First experimental observation of both microscopic anisotropy (uA) and compartment shape anisotropy (CSA) in randomly oriented biological cells using double-PFG NMR

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Introduction. Single-pulsed-field-gradient (s-PFG) methods have been widely employed in diffusion NMR and MRI to characterize microstructures in the central-nervous-system. Notably, diffusion tensor imaging (DTI) provides a means to quantify the orientation of coherently packed anisotropic compartments such as white matter fascicles[1]; the q-space approach offers a means to quantify the relative sizes of such structures[2]. However, DTI does not provide microstructural information about anisotropic compartments that are randomly oriented; q-space approaches necessitate extremely strong gradients to characterize only relative sizes. These inherent limitations of s-PFG limit its usefulness in, *inter-alia*, grey matter structures which are randomly oriented. Moreover, these methods do not provide a signature for compartment shape. For example, randomly oriented ellipsoids or cylinders specimens produce the same qualitative signal decay in s-PFG, as do spherical compartments.

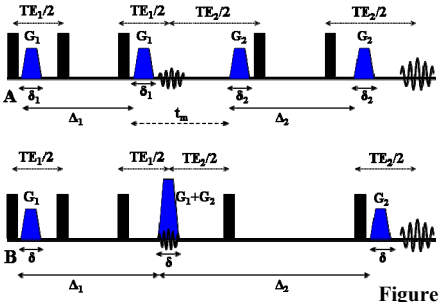

Figure 1

Double-PFG (d-PFG) is emerging as a new powerful tool for studying restricted diffusion, especially where s-PFG is inherently limited. The d-PFG, first proposed by Cory et al. in 1990[3] is an extension of s-PFG, and employs two gradient pairs $\mathbf{G_1}$ and $\mathbf{G_2}$ which are separated by a mixing time (t_m) (Fig. 1A). Another variant of d-PFG was recently introduced, in which the middle gradients are superimposed, so that t_m=0ms, a desirable property for some applications (Fig 1B). The angular d-PFG experiment, in which the angle ψ between the gradients applied in each block is varied at a defined q-value, results in characteristic curves from which the size of the compartment could be extracted, even at low q-values[4,5], thus obviating the use of high q-values required in s-PFG MR. Recent theoretical studies provided a general framework for characterizing restricted diffusion in multiple-PFG, and especially d-PFG experiments[6], from which very accurate compartmental dimensions were extracted on samples in which the ground-truth is know *a-priori*[7,8]. One striking feature of the new theoretical work[9] is that it predicts that the d-PFG angular experiment conducted on randomly oriented compartments is capable of distinguishing between microscopic anisotropy (μA) (that arises from the boundaries of the restricting compartment) and compartment shape anisotropy (CSA) (that arises from the shape of the compartment). This theory offers a powerful means to obtain microstructural information (namely compartment shape and size) that is not available from current methodologies.

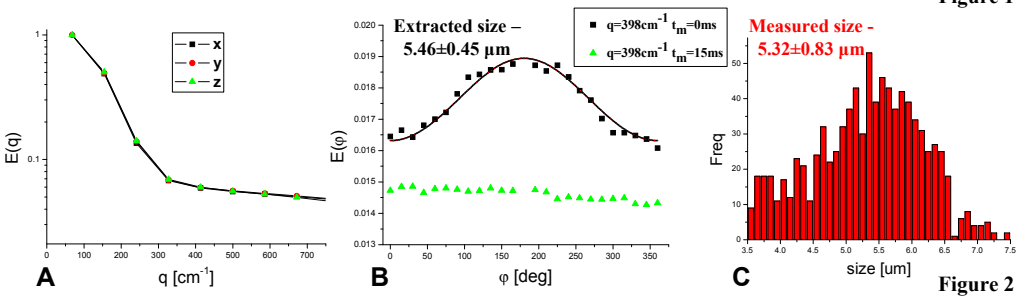

Figure 2

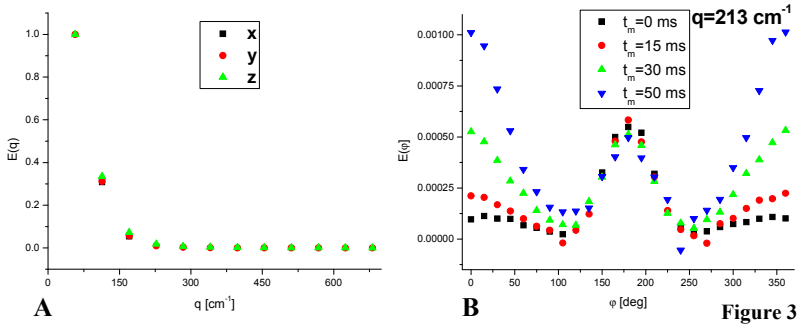

Figure 3

Objectives. Here, we show that: (1) using this new theory, d-PFG experiments can yield accurate sizes of randomly oriented biological compartments at relatively low q-values and (2) the d-PFG signal decay for various mixing times can be used to differentiate between different compartment shapes.

Methods. The angular d-PFG experiment, described extensively in [7], was performed on fixed Baker's yeast cells (*S. cerevisiae*), on cyanobacteria (*Fischerella*) and on fixed human and rat mesenchymal stem cells (hMSCs and rMSCs respectively). The angular experiment was performed using the d-PGSTE sequences shown in Fig 1: for the yeast specimen and cyanobacteria, the following parameters were used: $\Delta_1=\Delta_2$=250 ms and $\Delta_1=\Delta_2$=150 ms respectively and for both specimens $\delta_1=\delta_2=\delta_3$=2ms with varying mixing times. s-PGSTE experiments were performed to compare the microstructural information that can be obtained using the two methods with the following parameters: Δ/δ=250/2ms and 150/2ms for yeast and cyanobacteria specimens respectively. Light microscopy was then performed on the specimens, and the characteristic shapes and sizes were determined.

Results. Light microscopy revealed that the yeast cells are almost completely spherical, cyanobacteria are elongated and hMSCs are amorphous. Figure 2 shows results from fixed yeast cells. At low q-values, the s-PFG does not indicate the size and shape of the cells, yielding an isotropic signal decay (Figure 2A). The angular d-PFG experiment (black squares) at t_m=0ms is shown in Figure 2B. The expected bell-shaped function can be clearly seen, and the fit to the theory (solid black line) produces a compartment size of 5.46±0.45 μm, in excellent agreement with the mean size obtained from quantifying light microscopy images– 5.32±0.83 μm (Figure 2C shows the size distribution of yeast cells from which the mean size was quantified). Moreover, the loss of angular dependence at longer t_m (Figure 2B, green symbols) implies that the yeast are spherical, which is consistent with the microscopy results. Figure 3 shows the experimental results from Fischerella cyanobacteria, which are locally anisotropic. Figure 3A shows the s-PFG, which yields an isotropic signal decay providing no information about the eccentricity of the cyanobacteria. However, inspecting the d-PFG angular experiment data at various t_m (Figure 3B) clearly shows that the signal is not flat, but that the characteristic curve of compartment shape anisotropy is obtained. Therefore one can infer that the cyanobacteria are not spherical. Similar results were obtained for both human and rat fixed mesenchymal stem cells (data not shown), demonstrating that the angular dependence can be obtained for fixed mammalian cells as well.

Conclusions. This study demonstrates that d-PFG provides novel microstructural information in randomly oriented biological cell systems. We demonstrated that the cell size can be measured to within the accuracy of 0.2 μm in yeast, and moreover, the mixing time dependence reveals microstructural information about compartment shape; such results can even be obtained on mammalian cells which do not have a cell wall. Future studies will focus on d-PFG imaging and its ability to provide new contrasts in grey matter and in pathological conditions.

References. [1] Basser PJ and Jones DK. NMR Biomed. **15** (2002) 456. [2] Cohen Y and Assaf Y. NMR Biomed. **15** (2002) 516. [3] D. G. Cory, et al., Polym. Preprints. **31** (1990) 149. [4] P. P. Mitra, Phys. Rev. B **51** (1995) 15074. [5] E. Özarslan and P. J. Basser, J. Chem. Phys. **128** (2008) 154511. [6] E. Özarslan, et al., J. Chem. Phys. **130** (2009) 104702. [7] N. Shemesh et al., J. Magn. Reson. **198** (2009) 15. [8] N. Shemesh et al., J. Magn. Reson. **200** (2009) 214. [9] E. Özarslan, J. Magn. Reson. **199** (2009) 56.