The effect of axon shape and myelination on diffusion signals in a realistic Monte Carlo simulation environment

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Synopsis

The cylindrical models often used in Monte Carlo diffusion simulations do not resemble the shape of axons very well. In this work, a more realistic substrate derived from electron microscopy data is used to investigate the influence of axon shape and myelination on the diffusion signal. In the DifSim simulation environment, diffusion signals from EM-derived substrates are compared to those from cylindrical substrates matched for volume fraction. Furthermore, the effect of removing the impermeable myelin sheath from the substrate is assessed.

Introduction

The application of microstructure techniques in diffusion imaging, such as axon diameter[1] and membrane permeability estimation[2], are often validated using Monte Carlo simulations. The geometric substrates are usually highly simplified cylindrical models that have a limited number of compartments. This work investigates a more realistic substrate derived from electron microscopy data and aims to showcase how the influence of shape and compartments on the diffusion signal can be investigated.

Methods

Simulation substrates

To create a realistic substrate, a serial blockface electron microscopy dataset (4000x4000x500; 29.2x29.2x25.0 µm) was acquired from the genu of the corpus callosum of a mouse brain in sagittal sections. Acquisition used a Zeiss Merlin Compact Scanning Electron Microscope with a Gatan 3View system. For the present purpose, a single section was segmented by tracing cell membranes and myelin sheaths manually (Figure1). Six compartments were distinguished: unmyelinated axons (UA), myelinated axons (MM and MA), glial bodies (GB) and glial processes (GP). Extracellular space (ECS) was enforced around all objects (no touching fibres). Two EM substrates were created to yield ECS volume fractions of 0.19 and 0.23 (EM1 and EM2) by eroding the fibres. Cylindrical substrates were obtained by tightly packing circles with equivalent radii (CT1/CT2; ECS volume fraction 0.14). To create cylindrical substrates matching EM packing densities, the circle centroids were shifted (CM1/CM2). A substrate without myelin was created from EM1 by replacing

myelinated with unmyelinated axons with the shape of the MM outer boundary. Dilation of substrates by 0.0292 µm resulted in a container fitting tightly around the substrate. Substrates had a z-dimension (10 µm) to meet the 3D nature of the simulation environment, but diffusion was periodic and free in the—constant—z-dimension.

Monte Carlo simulations

Monte Carlo simulations were performed with DifSim[3] tracking the phase of the particles and calculating the diffusion MR signal. Diffusivities for ECS, intracellular compartments and myelin were set to [2.0;0.75;0.03] $\mu m^2/ms$ and the base rate of 35 particles $/um^3$ was scaled by concentration factors [0.95;0.88;0.50][4]. Simulation step size was 1 µs.

The effect of cross-sectional axon shape and myelination on the diffusion signal was assessed for a PGSE sequence using a range of diffusion times (Δ=[2-160] ms), b-values (100-32000 s/mm^2) and 30 diffusion gradient directions in a semicircle in the xy-plane.

Two simulation experiments were conducted to assess the effect of geometry and permeability. (i) Shape: For assessing the effect of shape and volume fraction, substrates EM1, EM2, CT1, CT2, CM1 and CM2 were compared. In this experiment, membranes were impermeable. (ii) Permeability: Myelination has a profound effect on the exchange between axons and ECS, therefore permeability was included for the comparison between the myelinated and unmyelinated substrate.

Results

Shape

The diffusion signal averaged over directions is similar for EM1/2 vs. CM1/2 substrates (Figure2). The main variation is due to volume fraction, as also demonstrated by smaller attenuation for the CT substrates. The ECS signal is different between the two CM substrates, indicating that unequal spacing around objects interacts with volume fraction (arrow). Two regimes where shape might become a relevant factor (Figure3:"ALL"): i) very short Δ, where lower signal in EM1 appears driven by restricted (quasi-)cylindrical compartments (MA/UA); ii) for the lower b-values across Δ's where the ECS signal is dominant.

Myelination

With low permeability, no effect of myelination on the aggregate diffusion signal is seen (Figure4:"o"). Higher permeabilities ("+") induce loss of signal in the unmyelinated vs. the myelinated substrate as there is both less restriction in intracellular compartments as well as increased diffusion across membranes.

Discussion

The EM-based substrate and simulation environment presented here has been developed to provide researchers with a flexible tool for investigating the role of a range of tissue features. Here, we present the two examples of shape and permeability.

Considering the modest change in signal between EM-derived and cylindrical substrates, our results suggest that the circular cross-sectional shape is a valid approximation for white matter tissue, for example as deployed for axon diameter mapping. On the other hand, the minor effect myelination had at low permeability suggests higher exchange rate estimates[2] in grey compared to white matter might be due to abundance of highly permeable glia, rather than degree of myelination.

The EM-derived substrate is somewhat oversegmented as connections between objects occuring outside the segmented section were ignored. Future 3D tissue modeling efforts will address this shortcoming. Further compartmentalisation and variations in compartment properties (e.g. mitochondria and separate permeabilities of glia and neurons) will add to the accuracy of this realistic model. Fortunately, these improvements are incorporated in the DifSim environment in a straightforward manner.

Acknowledgements

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References

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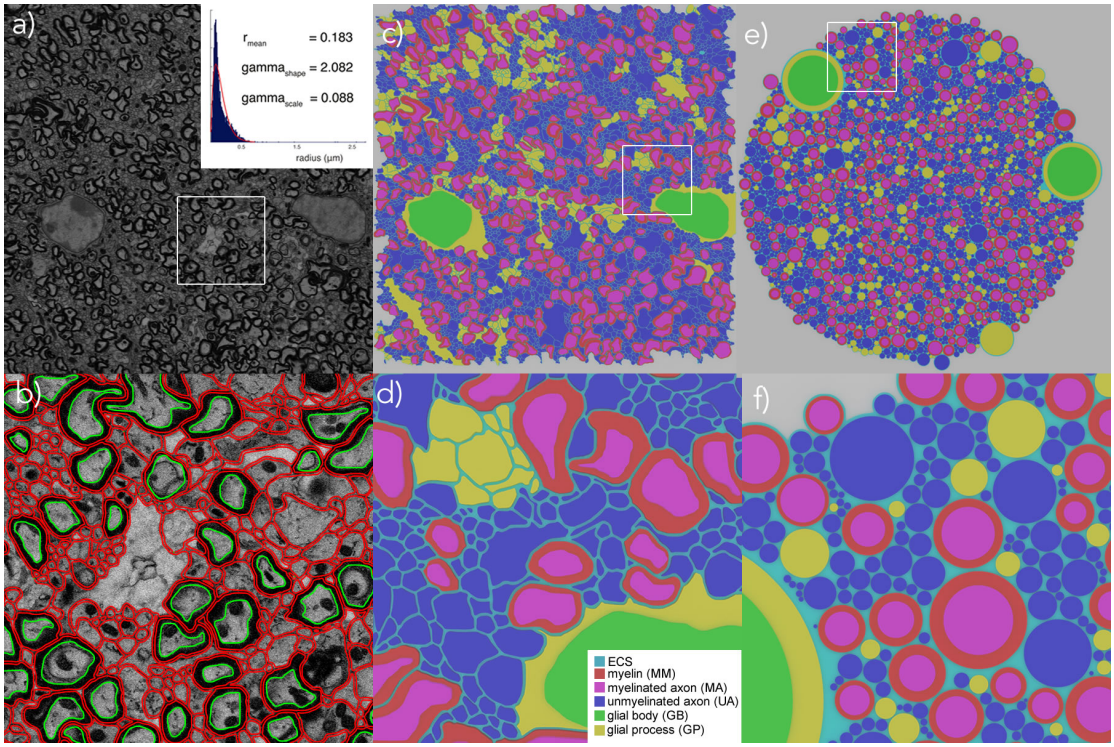

Figure 1.

Electron microscopy segmentation. a) original image (29.2x29.2 µm); b) boundaries of objects in contact with ECS (red) and myelinated axons (green); c) final segmentation (counts: 3329 UA; 445 MM/MA; 2 GB; GP:461); d) a closer look at the compartments; e) circle packing with equivalent radii; f) region in e.

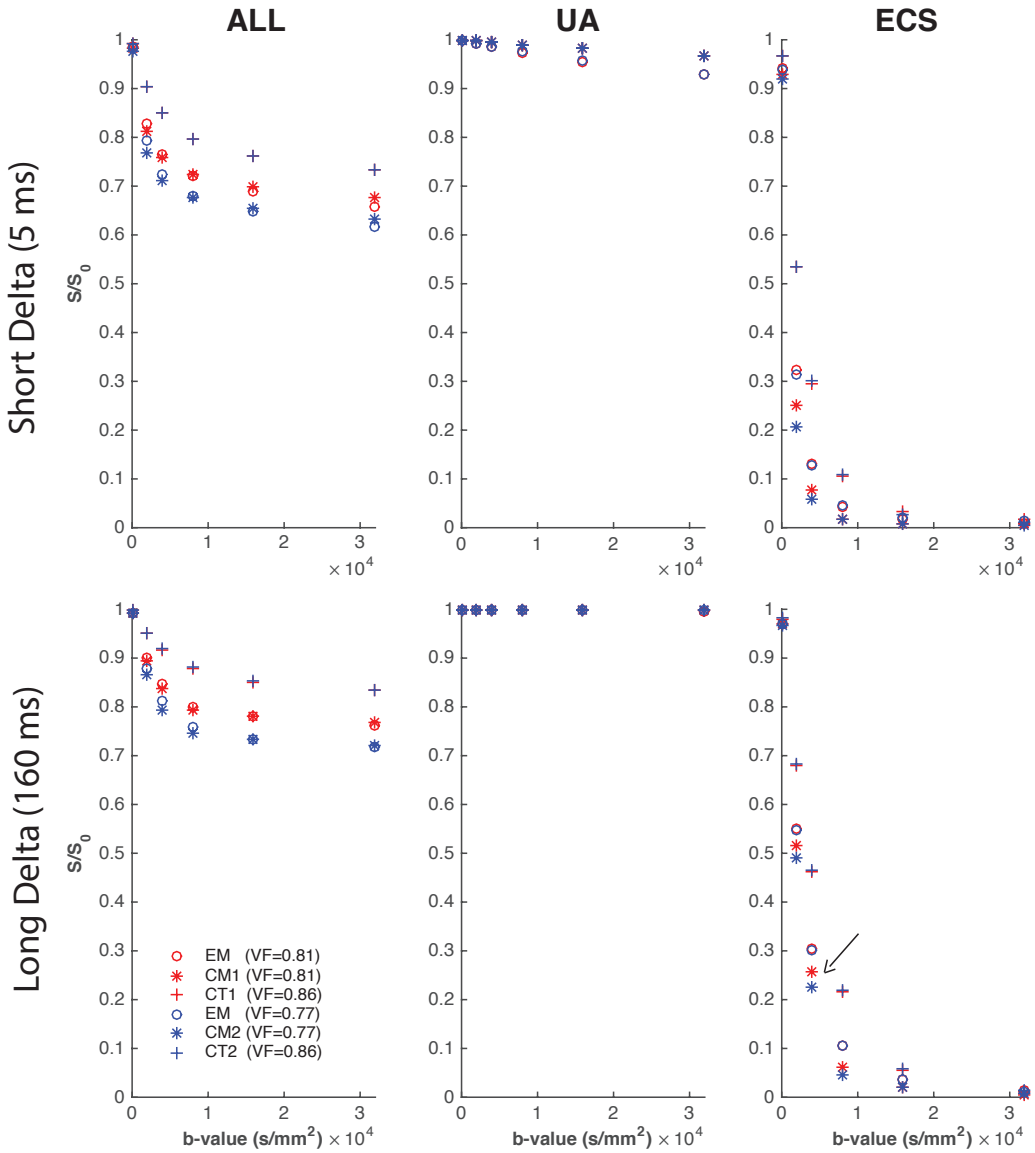

Figure 2.

Simulated signals vs b-value averaged over perpendicular directions for EM and cylindrical substrates. Aggregate (ALL) and UA / ECS compartments signals are shown for long and short diffusion time. Red / blue traces are for EM substrates with different packing densities; symbols indicate the EM (o), CM (*) and CT (+) substrates.

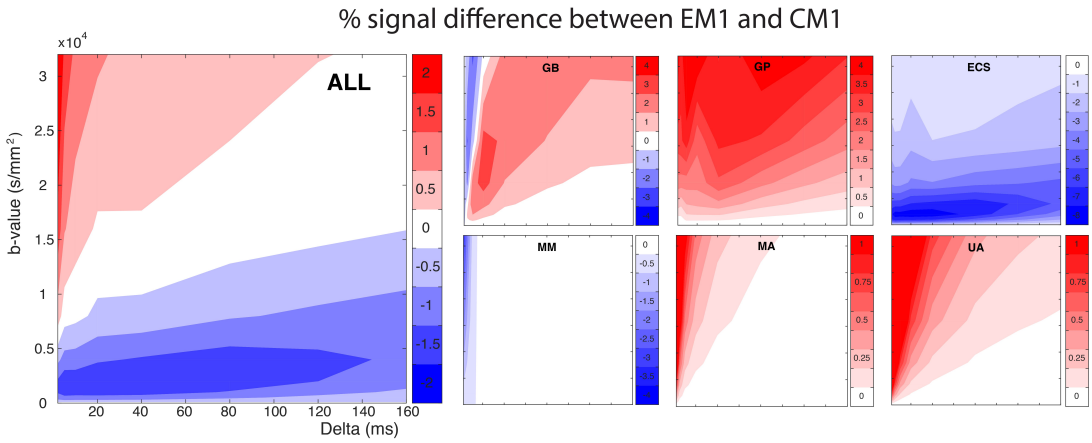

Figure 3.

Signal difference between shaped (EM1) and matched cylinder (CM1) model for the aggregate signal and the signal from the six compartments.

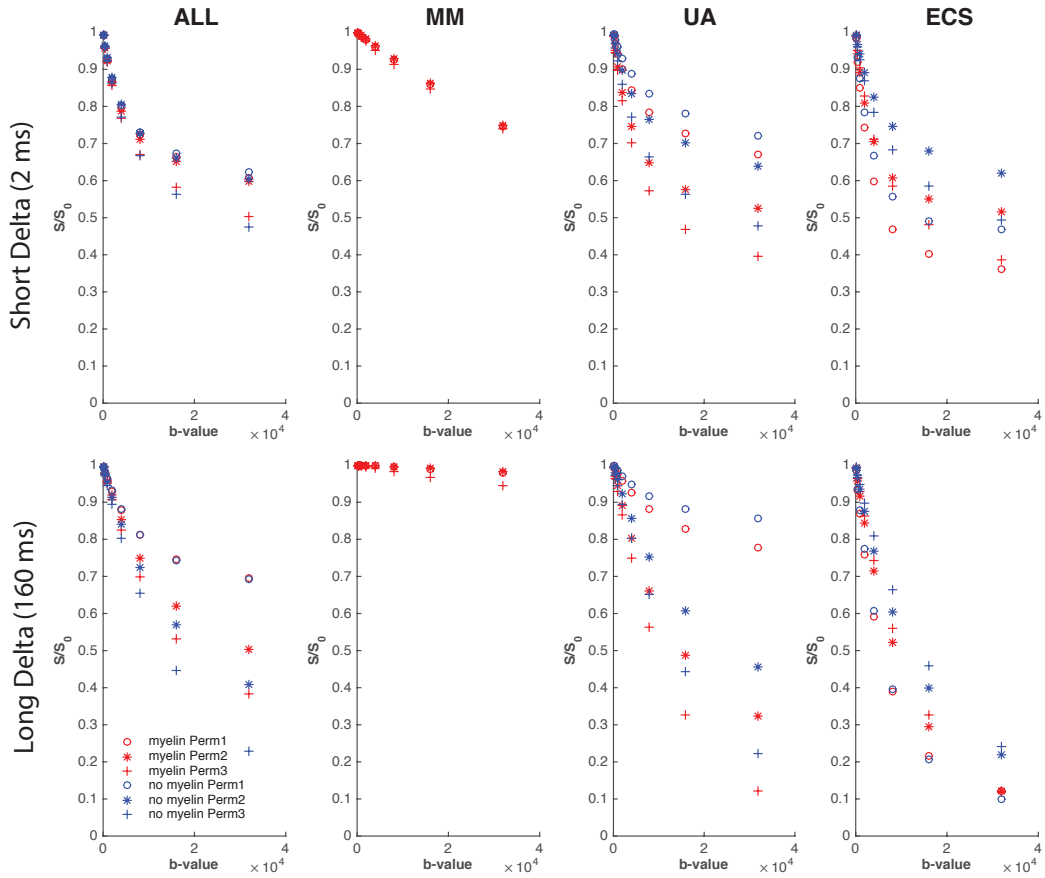

Figure 4.

Signal behaviour for diffusion in substrate EM1 with myelin (red) and without myelin (blue). Different symbols indicate various permeabilities: Transition probabilities of [1e-5,1e-4,1e-3] for UA to ECS with the other probabilities adapted accordingly. Exchange into the myelin sheath was chosen two orders of magnitude lower than for unmyelinated axons.