Quantification of mean cell size and intracellular volume fraction using temporal diffusion spectroscopy

Xiaoyu Jiang[1], Hua Li[1], Ping Zhao[1], Jingping Xie[1], John C. Gore[1], and Junzhong Xu[1]
[1]*Institute of Imaging Science, vanderbilt university, nashville, Tennessee, United States*

Target audience: Investigators interested in diffusion-weighted MRI and its application in cancer.

Purpose: A new approach has been developed to accurately quantify mean cell sizes and intracellular volume fractions using temporal diffusion spectroscopy with diffusion-weighted acquisitions over a broad range of diffusion times.

Theory and Methods:

Theory: Temporal diffusion spectra may be used to characterize tissue microstructure by measuring the effects of restrictions over a range of diffusion times. The conventional pulse gradient spin echo (PGSE) acquisitions use relatively long diffusion times to sample the low frequency region of temporal diffusion spectra, and hence is capable of measuring relatively long length scale (e.g. large cell sizes) although their ability to detect small length scales (e.g. intracellular structure) is limited[1]. By contrast, oscillating gradient spin echo (OGSE) methods provide a means to probe variations in tissues at cellular and subcellular scales, but their ability to accurately measure larger cell sizes (e.g. than 10 μm) is limited because of the relatively short diffusion times used[2]. By combining OGSE measurements with PGSE measurements of a single, long diffusion time, a broad range of diffusion times (~ 3 – 52 ms) can be achieved, and hence mean cell sizes (up to 20 μm) and intracellular volume fractions can be accurately quantified using a simple two compartment model (incorporating intra- and extracellular spaces).

in vitro cell culture experiments: Two different types of leukemia cells, K562 (human) and MEL (murine), were cultured to form cell pellets for NMR experiments. Three different cell densities, i.e. high, medium and low, were prepared for each cell type. By such a means, the influence of cell density on cell size fitting was investigated.

in vivo mouse xenograft experiments:MDA-MBA-231 (a human breast cancer) cells were injected to the hind limbs of female nude mice to form xenografts. Multiple-slice diffusion MRI acquisitions were performed to cover the whole xenograft with the slice thickness = 1 mm. [can add some experimental details here if word count is limited]

Data analysis: The diffusion MR signals were modeled as the sum of signals arising from intra- and extra-cellular spaces without water exchange between different compartments. The intracellular signals were described using previously published analytical expressions for water diffusion in perfectly impermeable spheres, while the extracellular signals were determined by a varying extracellular diffusion coefficient, which is linearly dependent on diffusion gradient frequency[2].

Results: Figure 1 shows the fitted mean cell sizes and intracellular volume fractions of cell culture samples using a combination of OGSE and PGSE measurements. The mean cell sizes for K562 and MEL cells are close to the microscope-derived diameters (20.94±1.08 and 11.74±1.30 μm for K562 and MEL, respectively). Note that fitted values were insensitive to variations in cell density ($p>0.05$), indicating this approach can accurately measure cell size independent of varying cell densities in practice. Moreover, the fitted intracellular volume fractions for cell samples of high density are significantly higher than that of low-density samples ($p=0.02$ and 0.03 for K562 and MEL, respectively). Figure 2 shows parametric images of mean cell size (top) and intracellular volume fraction (bottom) for three contiguous slices from a MDA-MBA-231 tumor in a representative mouse, which displays significant structural inhomogeneity within the tumor. The center region in the tumor has a small mean cell size and low intracellular volume faction, suggesting that this region is possibly in the process of forming a necrotic core. Efforts to verify the *in vivo* findings by histology are ongoing.

Conclusion: Combinations of OGSE and PGSE acquisitions can sample a larger region of temporal diffusion spectra over a broader range of diffusion times than any single method on its own, and hence allow an accurate quantification of intracellular volume fractions and relatively large mean cell sizes non-invasively.

References: 1. Gore JC, et al. NMR Biomed 2010;23:745–756. 2. Xu J, et al. Neuroimage 2014;103:10–19.

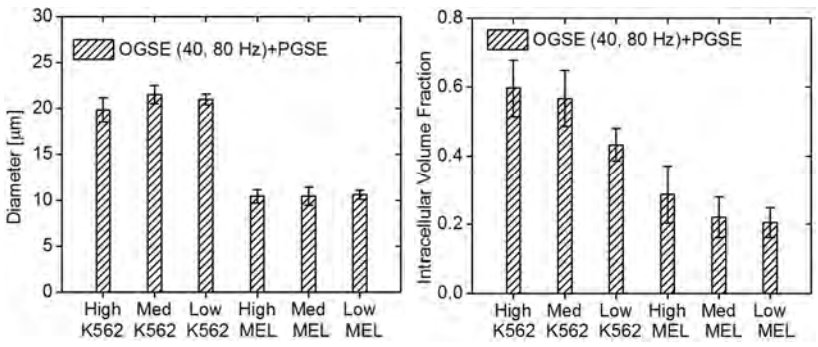

Figure 1. Fitted mean cell sizes (left) and intracellular volume fractions (right) at three different densities for two different types of cells (K562 and MEL).

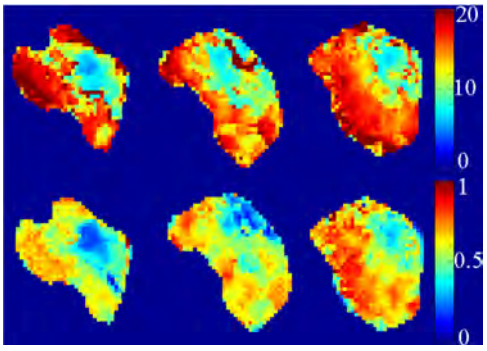

Figure 2. Maps of mean cell size (top) and intracellular volume fraction (bottom) for three contiguous slices from a MDA-MBA-231 tumor in a representative mouse.