

# The effect of diffusion in the extracellular space on double-PFG measurements of axon size: Insights from Monte-Carlo simulations

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**Introduction** Multiple pulsed field gradient (PFG) measurements can be employed to probe restricted diffusion of water molecules within the tissue. The double pulsed field gradient (double-PFG) technique employs two pairs of diffusion sensitizing gradients, and is simple enough to be included in a standard imaging sequence. Such techniques have attracted considerable interest in recent years due to their potential to enable the estimation of microstructural parameters such as cell size and shape. Accurately estimating the microstructural parameters demands models for extra- as well as intra-cellular spaces. Although there are analytical models [1,2] for diffusion within simple pore shapes, no such model exists for the extra-cellular medium. Therefore, bicompartamental models [3] have typically assumed diffusion in the interstitial medium to be Gaussian. Here, we present Monte-Carlo simulations of diffusion within hexagonally packed arrays of cylinders mimicking the white-matter, and report the signal behavior for double-PFG experiments with zero mixing times (see Figure 1). We investigate the accuracy of the axonal diameter estimates when a bicompartamental model that assumes Gaussian diffusion outside the cells is employed.

**Simulations** We used the recently developed DiffSim simulation environment [4] to compute the signal intensities associated with the pulse sequence depicted in Figure 1 for the geometry shown in Figure 2. Periodic boundary conditions were imposed at the boundaries of the box. The geometry was specified by the inner diameter (ID) and spacing (s). In the simulations we took ID=8  $\mu\text{m}$ , and investigated the signal response for s=0, 2, 4, 6, and 8  $\mu\text{m}$ . The diffusion coefficient was taken to be  $D_0=2.0 \times 10^{-3} \text{ mm}^2/\text{s}$ . Simulated experimental protocol involved the following timing parameters:  $\Delta = 40 \text{ ms}$ ,  $\delta = 2 \text{ ms}$ . We ran simulations with 7 values of  $q$  ranging from 15 to 105  $\text{mm}^{-1}$ , and 12 values of the angle between the two gradients of the double-PFG sequence ranging from  $0^\circ$  to  $330^\circ$ .

**Model Fitting** The bicompartamental model used to fit to the simulated data is given by the expression  $E(q) = f_{\text{free}}E_{\text{free}}(q) + f_{\text{rest}}E_{\text{rest}}(q)$ , where  $f_{\text{free}}=S_{\text{free}}/(S_{\text{free}} + S_{\text{rest}})$  and  $f_{\text{rest}}=S_{\text{rest}}/(S_{\text{free}} + S_{\text{rest}})$  satisfy the relationship  $f_{\text{free}}+f_{\text{rest}}=1$ . Here,  $S_{\text{free}}$  and  $S_{\text{rest}}$  are, respectively, the free and restricted diffusion contributions to the signal at  $q=0$ . The component of the signal due to restricted diffusion was computed using the generalized framework [2], which is an extension of the multiple correlation function (MCF) method [5] to arbitrary angular variations between the gradients. The Gaussian (free) diffusion component was estimated using the approach in [6], which is consistent with the MCF method.

**Results and Discussion** Figure 3 illustrates the simulation results for the cases  $s=0$  and  $s=2 \mu\text{m}$ . Each line includes seven angular modulations, each of which corresponds to a particular  $q$ -value. Clearly, for tightly-packed geometries and at larger  $q$ -values, diffusion in the extracellular space leads to angular variations similar to those for intracellular space. However, those effects quickly disappear as the spacing is increased. In Figure 4, we show the fitting results for the tightly-packed geometry. The model accurately describes the simulated values for all spacings. In fact, the fits are more satisfactory at larger values of  $s$ . The parameters estimated from the fit are tabulated in Table 1. The ground truth values are: ID=8  $\mu\text{m}$ ,  $D_0 = 2.0 \times 10^{-3} \text{ mm}^2/\text{s}$ ,  $S_0 = 1$ , and  $f_{\text{rest}}=\{0.91, 0.58, 0.40, 0.30, 0.23\}$ , for increasing values of  $s$ . It is clear that the estimated  $f_{\text{rest}}$  and ID values are very accurate. For the tightly-packed configuration, diffusion in the interstitial space is also restricted. Hence, the estimated ID represents a weighted average of two pore sizes, which explains why the estimate is about 1  $\mu\text{m}$  below the ground truth ID value.  $D_0$  values are substantially lower than its ground truth value for all geometries presumably due to tortuosity of the extracellular space, and weak dependence of the signal on  $D_0$  in the intracellular space.

**Conclusion** We investigated the effects of diffusion in the extracellular space for a periodic array of cylinders featuring hexagonal packing on the double-PFG measurements of cell size. A bicompartamental model that assumes a Gaussian compartment for the extracellular space yields accurate estimates of the cylinder diameter when there is some spacing between the compartments. For tightly-packed geometries, some underestimation of ID should be expected. Despite the substantially underestimated bulk diffusivity values, volume fraction estimates are also adequately captured by the bicompartamental model.

**References** [1] Ozarslan & Basser., J. Chem. Phys. 128:(2008), [2] Ozarslan, et.al., J. Chem. Phys. 130:(2009), [3] Shemesh et al., J. Magn. Reson. 200:(2009), [4] Balls & Frank, Magn Reson Med 62:(2009), [5] Grebenkov, Rev. Mod. Phys 79: (2007), [6] Ozarslan & Mareci, Proc. Int'l. Soc. Mag. Reson. Med. 21:(2013).

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Table 1: Estimates obtained by fitting the bicompartamental model to simulated data for different geometries.

s	0 $\mu\text{m}$	2 $\mu\text{m}$	4 $\mu\text{m}$	6 $\mu\text{m}$	8 $\mu\text{m}$
ID ( $\mu\text{m}$ )	$6.9959790 \pm 0.068141665$	$7.9431597 \pm 0.0076097005$	$7.9409514 \pm 0.011278582$	$8.0199007 \pm 0.015961476$	$8.1353940 \pm 0.028460198$
$S_0$	$1.0026757 \pm 0.022945414$	$0.99962715 \pm 0.001855296$	$0.99561708 \pm 0.0018942293$	$0.99540504 \pm 0.0020016274$	$0.99774205 \pm 0.0027797727$
$f_{\text{rest}}$	$0.92729421 \pm 0.025188815$	$0.59244848 \pm 0.0016634922$	$0.41437376 \pm 0.0015224839$	$0.31316371 \pm 0.0015114065$	$0.24589385 \pm 0.0020119623$
$f_{\text{free}}$	$0.072705785 \pm 0.018500860$	$0.40755152 \pm 0.0015680239$	$0.58562624 \pm 0.0017789801$	$0.68683629 \pm 0.0020157719$	$0.75410615 \pm 0.0029321724$
$D_0 (10^{-3} \text{ mm}^2/\text{s})$	$0.32189651 \pm 0.035700160$	$0.47747504 \pm 0.0024328616$	$0.53624581 \pm 0.0022570055$	$0.58458848 \pm 0.0023715479$	$0.62904913 \pm 0.0033625050$
$s=0 \mu\text{m}$					

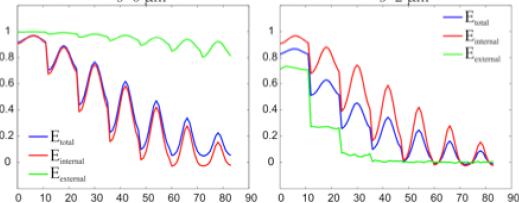


Fig 3. Simulated signal values plotted against the experiment index for tightly packed cylinders (left) and with spacing  $s=2 \mu\text{m}$  (right). Each curve comprises 84 points corresponding to 7  $q$ -values and 12 angles for each  $q$ -value.

Fig 4. The lines depict the model curves fitted to the simulated data points (symbols) for the case  $s=0 \mu\text{m}$ .

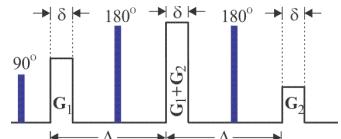
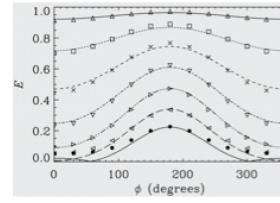


Fig 1. The double-PFG pulse sequence with zero mixing time.

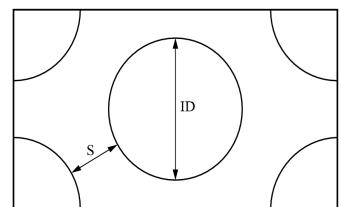


Fig 2. Hexagonally packed array of cylinders. Inner diameter (ID), distance between cylinder boundaries (S).