

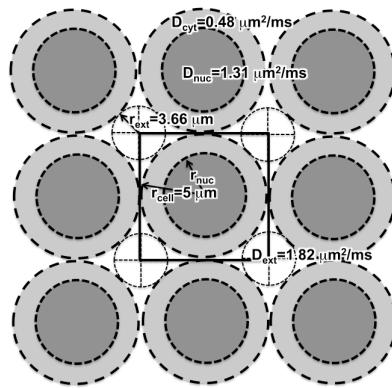
## Nucleus Size Determination in Q-Space Analysis of Three-Dimensional Cells

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**Introduction:** Diffraction patterns found in a one-dimensional q-space analysis of diffusion in a restricted geometry have offered hope of being able to infer nucleus size directly from the locations of signal minima. Nucleus size can be the sole indicator of cancer in its earliest stages. The impulse-propagator (matrix) formalism allows one to extend diffraction results with simple delta functions to realistic PGSE and OGSE sequences, but has not heretofore been applied to restricted flow in three-dimensional compartments. Here we extend previous results with one-dimensional geometries to three dimensions, so as to allow an idealized representation of nucleus, cytoplasm, and extracellular fluid in a random array of identical cells.

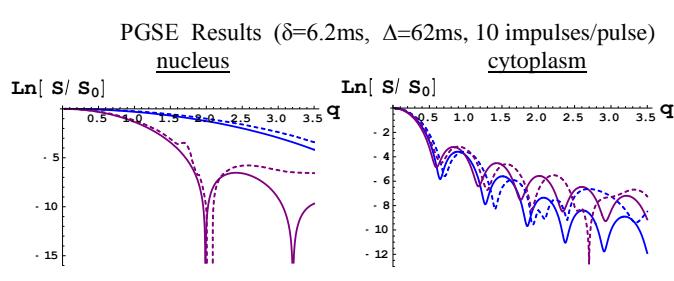
**Methods:** We consider a collection of spherical cells containing concentric spherical nuclei, with all membranes initially assumed impermeable. Our



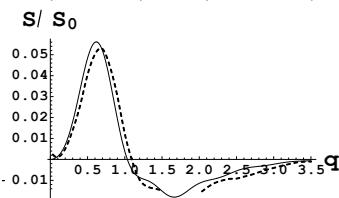
models of the extracellular region range from free diffusion to flow in a collection of spheres fitted to the interstitial spaces. The latter model gives a *worst-case* scenario in which constructive interference<sup>1</sup> from neighboring extracellular spheres would give maxima at the same q-values where destructive interference from the cells would give minima. In the impulse-propagator formalism<sup>2</sup>, the normalized signal from each compartment is approximated as arising from a series of delta-function impulses, with q-values  $q_1, q_2, \dots, q_N$ .

The normalized signal is:  $S/S_0 = S(q_1)RA(q_2)RA(q_3)\dots RA(q_N)$  where the A's are matrices expressing the effects of the individual impulses, the S's are vectors representing the first and last impulses, and R is a diagonal matrix representing diffusion between impulses. The elements of the A's, S's, and R are derived using a basis of eigenfunctions of the diffusion operator  $D\nabla^2$  with boundary conditions appropriate for the compartments. For spherical compartments, these eigenfunctions are of the form  $u_{klm}(r, \theta, \phi) = F^l(c_{kl} r) Y^l_m(\theta, \phi)$ , where  $F^l$  is a linear combination of spherical Bessel functions of the first and second kind, both of order l, the constants  $c_{kl}$  are chosen so as to satisfy the boundary conditions at the membranes, and  $Y^l_m$  is a spherical harmonic. To achieve a spatial resolution comparable to that used in the previous one-dimensional analysis<sup>2</sup>, about 1000 modes  $u_{klm}$  are needed, defining matrices of dimension 1000x1000, each element given by a three-dimensional numerical integration. (The same basis has been used in a Gaussian Phase Distribution (GPD) approximation<sup>3</sup>, giving a signal that steadily decreases with q, and not a diffraction pattern, thus requiring only O(1000) integrations.) The expensive computation is streamlined using methods that avoid redundancy, as in a Fast Fourier Transform.

In the case of PGSE, nuclear membranes are permeable on the diffusion time scale. The impermeability assumption is simply a step in the development of the method – results were verified by comparing with those of a Monte Carlo simulation for the same geometry with impermeable membranes. On the shorter diffusion time scales of OGSE, all membranes can be assumed impermeable.<sup>3</sup>



contrast in total signal:  
 $S/S_0(N/C=22\%) - S/S_0(N/C=6.2\%)$



on PGSE sequences to assess cytoplasmic geometry, but the effects of nuclear membrane permeability remain to be analyzed. Also, the irregularity of the placement of the nucleus within the cell will blur any results that rely on cytoplasm shape. Further study, especially with Monte Carlo simulations, is needed to determine the extent to which the results with regular internal cell geometry are degraded.

3) In regard to methodology, it is expected that there are many other situations in which an impulse-propagator analysis in q-space, with mild assumptions of regularity, will give exact results as a reference point to which more realistic simulations or experiments can be compared.

**References:** 1. Callaghan PT, *Principles of Nuclear Magnetic Resonance Microscopy*, Oxford Univ. Press, Oxford/NY 1991; 2. Callaghan PT, J Mag Res 129: 74-84 (1997); 3. Xu J, Does MG, Gore JC, J Mag Res 200:189-197 (2009); 4. Walters BR, Kim JK, ISMRM (2011).

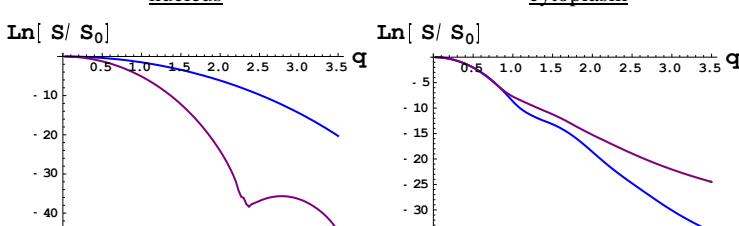
**Results:** Normalized signal vs. q (in units of  $1/R_{cell}$ ) is shown for nucleus and cytoplasm, with results displayed for  $r_{nuc}=2\mu\text{m}$  (red) and for  $r_{nuc}=3\mu\text{m}$  (purple). The contrast between the two cases is also shown. A strong diffraction pattern is observed with a PGSE sequence for both cytoplasm and nucleus. In the worst case, a diffraction pattern is absent in the total 3-compartment signal (not shown) but there is significant contrast between the two cases that can be traced to the first minimum in the cytoplasm pattern. Results compare favorably with those of corresponding Monte Carlo simulations<sup>4</sup> (dashed lines), especially for low q values. With square-wave OGSE, the shorter diffusion time scales appear to

emphasize the nuclear diffraction pattern, so that the total signal is decreased for larger nuclei, but the expected contrast is smaller in magnitude than in the PGSE case where cytoplasm dominates.

**Discussion:** 1) The difference between the OGSE and PGSE results support the common suggestion that OGSE sequences are appropriate for probing nuclear scales.

2) The maximum contrast achieved in the two cases suggests that the S/N ratio required will be reduced by a factor of 5 if we rely

**OGSE Results (f=80Hz, Δ=62ms, 3 cycles/pulse, 10 impulses/half-cycle)**



contrast in total signal:  
 $S/S_0(N/C=22\%) - S/S_0(N/C=6.2\%)$

