Measurements of Mean Nuclear and Cell Sizes Using Ultra-Short Diffusion Times

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Introduction:
Tumor cell nuclear size is a diagnostic indicator of tumor malignancy (1, 2). A larger cell nuclear size usually corresponds to a more aggressive (high grade) tumor (3), and so measurements of nuclear size are of interest for clinical cancer diagnosis. However, at present tumor cell nuclear size can be found only by invasive biopsy. Diffusion-weighted magnetic resonance imaging (DWI) provides a unique means to obtain quantitative micro-structural information about biological tissues non-invasively, and it may have the potential to detect tumor cell nuclear size *in vivo*. However, due to the limitations of gradient strength and slew rate of normal gradient systems, conventional pulsed gradient spin echo (PGSE) methods employ relatively long diffusion times and thereby are insensitive to intracellular structures, such as nuclear size (4). In the present work, an oscillating gradient spin echo (OGSE) method has been applied to probe ultra-short diffusion times as low as ~0.13ms, corresponding to characteristic diffusion lengths ~0.7μm, which is much shorter than nuclear dimensions. In this work, simulations and experiments were performed to show how this approach may be used to measure mean cell and nuclear sizes.

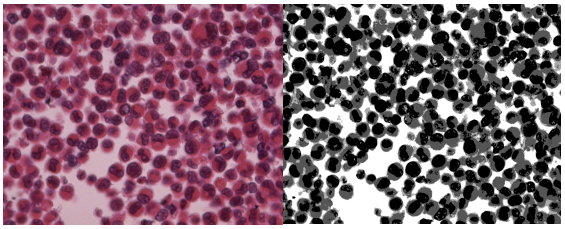
Methods:
Modeling: Tissues were modeled as highly packed spherical cells with centric spherical nuclei. There are three distinct diffusion compartments: nuclear, cytoplasmic and extracellular space. The computer simulation predicts that, when the diffusion time is ultra-short (<1ms), the influence of water exchange between different compartments on MR signals is negligible (<2%) (5). Hence, the total signals can simply be expressed as the sum of signals arising from each compartment, namely

$$E = f_{nuc}\exp(-\beta_{nuc}) + f_{cyto}\exp(-\beta_{cyto}) + (1 - f_{nuc} - f_{cyto})\exp(-bD_{ex}) \quad [1]$$

The analytical expressions which describe how the OGSE signals depend on specific structures have been derived in a previous study (5). Micro-structural parameters, including nuclear size and cell size, can be obtained by fitting the experimental signals to Eq.[1].

Diffusion experiments: Packed HL-60 cell samples were pelleted and centrifuged into ~10million cells pellets and studied using a 7.0-T, 16-cm bore Varian INOVA spectrometer (Varian Inc. Palo Alto, CA) equipped with a Doty PFG/diffusion z-gradient coil (Doty Scientific Inc. Columbia, SC) with strength up to 1500G/cm. A cosine-modulated OGSE non-imaging pulse sequence was used with three frequencies (500Hz, 750Hz, 1kHz) and eleven b values evenly ranging from zero to 1000s/mm^2. TR=3s, TE=64ms and each gradient duration=20ms.

Cell analysis: Cells were resuspended with PBS and fixed with 4% paraldehyde after MR scanning. The fixed cells were then encapsulated in Histogel, and cut into 10μm thickness slices. Each slide was then stained with H&E and photographed using light microscopy (see Fig.1a). The obtained digital images were then analyzed by using NIH ImageJ and a 3-compartment segmentation (Fig.1b). The touching cells were separated by the watershed algorithm. The mean nuclear and cell sizes were then calculated from the averaged area of all sections excluding the edge cells.



(a) (b)

Fig.1 (a) H&E stained cell slice. (b) Segmented image. (black: nuclei, gray: cytoplasm and white: extracellular space)

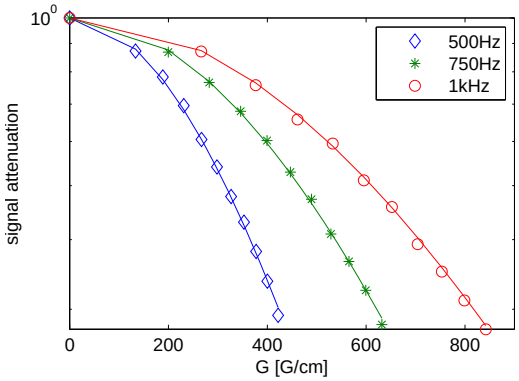


Fig.2 Experimental (markers) and fitted (lines) signal attenuation as a function of diffusion gradient amplitudes and frequencies.

Results:
The fitted mean nuclear size and cell size from diffusion measurements are 7.52±2.16μm and 11.18±0.02 μm (errors represent 95% confidence interval from non-linear least square fitting). These values are close to the values obtained from the light microscopy data, 8.37±3.91μm and 10.22±2.44μm (mean ± standard deviation averaging over 215 nuclei and 249 cells). For reference, the mean HL-60 cell size was reported to be 11.0μm in a previous study using light microscopy (6).

Discussion and Conclusion:
DWI measurements with conventional PGSE methods have previously been successfully performed to estimate axon size distribution (7) and cell size (8). Those measurements usually assume slow water exchange between different compartments. However, conventional PGSE diffusion measurements in cells are relatively insensitive to intracellular structures (4). In the present work, a novel approach has been developed to measure nuclear size with ultra-short diffusion times, in which the influence of water exchange across the highly permeable nuclear envelope is negligible. The results obtained from OGSE diffusion measurements are consistent with light microscopy, proving the feasibility of our method. This new approach provides structural parameters which may be helpful for the assessment of tumor malignancy, tracking intracellular changes in tissues, and potentially monitoring tumor response to treatment *in vivo*.

References: (1) Arai et al. Prostate. 2001 (2) Hsu et al. Hum Pathol 2005 (3) Zink et al. Nat Rev Cancer. 2004 (4) Xu et al. Magn Reson Med 2009 (5) Xu et al. J Magn Reson 2009 (6) Matsuhisa T. Cell Biol Int. 1995 (7) Assaf et al. Magn Reson Med 2008 (8) Sagi et al. Proc Intl Soc Magn Reson Med 2009