit ball, and 2) ACG distribution (Tyler, 1987)

\[ A(x; M) = CM^{-\frac{1}{2}} (x^T Mx)^{-\frac{1}{2}}, \]

where \( M \) is a 3 × 3 rank-2 matrix, which was eigen-decomposed to determine the distribution mean, and two orthogonal dispersion directions on a unit ball along with eigenvalues (\( \lambda_1 \geq \lambda_2 \geq \lambda_3 \)) that were used as dispersion estimates. Whereas the Watson distribution is axially symmetric, the ACG has two parameters for modelling the distribution asymmetry. In addition to a single-fitted distribution, we fitted a mixture of two distributions to all sample distributions. Maximum likelihood estimates were computed by applying an expectation maximisation algorithm to both the Watson (Bijral et al., 2007) and ACG (Ito et al., 2016) distributions modified for real-valued mixtures. In addition to the fitted distributions, we produced a histogram distribution of orientations from the minor eigenvectors to visualise the ground truth distribution. All distributions were visualised using a mesh of triangles with 8000 points, where the points were evenly scattered on the unit sphere using an electrostatic repulsion algorithm.

Furthermore, the 3D-ST histogram data was decomposed using SH (Talman, 1968)

\[ f(\omega) = 1 + \sum_{l=2,4} \sum_{q=-l}^l f_q^l Y_q^l(\omega), \]

where \( f_q^l \) is the constant for harmonic \( q \) for level \( l \), \( f \) is the orientation distribution, \( Y_q^l \) is the spherical harmonic basis function for harmonic \( q \) of level \( l \), and \( \omega \in S^2 \).

Three dispersion estimates were computed for Watson, ACG, and SH fittings for the SBEM orientation distributions. For the Watson distribution, we used the orientation dispersion index: \( ODI = \sum_{l,k} |l|/|k| \) (Zhang et al., 2012), and for the ACG distribution the eigenvalues of \( M: OD_{ACG} = \lambda_2/\lambda_1 \). As with OD, \( OD_{ACG} \) has property \( 0 \leq OD_{ACG} \leq 1 \), where \( OD_{ACG} = 0 \) means fully coherent distribution and \( OD_{ACG} = 1 \) means evenly distributed distribution. The angular dispersion for SH was computed from the normalized 2nd level SH rotational invariants (Novikov et al., 2018; Lee et al., 2019) as

\[ \theta_p = \cos^{-1} \sqrt{\frac{2p_z + 1}{3}}, \]

where

\[ p_z = \frac{1}{2} \sum_{q=-2}^2 |f_q^2|^2, \]

(4)

(5)

where \( N_x \) is a scaling factor chosen such that \( p_z \equiv 1, f_2^2 \) is the spherical harmonic \( q \) of level 2, and \( p_z \) is the 2nd, and \( p_z \) is the 0th level rotational invariant. ACG has two dispersion parameters that characterize dispersion on the unit sphere in two orthogonal directions. We used this feature to compute the asymmetry (flatness) of the distribution as \( a = \lambda_2/\lambda_1 \). The CSD angular dispersion in each voxel was computed using CSD SH coefficients and Eqs. (4) and (5).

Correlations between dispersion values extracted from SBEM models and dMRI reconstruction were assessed using a Pearson’s linear correlation (2-tailed) with GraphPad Prism (Version 5.03, GraphPad Software Inc., La Jolla, CA, USA).

We defined the angular difference as the difference in angle between the central direction vectors of any two primary or secondary distribution. In SBEM data, these central vectors were the distribution mean of the Watson model, and the first eigenvector of the ACG model. In NODDI, the central vector was the Watson distribution mean, and in CSD, the sub-distribution peak direction. The central direction of the SH was estimated as the direction of the maximum of the SH distribution. The angular difference ranges from 0 to 90°.

Adding parameters, by way of additional variables and/or mixtures, into the model generally improves the model fit with the expense of more complex model, leading to potentially overfitting the data. Therefore, we assessed the trade-off of adding model parameters and better model fit using the information theory-based Bayesian information criterion (BIC) (Schwarz, 1978),

\[ BIC = k \ln(n) - 2 \ln(\hat{L}). \]

(6)

where \( k \) is the number of variables in the model, \( n \) is the number of samples, and \( \hat{L} \) is the maximized likelihood value of the model. The BIC is a criterion for model selection and it answers to two questions in our study: does modelling the shape of the distribution add information, and does adding mixture of multiple distributions make the fit better without overfitting? We used BIC in all seven VOIs and to the four probability distribution models we used: single Watson (2 variables), single
ACG (5 variables), mixture Watson (5 variables), and mixture ACG (11 variables).

Results

The location of selected areas in the FOD-based DEC map of the brain are shown in Fig. 1A, and representative images of SBEM datasets from the same areas and the VOIs for analysis are shown in Fig. 1B–F. We analysed VOIs from four white matter areas: the corpus callosum (Fig. 1B), cingulum (Fig. 1B), and internal (Fig. 1C) and external capsule (Fig. 1E); and from three grey matter areas: the ventroposterior thalamic nucleus (Fig. 1D), layer VI of the primary somatosensory cortex (Fig. 1E), and the dentate gyrus (Fig. 1F). These areas show tissue microstructures with different cell types, architecture, organisation, and orientation. SBEM images from white matter, such as the cingulum, corpus callosum, and internal and external capsules, revealed clear differences in the organisation of the fibres, including fanning of the fibre bundles or scattered individual axons (Fig. 1B, C and E, and Fig. 2A, A′, A″, B and C′). Note that Videos 1, 2 and 4 allow the 3D visualization of these white matter structures. Grey matter areas exhibited a high degree of organisation such as the granule cells and their dendritic arborisation in the dentate gyrus, highly organised fibre bundles between neurons such as in the ventroposterior thalamic nucleus combination of cell bodies, and neurites with different density and organisation such as in the somatosensory cortex (Fig. 1D–F, and Fig. 2B′, C, D′ and D″). Note that Videos 3–5 allow the 3D visualization of these grey matter structures.

White matter

The main cellular components in the white matter are myelinated axons forming fibre bundles with a minor presence of oligodendrocytes, glial cells, non-myelinated axons, and vessels (Fig. 1B, C and E, and Fig. 2A, A′, A″, B′ and C%). The corpus callosum showed organised fibre bundles running along the xy-plane or the imaging plane, but these bundles fanned in the x-plane or through the imaging plane (Fig. 1B, Fig. 2A and Video 1). The cingulum presented highly parallel axons perpendicular to the xy-plane with a small number of individual axons running in the xy-plane (Fig. 1B, Fig. 2A and Video 1). Major and small fibre bundles in the internal capsule ran across the xy-plane, but the bundles ran with different orientations to one another (Fig. 1C, Fig. 2B′ and Video 2). The external capsule showed the majority of fibre bundles running in a transverse plane to the xy-plane with a small number of fibre bundles running in a different orientation compared with the main population (Fig. 1E, Fig. 2C″ and Video 4). Despite the differences in organisation and orientation between the white matter areas, all the areas were characterised by either fibre bundles in one orientation or fibre bundles fanning within a small angle. Additionally, we observed a minor presence of cell bodies corresponding mainly to oligodendrocytes, non-myelinated axons, and vessels in comparison with the number of myelinated axons.

The orientation histograms of the selected white matter areas showed a major distribution corresponding to the main population of myelinated axons observed in the EM datasets (Fig. 3A1–D1). Secondary small distributions were distinguishable in the external and internal capsule histograms (Fig. 3C1 and D1).

The Watson and ACG models fitted with a single distribution and SH decomposition visualised in the histograms in all white matter areas (Fig. 3A2–D2, A3–D3 and A6–D6). The low dispersion values of the Watson, ACG and SH models reflected the highly organised nature of the white matter (Table 1). Comparison of the dispersion values between white matter areas showed that the cingulum was the area with less dispersion (0.052 with Watson fitting and 0.018 with ACG fitting; 18.5° in SH decomposition), resembling the organisation of highly parallel axons observed in the SBEM images (Video 1). In the corpus callosum, and internal and external capsules (Videos 1, 2 and 4), the fanning of the fibre bundles slightly increased the dispersion values in both the Watson (0.077–0.100) and ACG (0.039–0.044) models and in the SH decomposition (22.3°–25.2°) (Table 1).

While the Watson model produced circular distributions, the ACG model generated distributions with dispersion values deviating from circularity to asymmetry (Fig. 4). The ACG distributions ranged from round-shaped in the cingulum distribution to flat-shaped in the corpus callosum, with the external and internal capsule distributions as intermediate cases. The asymmetry of the ACG model was 4.7 for the corpus callosum (Fig. 4A), 2.8 for the external capsule (Fig. 4D), 1.7 for the internal capsule (Fig. 4C), and 1.5 for the cingulum (Fig. 4B).

The Watson and ACG models fitted with a mixture of two distributions generated two-lobed FODs (Fig. 3A4–D4 and A5–D5). The secondary distribution in the Watson model showed a lower dispersion value than the primary distribution in the corpus callosum (0.088 vs 0.004; Table 1). In contrast, the secondary distribution showed higher dispersion values than the primary distribution in the cingulum (0.024 vs 0.880), and external (0.021 vs 0.189) and internal capsule (0.040 vs 0.746) (Table 1). The ACG model split the FODs into two distributions with the same dispersion in the cingulum (0.0011; Table 1). The secondary distribution showed higher dispersion values than the primary distribution in the corpus callosum (0.026 vs 0.035), and external (0.025 vs 0.051) and internal capsule (0.031 vs 0.045) (Table 1).

Comparison of the 3D-CT and dMRI methodologies revealed small angular differences between their distributions in the corpus callosum (3–13°; Fig. 5A). In the cingulum and external capsule, all the angular differences between the 3D-CT and dMRI methods were small (5–10°), except for the Watson secondary distribution, which showed angular differences of 79° in the cingulum (Fig. 5B) and 89° in the external capsule (Fig. 5C). In the internal capsule, the angular differences between 3D-CT and dMRI were small (7–21°; Fig. 5D) when comparing 3D-CT with the NODDI or CSD primary distributions, except for the Watson secondary distribution, which showed moderate angular differences of 48° with the NODDI and 51° with the CSD primary distributions (Fig. 5D). Table 1 shows the individual weights in the mixture distributions in the white matter areas. We found that the secondary Watson distribution (0.11–0.36) was small in comparison to the primary one. Primary and secondary ACG distributions were similar in weight (0.47–0.50) and close to each other as demonstrated by their angular differences. The CSD secondary distribution also showed moderate angular differences when compared with 3D-CT methods (38–47°; Fig. 5D).

The dispersion values of dMRI-FODs showed a trend similar to that of the dispersion values extracted from SBEM data in white matter areas (Table 1 and Fig. 3A6–D6 and A7–D7). NODDI-FODs presented only a single distribution. We obtained the lowest dispersion values in the cingulum and external capsule (0.10; Table 1), followed by the internal capsule (0.26; Table 1) and corpus callosum (0.30; Table 1). CSD produced only a single distribution in FODs in the corpus callosum, cingulum, and external capsule. The external capsule was the area with the lowest dispersion (0.65; Table 1), followed by the cingulum (0.72; Table 1) and the corpus callosum (0.85; Table 1). The internal capsule showed the highest CSD dispersion value of white-matter areas (1.27) with a CSD-FOD splitting into two distributions with approximately the same dispersion (0.62 and 0.65; Table 1).

The BIC was the smallest for the mixture ACG in the corpus callosum, cingulum, and internal capsule, and for the single ACG in the external capsule (Table 2). The ability of the single ACG to model asymmetry of the distribution outperformed single Watson in all cases in the white matter areas included in this study (Table 2).

Grey matter

Neurons and their neurites are the dominant feature in the grey matter, but glial cells and myelinated axons are also present as demonstrated by SBEM datasets (Fig. 1D–F and Fig. 2B–D). The ventroposterior thalamic nucleus has numerous neurons mainly projecting from the soma.
running in the xy-plane (Fig. 1D, Fig. 2B'' and Video 3). We also observed loosely packed bundles of myelinated axons running perpendicularly to the xy-plane, as well as individual myelinated axons running in the xy-plane (Fig. 1D, Fig. 2B'' and Video 3). The somatosensory cortex showed numerous neurons and their neurites with no preference for any orientation (Fig. 1E, Fig. 2C' and Video 4). Similarly, we observed widely distributed individual myelinated axons running both in-plane and perpendicular to the xy-plane (Fig. 1E, Fig. 2C' and Video 4). The architecture of the dentate gyrus differed from that in other grey matter areas (Fig. 1F and Fig. 2D). The granule cell layer consists of approximately seven rows of densely packed cell bodies (Fig. 1F and Fig. 2D'').

The main component of the adjacent molecular layer is the dendritic tree emerging from the granule cells to the molecular layer with few scattered cell bodies and randomly organised myelinated axons (Fig. 1F, Fig. 2D' and Video 5).

The histogram of the ventroposterior thalamic nucleus showed two distributions (Fig. 6A1): an asymmetric distribution and a round-shaped distribution approximately 45° to the first. The histogram of the somatosensory cortex showed two asymmetric distributions (Fig. 6B1). The histogram of the dentate gyrus generated a round-shaped distribution (Fig. 6C1). Also, we separately obtained a histogram of the molecular and granule cell layer to study the effect of high cell- and dendritic-
Table 1
Dispersion values (ODI) from Watson and ACG models and NODDI reconstruction, and dispersion angles (°) from SH decomposition and CSD reconstruction.

| Brain area       | SBEM          | dMRI          |
|------------------|---------------|---------------|
|                  | Single fitted distribution |                |
| Watson | ACG | Watson | ACG | SH | NODDI | CSD |
|-------------------------------------------------|
| Corpus callosum | 0.077          | 0.044         | 0.088 (0.88) | 0.026 (0.51) | 22.3 | 0.30 | 23.6 |
| Cingulum         | 0.052          | 0.018         | 0.024 (0.89) | 0.011 (0.50) | 18.5 | 0.10 | 22.8 |
| External capsule | 0.100          | 0.039         | 0.021 (0.64) | 0.025 (0.53) | 25.2 | 0.10 | 25.6 |
| Internal capsule | 0.099          | 0.042         | 0.189 (0.36) | 0.051 (0.47) | 25.1 | 0.26 | 27.2 |
| Thalamus         | 0.717          | 0.856         | 0.746 (0.20) | 0.045 (0.48) | 47.5 | 0.88 | 39.0 |
| Cortex           | 0.235          | 0.670         | 0.448 (0.18) | 0.492 (0.45) | 46.1 | 0.91 | 39.6 |
| Dentate gyrus    | 0.361          | 0.290         | 0.345 (0.92) | 0.159 (0.60) | 43.1 | 0.33 | 27.2 |

Values in parentheses represent the individual mixture weights in the mixture distribution.

Fig. 4. Orthographically projected views of the histogram distributions of the corpus callosum (A), cingulum (B), external capsule (C), internal capsule (D), ventroposterior thalamic nucleus (E), Layer VI of the primary somatosensory cortex (F), and dentate gyrus (G), where the distribution mean points to the viewer. The asymmetry of each distribution is reflected by the histogram distribution flatness and the ACG-derived asymmetry value below each histogram. The sizes of the histogram distributions also reflect the general dispersion of the distributions.

Table 2
Bayesian information criterium (BIC) ranking of the single Watson, single ACG, mixture of two Watson, and mixture of two ACG probability distribution fittings, where the methods are ranked from 1st to 4th according to BIC.

| Brain area     | BIC rank | Corpus callosum | Cingulum | External capsule | Internal capsule | Thalamus | Cortex | Dentate gyrus |
|----------------|----------|-----------------|----------|------------------|------------------|----------|--------|---------------|
| 1st            | ACG mixture | ACG mixture | ACG single | ACG mixture | ACG single | ACG single | ACG single | ACG single |
| 2nd            | ACG single | Watson mixture | ACG mixture | Watson mixture | Watson mixture | ACG single | Watson single | Watson single |
| 3rd            | Watson single | ACG single | Watson single | Watson mixture | Watson mixture | Watson single | Watson single | ACG mixture |
| 4th            | Watson single | Watson single | Watson single | Watson single | Watson single | Watson single | Watson single | ACG mixture |

Watson fitting model is colour-coded in blue and ACG in yellow. Single distribution model is represented with lighter colour and mixture with darker colour.

density in the dentate gyrus VOI (Fig. 7). We observed a similar distribution when analysing the molecular layer and the granule cell layer together or separately.

The Watson and ACG models fitted with a single distribution and SH decomposition captured the histograms of the grey matter areas (Fig. 6A2–C2, A3–C3 and A6–C6). The ventroposterior thalamic nucleus showed the highest dispersion of the three grey matter areas in both the Watson and ACG models (0.717 with Watson and 0.860 with ACG, and 47.5° with the SH decomposition; Table 1), which matched with the organisation of neurons and their projections and axonal bundles in this area (Fig. 1D and Video 3). The Watson model generated a lower dispersion value in the somatosensory cortex (0.235; Table 1) than in the dentate gyrus (0.361; Table 1). In contrast, the ACG model yielded a higher dispersion value from the somatosensory cortex (0.670;
The distributions obtained from the ACG model in grey matter areas were flat-shaped for the thalamus and cortex, and round-shaped in the dentate gyrus (Fig. 4). The asymmetry of the ACG model was 2.6 for the thalamus (Fig. 4E), 3.2 for the cortex (Fig. 4F), and 4.4 for the dentate gyrus (Fig. 4G).

The Watson and ACG two-component mixture models produced secondary distributions corresponding with those observed in the histograms of the thalamus and cortex (Fig. 6A–A5 and B4–B5). The models generated secondary distributions in the dentate gyrus, however, which cannot be seen in the histograms (Fig. 6C4 and CS). The primary and secondary distributions in the Watson model showed similar dispersion values in the thalamus (0.476 vs 0.448) and cortex (0.225 vs 0.254), while the dispersion values of the secondary distribution in the dentate gyrus were higher than those of the primary distribution (0.345 vs 0.532) (Table 1).

When comparing CSD, the primary distribution showed moderate to high angular differences (39°–65°), and the second, third, and fourth distribution ranged from 19° to 89° (Fig. 5E). We obtained a similar variety of angular differences in the somatosensory cortex when comparing the 3D-ST and dMRI methods (Fig. 5F). The angular differences between NODDI and the 3D-ST distributions varied from 4° to 84°, and between CSD and the 3D-ST distributions from 11° to 89° (Fig. 5F). The angular differences in the dentate gyrus were small between the 3D-ST and dMRI methodologies (11°–13°; Fig. 5G). In the grey matter areas, the individual weights in the mixture distributions varied from small in the ventroposterior thalamic nucleus (0.18) and dentate gyrus (0.08) to equal in the somatosensory cortex (0.44) in the Watson mixture model, and equal in the ACG mixture model (0.40–0.46) (Table 1).

The NODDI-FODs showed higher dispersion values in the grey matter areas compared with the white matter areas (Table 1 and Fig. 6A6–C6). We obtained the highest dispersion values in the thalamus (0.88) and cortex (0.91), and a low dispersion value in the dentate gyrus (0.33), close to the white matter dispersion values (Table 1). The dispersion in the CSD-FODs oscillated from high to low values in the thalamus (1.00 to 0.26), had the same value for the three distributions in the cortex (0.60), and a single value for the distribution in the dentate gyrus (0.93) (Table 1 and Fig. 6A7–C7).

The BIC showed that single ACG performed the best in the thalamus, cortex, and dentate gyrus, whereas single Watson showed worst performance in the thalamus and cortex (Table 2). The only location where mixture ACG performed worse than the other models was in the dentate gyrus. The single ACG ability to model asymmetricity of the distribution outperformed single Watson in all grey matter VOIs (Table 2).

Fig. 8 shows the correlations of dispersion values obtained with 3D-ST models and dMRI reconstructions. We found significant correlations between the ACG and NODDI dispersion values (R = 0.96; p-value = 0.0008; Fig. 8B), and between the ACG dispersion values and

| Table 1 | than from the dentate gyrus (0.290; Table 1). Similar to the ACG fitting, the SH decomposition showed a higher dispersion angle in the somatosensory cortex (46.1°; Table 1) than from the dentate gyrus (43.1°; Table 1). While both models reflected the grey matter nature of these areas, the ACG dispersion values and the SH dispersion angles better fitted with the more organised environment of the dentate gyrus (Fig. 1F and Video S) and the more random appearance of the cortex (Fig. 1E and Video 4).

The distributions obtained from the ACG model in grey matter areas were flat-shaped for the thalamus and cortex, and round-shaped in the dentate gyrus (Fig. 4). The asymmetry of the ACG model was 2.6 for the thalamus (Fig. 4E), 3.2 for the cortex (Fig. 4F), and 4.4 for the dentate gyrus (Fig. 4G).

The Watson and ACG two-component mixture models produced secondary distributions corresponding with those observed in the histograms of the thalamus and cortex (Fig. 6A–A5 and B4–B5). The models generated secondary distributions in the dentate gyrus, however, which cannot be seen in the histograms (Fig. 6C4 and CS). The primary and secondary distributions in the Watson model showed similar dispersion values in the thalamus (0.476 vs 0.448) and cortex (0.225 vs 0.254), while the dispersion values of the secondary distribution in the dentate gyrus were higher than those of the primary distribution (0.345 vs 0.532) (Table 1). The ACG model showed a higher dispersion value in the primary distribution compared with the secondary distribution in the thalamus (0.855 vs 0.492), similar dispersion values for both distributions in the somatosensory cortex (0.214 vs 0.242), and a lower dispersion value in the primary distribution as compared with the secondary distribution in the dentate gyrus (0.159 vs 0.428) (Table 1).

In the ventroposterior thalamic nucleus, we found moderate angular differences between NODDI and the two 3D-ST models (35°–52°), but the angular difference was high compared with NODDI and the Watson primary distribution (70°), and the SH decomposition (60°) (Fig. 5E).
Fig. 6. Orientation distributions in grey matter areas. Histogram distributions (1) and fibre orientation distributions (FOD) from Watson (2) and angular central gaussian (ACG) (3) fitted with a single distribution and from Watson (4) and ACG (5) fitted with two distributions, spherical harmonic (SH) decomposition (6), neurite orientation dispersion and density imaging (NODDI) (7) and constrained spherical deconvolution (CSD) (8) of the ventroposterior thalamic nucleus (A), Layer VI of the primary somatosensory cortex (B), and dentate gyrus (C). Colour coding: red, medio-lateral orientation; green, dorso-ventral orientation; blue, rostro-caudal orientation. Note that the distributions are rotated to allow for visualisation of their shape and orientation. The same rotation was applied to every distribution in the figure.

CSD dispersion angles ($R = 0.91$; $p$-value = 0.0008; Fig. 8E). SH dispersion angles correlated to both NODDI dispersion values ($R = 0.85$; $p$-value = 0.015; Fig. 8C), and CSD dispersion angles ($R = 0.75$; $p$-value = 0.012; Fig. 8F). Additionally, SH dispersion angles correlated to Watson dispersion values ($R = 0.82$; $p$-value = 0.023; Fig. 8G), and ACG dispersion values ($R = 0.91$; $p$-value = 0.0044; Fig. 8H), as well as Watson and ACG dispersion values also correlated ($R = 0.91$; $p$-value = 0.0044; Fig. 8I).

Discussion

We utilised seven SBEM datasets from the rat brain to serve as ground truth for evaluation of the tissue microstructure of white and grey matter areas. SBEM is particularly suitable for visualisation of cellular membranes, the strongest component modulating diffusion MRI signal, with an imaging volume reaching the MRI voxel size. Furthermore, we implemented 3D-ST analysis using the Watson and ACG distribution models and SH decomposition to estimate and parameterise the orientation distribution on the SBEM data. The orientation and dispersion properties obtained from the two 3D-ST models were compared to parameters obtained from two dMRI reconstructions, CSD and NODDI. The main results of this work are: 1) the Watson, ACG and SH models implemented on the SBEM data could differentiate between distinct tissue microenvironments, consistent with observations of the cellular architecture in the SBEM images; 2) the orientation distributions obtained from the dMRI reconstruction methods in highly homogeneous white matter microenvironments matched well with the orientation distributions obtained from the 3D-ST analyses, whereas the agreement was less evident in the more complex grey matter microenvironments; 3) the dispersion values obtained from CSD well captured the differences between tissue environments, whereas NODDI could only moderately distinguish orientation distribution features evident in the SBEM; 4) dMRI dispersion values correlated better with ACG and SH angular dispersion than Watson dispersion; and 5) in contrast to the common modelling assumption of axial symmetry, the microstructure-derived orientation distributions often appeared asymmetric.

The incorporation of 3D microscopy imaging and advanced analysis methods on histological preparations allows for direct comparison between the ground truth and dMRI, as shown in previous studies, where combining 3D tissue imaging modalities and ST analysis improved the validation of advanced dMRI (Khan et al., 2015; Schilling et al., 2016, 2018; Wang et al., 2015). Light microscopy methods in 3D, such as confocal microscopy imaging, offer whole brain coverage, which facilitates good co-registration between imaging modalities and the selection of a large number of VOIs for robust statistical analysis. When working with 3D-EM methods, the analyses require a more elaborate
co-registration to guide the selection of the dMRI voxels due to the limited sample size versus the whole brain dMRI maps. The limited sample size may produce small to moderate sources of error when comparing SBEM and dMRI, e.g. distribution main directions, due to trimming or mounting of the blocks during sample preparation, or partial-volume effect in dMRI voxels. Increasing the sample size will overcome uncertainties, such as the angular differences between SBEM and dMRI models in the grey matter areas, where the heterogeneity of the tissue microenvironment in SBEM and the partial volume effect in dMRI voxel might affect the outcomes, e.g. in the ventroposterior thalamic nucleus. On the other hand, EM techniques offer high-resolution and excellent tissue contrast to visualise cellular membranes, which are the main physical barriers for the water molecule motion. Lipophilic carbocyanine dyes used in previous studies stain membranes, but light microscopy cannot reliably resolve individual membranes. The nominal resolution in light microscopy imaging can be 2- to 3-orders of magnitude greater than that in EM imaging with approximately the same volume of tissue (Schilling et al., 2018). Information extracted from histological assessments of light microscopy images, however, remains at the “macrostructural level” (Khan et al., 2015; Schilling et al., 2016, 2018). The high spatial resolution of the photomicrographs can resolve finer detail of the tissue microenvironment, e.g. crossing fibres, which will be confounded in low spatial resolution images. In addition, light microscopy allows the selection in the same preparation of both different brain areas and a variety of VOI sizes for the ST analysis to test the influence of specific microstructural features (Schilling et al., 2017). Both electron and light microscopy techniques required sample preparation and imaging, which might confound the 3D-ST results in certain extend. For example, tissue shrinkage by the staining and embedding processes can affect the correlation between MRI and histologic quantitative analyses. To assess how isotropic or anisotropic the tissue shrinkage is, future studies are required to estimate the reduction of the tissue volume before and after sample preparation in both global and cellular levels (Korogod et al., 2015).

Parameterisation of the antipodal orientation distributions allows for straightforward quantitative analysis of the distribution properties, such as the orientation and dispersion, and thus comparison with the corresponding dMRI parameters. A number of studies have parameterised the histological orientation distributions. The von Mises distribution, which provides parameterisation using the population mean and concentration, is the most frequently used method to model orientation distribution (Bock et al., 2010; Budde and Frank 2012; Ronen et al., 2014; Schilling et al., 2017). Confocal light microscopy images have also been parameterised (Schilling et al., 2018) by fitting the Watson distribution on the orientation distribution populations after estimating the number and orientations of the separate populations using the Mrtrix peak-finding algorithm (Jeurissen et al., 2013; Tournier et al., 2004). Using this approach, Schilling and collaborators reported low to moderate correlations between histological and dMRI orientation dispersion indices, similar to what we report in the present study in white matter VOIs. Adding a larger range of dispersion values in the form of increased tissue complexity, such as in crossing fibres and grey matter, may further challenge the correlation between the Watson model and dMRI dispersion indices. Another model utilised for the validation of dMRI is the Bingham distribution, which has been fitted to 2D-ST distributions in polarised light microscopy images (Mollink et al., 2017), also revealing a moderate correlation between the histologic and dMRI distributions. It is interesting to note that the angular dispersion using SH decomposition led to a value of 22.3° in the corpus callosum, which was consistent with values reported by Ronen and collaborators obtained using silver impregnation on human samples (Ronen et al., 2014), Lee and collaborators using SBEM in mouse brain (Lee et al., 2019), as well as other diffusion MRI studies of human brain (Novikov et al., 2018; Veraart et al., 2018).

The shortcoming of the Watson model is that the distribution is axially symmetric around the mean. As seen in the histograms obtained from our SBEM data, however, the orientation distributions were hardly ever axially symmetric and rather exhibited varying degrees of asymmetric dispersion. We implemented ACG to model the asymmetries observed in the histogram distributions (Tyler, 1987). ACG is a distribution similar to the Bingham distribution (Sotiropoulos et al., 2012) in that it parameterises the orientation distribution with a $3 \times 3$ matrix describing mean orientation and two orthogonal concentration parameters of the distribution on the unit sphere. There are some advantages of the ACG parameterisation over the Bingham, such as simple geometrical interpretability, transformability to uniform distribution (which allows for testing whether observations arise from the uniform distribution), and simple computation of the scaling constant (Tyler, 1987).

While both Watson and ACG distributions captured the histogram orientations well, the ACG distribution more faithfully fit the distributions and was therefore able to capture the asymmetric flatness of the SBEM histogram distributions. Furthermore, we used SH to analyse the distribution with a complete base decomposition. While Watson and ACG have few parameters, SH has tens-to-hundreds of parameters depending on the decomposition level. SH decomposition was able to represent the histogram well due to its additional number of parameters. Given the abundance of asymmetry in our SBEM datasets, we postulate that the often-used dMRI assumption of axial symmetry of tissue microstructure populations may differ from the ground truth. Furthermore, as single ACG outperformed single Watson in all of our VOIs, the asymmetricity of the distributions may reflect valuable information related to the tissue microstructure or even improve reconstructions such as tract tracing (Sotiropoulos et al., 2012; Tariq et al., 2016), if this property can be reliably teased out from the dMRI signal. Additional studies focused on
Fig. 8. Correlation between dispersion values/angles generated from the neurite orientation dispersion and density imaging (NODDI) reconstruction and Watson (A), angular central Gaussian (ACG) (B) model fitted with a single distribution and the spherical harmonics (SH) decomposition (C); between constrained spherical deconvolution (CSD) reconstruction and Watson (D), ACG (E) and SH (F); and between the models applied to SBEM data (G–I). Each point corresponds with dispersion values extracted from selected white and grey matter areas. The linear correlation (dashed line), Pearson’s coefficients (R) and p-values are included in the graphs. Abbreviations: cc, corpus callosum; cg, cingulum; Cx, somatosensory cortex; DG, dentate gyrus; dMRI, diffusion magnetic resonance imaging; ec, external capsule; ic, internal capsule; SBEM, serial block-face scanning electron microscopy; Tha, ventroposterior thalamic nucleus.

the asymmetric distributions in both histology and dMRI are needed to evaluate the potential of analysing this property.

As all selected VOIs also contained orientation populations other than the principal orientation population, we also fitted two-component mixture models with both the Watson and ACG models. The orientations of the principal population in coherently organised VOIs were similar in both the Watson and ACG methods, whereas the orientations of the secondary populations differed. The benefits of using mixture model fitting were evident in the sense that the mixture ACG outperformed single ACG in several VOIs and mixture Watson outperformed single Watson fitting in all VOIs. In our datasets, modelling asymmetric dispersion captured a vast amount of the variability in the orientation distributions, but the benefits of mixture modelling were also evident. An appropriately chosen distribution model may reduce the need to apply mixture modelling.

Advanced dMRI algorithms can resolve multiple fibre populations within a voxel, but these algorithms differ in several aspects, including requirements of the dMRI acquisition, fundamental assumptions of the diffusion process, representation of orientation information, and intended area of application. These inherent differences result in diverse outcomes, which limits comparisons across methods and, more importantly, must be considered when validating these methods. The differences between NODDI and CSD were demonstrated in this study by how well they captured and/or failed to reflect the brain tissue architecture in distinct microenvironments. Previous studies demonstrated the potential of these methods to extract orientation and dispersion in the single- and crossing-fibre white matter (Mollink et al., 2017; Schilling et al., 2018). In this study, we included distinct tissue microenvironments, such as areas with fanning fibre bundles, mixtures of white and grey matter, and highly organised grey matter, to explore the potential of the dMRI reconstructions. Our results indicate that both NODDI and CSD captured well the primary orientation of the main population within the voxel, whereas secondary populations in the more complex architectures, such as crossing fibres and grey matter, were a challenge for those methods. Similarly, Schilling and collaborators found that dMRI reconstructions generally captured the nature of the ground truth in coherently oriented fibre bundles in the white matter (Schilling et al., 2018), but none of the reconstruction methods tested were able to successfully extract FODs in crossing fibres. The indication of our study, that CSD overcome NODDI to model the underlying tissue microstructure orientation distribution features, can be due to CSD SH-based decomposition having more degrees of freedom than Watson-based NODDI. The asymmetry of the tissue microstructure orientations could be better modelled by the Bingham-based NODDI, a feature not currently available and required for future comparisons.
Similar to anisotropy and diffusivity in the classical diffusion tensor model, dispersion represents important properties of the tissue microstructure. Few studies have explored the dispersion obtained by dMRI methods and included extensive validation from histology (Bock et al., 2010; Jespersen et al., 2011; Mollink et al., 2017; Schilling et al., 2018). The pattern of the dispersion values derived from 3D-ST analyses well followed the complexity of the brain areas included in our study. Mollink and collaborators found a similar pattern of dispersion values when comparing crossing and single fibre regions in dMRI and histology (Mollink et al., 2017). These authors also reported considerably lower dispersion values from the 2D-ST analysis performed on polarised light microscopy than dispersion values derived from dMRI. However, as demonstrated by Lee and collaborators, the dispersion angle extracted from the 2D-ST analysis was inherently smaller than that from the 3D dMRI analysis (Lee et al., 2019). Despite including the third dimension, we observed that the Watson and ACG fitting models extracted lower dispersion values than those obtained from dMRI, which might be associated to intrinsic differences between 3D-ST and dMRI analyses. The 3D-ST is an intensity-based analysis based on the staining with heavy metals into cellular structures, while dMRI is sensitive to restriction of diffusion of the water molecules caused by these structures. The differences in the dispersion values complicate comparisons between methods and interpretation of the results.

As mentioned above, Schilling and collaborators obtained a moderate correlation between dispersion derived from 3D-ST analysis and dMRI methods (Schilling et al., 2018); they found differences in the dispersion values between dMRI models, which were attributed to the presence of false-positive peaks in the FODs derived from dMRI as a potential source of discrepancy between methods (Schilling et al., 2018).

The Watson and ACG models implemented in this study differently modelled the SBEM data. Both models obtained good estimates of the FODs and their dispersion values differentiated between distinct tissue microenvironments. Our correlations between dispersion derived from dMRI and 3D-ST showed that ACG dispersion values better correlated with dispersion values derived from CSD and NODDI. This outcome together with the asymmetry of the ACG distributions indicates that this fitting model may have better potential for the validation of dMRI reconstructions.

An important limitation in the validation of dMRI with histology is the fundamental difference between ST analyses based on microscopy and dMRI. ST analyses obtain information from the intensity differences in SBEM photomicrographs, while dMRI methods are sensitive to diffusion of the water molecules within the tissue. This limitation can be overcome by performing numerical simulations, such as Monte Carlo simulations, on tissue models (Lee et al., 2019, 2020). Realistic tissue models based on segmentations in 3D-EM are the bottleneck, however, especially for large tissue volumes. Fully segmented 3D-EM datasets in combination with dMRI measurements of the same animal will enable fitting of biophysical models, such as composite hindered and restricted models of water diffusion (CHARMED) (Assaf et al., 2004) or standard model (Novikov et al., 2018). The validation of these models will require a set of tissue parameters, such as axonal diameter, soma size, intra- and extra-cellular volume fraction, which rely on the full segmentation of the cellular component present in the dataset. Future studies focused on the validation of dMRI methodologies will benefit from the development new strategies for automated and/or semiautomated segmentation pipelines of 3D-EM data (Abdollahzadeh et al., 2019a, 2019b; Kleinjnenhuis et al., 2020). The connection between dMRI and histology can also be investigated by deriving the response function of spherical deconvolution from the histological fibre orientation distribution (Schilling et al., 2019) as outlined in Supplementary Fig. S1 for our samples. Other 3D-EM techniques, such as the automated tape-collecting ultramicrotome, can image larger tissue volumes than our sample size and at an ultra-high resolution of up to 4 nm (Baena et al., 2019; Shibata et al., 2019). Larger tissue volumes may improve the selection of volumes in 3D-EM and co-localisation with dMRI. On the other hand, larger images greatly increase the imaging time from days to weeks, challenge the capacity of data storing from gigabytes to petabytes of data, and require higher computational capacity. More importantly, large imaging data increase the complexity and time for segmentation pipelines, computational time for numerical simulations, and other quantitative analyses. Nevertheless, segmentation of large 3D-EM data can be applied to determine the contribution of different cellular components to the dMRI signal in unprecedented detail.

Conclusion

This work demonstrates the potential of SBEM imaging as ground truth for the validation of advanced dMRI reconstructions. We performed 3D-ST analyses, probability distribution parameterisation and complete base decomposition, of seven high-resolution SBEM white and grey matter volumes. All our models, Watson, ACG, and SH, captured the nature of the orientation distributions, but ACG and SH outperformed Watson with their ability to detail the asymmetry displayed by the orientation distributions. When comparing distribution orientation and dispersion from SBEM and dMRI, NODDI and CSD reconstructed coherently orientated architecture well, whereas complex crossing-fibres and grey matter were far more challenging. In the future, the ability to image intricate details of the tissue microstructure may provide unprecedented possibilities to address detailed questions about fundamental properties of dMRI.

CRedit author statement

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Data and code availability statement

We will make the datasets publicly available in an open repository. Until then, the data are available upon request to the corresponding author.

The source code of the structure tensor analysis and parameterisations presented in this study is available at GitHub (https://github.com/omiar/SBEMST3D).

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2020.117529.
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