Estimation of Cell Size Using the Composite Hindered and Restricted Model of Diffusion

Y. Sagi[1], P. J. Basser[2], and Y. Assaf[1]

[1]Department of Neurobiology, Tel Aviv University, Tel Aviv, Israel, [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, The National Institutes of Health, Bethesda, MD, United States

Introduction

Diffusion imaging has become a tool for microstructural assessment of neuronal tissue, ranging from characterizing aligned neuronal fibers via diffusion tensor imaging (DTI) (1,2) to fine delineation of axonal density (with the composite hindered and restricted model of diffusion, CHARMED) (3,4), to measurement of the axon diameter distribution (with AxCaliber) (5) and local gray matter anisotropy (with the double pulsed gradient spin echo framework) (6). In the late 1980s, it was suggested that using the q-space approach one can extract the dimension of pores as Cory and Garroway have shown on yeast cells (7). The main problem in defining a framework that will measure neuronal cell size is that the exchange rate between extra- and intra-cellular matrices in unknown. The AxCaliber framework assumes that the exchange rate between intra- and extra-axonal spaces is slow compared with the measured diffusion time range. It is hypothesized that by acquiring a wide range of diffusion times, one will be able to probe restricted diffusion within cells. In this work we extended the CHARMED/AxCaliber framework to estimate cell size in samples of cell cultures with different dimensions.

Methods and theory

PC12 and COS7 cell cultures were prepared. The two cultures vary significantly in mean cell size (Figure 1). PC12 cells have a diameter of ~10-12 microns, while COS7 cells have an average diameter of ~20-25 microns. The cells were centrifuged, suspended and placed in an Eppendorf tube.

MRI was performed on a 7T MRI scanner (Bruker, Germany). The acquisition included a series of diffusion weighted stimulated echo (DW-STE) images with the following parameters: TR/TE=3000/17ms, δ=4.5ms, Δ varied from 12ms to 100ms, G was incremented linearly from 0 to 200 mT/m in equal steps to reach a maximal b value of about 5000 s/mm2. For each gradient amplitude, 15 gradient directions were acquired, resulting in a multi-shell acquisition as in the CHARMED framework (4). The total experimental time was about 1 hour for all diffusion times.

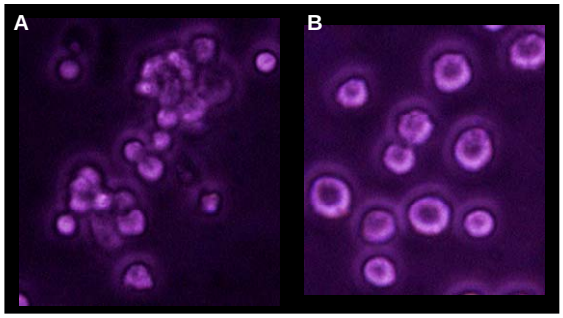

Figure 1: Light microscope of (A) PC12 cells and (B) COS 7 cells. The magnification is x20.

The CHARMED framework was modified to account for restricted diffusion within spherical cells. This can be done by modeling the restricted diffusion component in the cell culture system using an expression given by Callaghan for diffusion within impermeable spheres (8). To reduce computation complexity we did not include intra- and extra-cellular exchange or the distribution of cell sizes as in AxCaliber (since the cultured cells are homogeneous in size).

Results and discussion

The diffusion signal decay of the cell samples deviated from Gaussian behavior (i.e. appears multi-exponential) at b value of approximately ~2000 s/mm2 indicating multi-compartmentation or restricted diffusion. The large extra-cellular matrix that exhibits fast diffusion could be one source for compartmentation; however, one can not overrule restricted diffusion within the cells as a potential contribution to the multi-exponential signal decay. This signal decay at different diffusion times reveals that the slow diffusing component remains constant indicating the existence of restricted diffusion. This is in agreement with previous works utilizing the q-space approach for estimation of cell size dimension in cell cultures (7).

CHARMED analysis of diffusion signal decay at the different b values, gradient directions and diffusion times produces an estimate of the mean cell size of the PC12 as 11.9±2.0 microns and of the COS7 as 18.6±4.1 microns (at diffusion time of 100ms). These cell sizes are in good agreement with the real cell size as known from the literature and as shown under the microscope (Figure 1). The volume fractions of the cellular compartment were about 20% which is relatively low and can be attributed to the large extra-cellular matrix, exchange effects and T1/T2 weighting of the compartments signal.

Conclusions

This work shows that the CHARMED and AxCaliber frameworks, that were developed previously to infer microstrucutral features of white matter, can be adjusted to cope with a cellular spherical environment. The results presented here show that the methodology enables accurate estimation of cell size and other microstrucutral parameters at least for the investigated samples. Implementing this methodology for cell size analysis in the cortex might enable extraction of new microstrucutral features such as variation in cell size along the cortex and local cell size distribution. However, it might be that in order to provide accurate information on these parameters in the cortex *in vivo*, additional factors should be incorporated into the model such as exchange and partial volume effects from other tissues (CSF and white matter).

References:

1. Basser PJ, Mattiello J, Le Bihan D. Proc Intl Soc Magn Reson Med 1992;11:1222.
2. Basser PJ, Mattiello J, LeBihan D. Biophys J 1994;66(1):259-267.
3. Assaf Y, Freidlin RZ, Rohde GK, Basser PJ. Magn Reson Med 2004;52(5):965-978.
4. Assaf Y, Basser PJ. Neuroimage 2005;27(1):48-58.
5. Assaf Y, Blumenfeld-Katzir T, Yovel Y, Basser PJ. Magn Reson Med 2008;59(6):1347-1354.
6. Komlosh ME, Horkay F, Freidlin RZ, Nevo U, Assaf Y, Basser PJ. J Magn Reson 2007;189(1):38-45.
7. Cory DG, Garroway AN. Magnetic Resonance in Medicine 1990;14(3):435-444.
8. Codd SL, Callaghan PT. J Magn Reson 1999;137(2):358-372.