# Surface to Volume ratio mapping of mouse GBM using OGSE

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**TARGET AUDIENCE** – Researchers interested in quantitative metrics for brain tumors

**PURPOSE** – ADC dependence on diffusion time / oscillation frequency observed using PGSE/OGSE-DWI is an exciting microstructural contrast which remains not fully understood in tumors. The interpretation of $ADC_{PGSE}(t)$ or $ADC_{OGSE}(\omega)$ often relies on specific geometric models [1], which are often over-parametrized and rely on many assumptions. Empirical approaches have shown that the slope of $ADC_{OGSE}(\omega)$ versus frequency ω [2-3] may reflect microstructural changes or cancer treatment efficacy, but interpretation of the MR data again requires further assumptions on tumor microenvironment. In this study, we investigate whether the short diffusion times accessible with OGSE (high frequencies) belong to the Mitra regime [4-5] and can be used to interpret our measurements in GL261 mouse tumor model in terms of only two well-defined parameters: *free water diffusivity* $D_0$, and the *surface-to-volume ratio* $S/V$ of restrictions (membranes).

**METHODS** – GL261 cells ($10^6$ in 5μL) were injected into the subcortex of C57BL/6 mice (n=13, female, 6-8 week old) under anesthesia (air +3% isoflurane). In vivo DWI consisted of OGSE and PGSE measurements at 7T (Bruker Biospec, Etlingen), probing diffusion properties with 4 diffusion times = 6/8/16/31 ms for PGSE and 10 frequencies in the range of 60-225 Hz for the fast ramp cos-OGSE ($1 \le N_{OGSE} \le 5$). The SE-EPI MR parameters were: TR/TE=3000/70ms, Bandwidth 300kHz, 1 readout segment, 20 averages, NR=2, res. 250x250x1500 μm, matrix 80x80, b=[0,200,400] s/mm$^2$, dir. (1,1,1), TA=6 min. Total scan time was 84 min. The mice were scanned once between Day 14 and 28 after tumor implantation and sacrificed for histology. Linear fitting was performed on OGSE data to demonstrate the validity of the very short diffusion time regime [4,5] characterized by $D(\omega) = D_0 \times (1 - S/V \cdot c_{OGSE}(\omega)/d\sqrt{D_0/\omega})$ **(1)**, with $d$=3 and $c_{OGSE}$ being the system's dimensionality and correction coefficients for the gradient time-course [5,6]. Free diffusivity ($D_0$) and surface-to-volume ratio ($S/V$) maps were derived on a voxelwise basis and compared to conventional $ADC_{PGSE}$ obtained with Δ=31 ms. $S/V$ measurements were converted into an restriction scale indicator $L$ (in μm) based on a cubic lattice geometry: $L=6/(S/V)$.

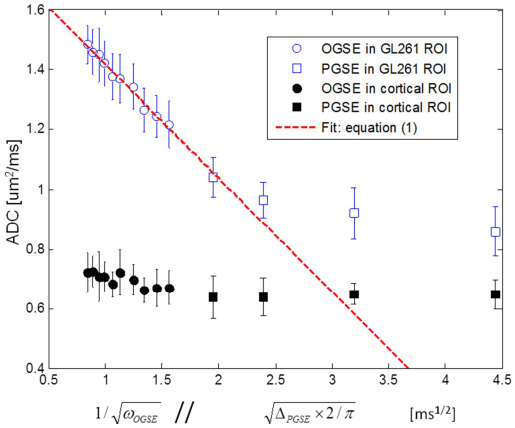


**Figure 1**. In vivo time dependence in the short time regime inside the tumor (blue) is much stronger than in normal region (black). In the tumor, $ADC_{OGSE}(\omega)$ (blue circles) decreases linearly with inverse square-root of oscillation frequency (red line, $R^2$=0.99), confirming the regime [4-5], and provides $S/V$=0.55μm$^{-1}$. Error-bars represent standard deviations over 13 animals.

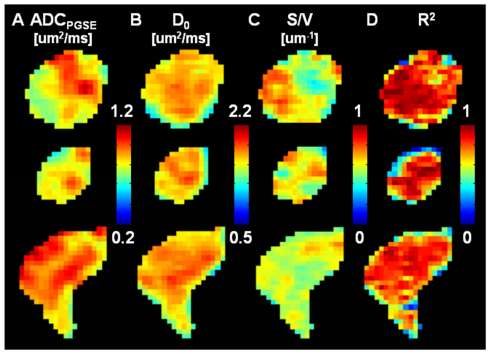


**Figure 2A.**$ADC_{PGSE}$, **B.**$D_0$, **C.**$S/V$ and **D.**$R^2$ parametric maps of three tumors. Systematic $S/V$ variations inside the tumor are stronger than those in $D_0$ and $ADC_{PGSE}$.

**RESULTS** – $ADC_{OGSE}(\omega)$ in contralateral cortical brain regions (black circles, Fig. 1) shows a relatively small residual frequency dependence, which is likely to fall into the long-time limit [7]. After correction for gradient duration in $c_{OGSE}$ ([4-14]% changes), excellent agreement is demonstrated with equation **(1)** on the average ADC in GL261 ($R^2$=0.99, see red curve, Fig. 1) and on individual tumors ($R^2$=0.93±0.04, N=13), confirming that *our OGSE measurement indeed falls into the short-time limit* and justifying the use of the theory [4-5]. This relationship did not extend to PGSE measurements beyond diffusion times Δ>6ms. Parametric maps (Fig. 2) show that the model **(1)** decouples the tissue parameters in the tumors: While the variations of the free diffusion coefficient $D_0$=1.7±0.3 μm$^2$/ms (Fig. 2B) are relatively weak (±17%) and do not exhibit anatomical features (as is perhaps expected), the relative $S/V$=0.60±0.26 μm$^{-1}$ variations are much more pronounced (±43%, Fig. 2C), and clearly demonstrate tumor heterogeneity, which may occur due to spatially varying cellularity. Indeed, $S/V$ and $ADC_{PGSE}$ are mildly negatively correlated (Spearman's $\rho$=–0.43, N=1300 voxels), with variation of $ADC_{PGSE}$=0.9±0.2 μm$^2$/ms of about ±22%. The correlation between $ADC_{PGSE}$ and $D_0$ is much less pronounced ($\rho$=0.20). The mean $S/V$ value of 0.6 μm$^{-1}$ suggests that restrictions happen on the $L$~10 μm scale (equivalent cubic lattice period), in agreement with EM data previously collected on the same cell line (12 μm average cell diameter).

**DISCUSSION AND CONCLUSION** – Our measurements coupled with the model [4-5] show that it is possible to characterize restrictions in vivo based on the membrane surface-to-volume ratio, measured using OGSE-DWI in glioblastoma in the very short diffusion time regime. This regime can be reached using oscillating gradients on preclinical systems with a commercially available gradient system (750 mT/m, rise time 100 μs), provided that the scale of restrictions is large enough, thus benefiting cancer studies. Diffusivities, nuclei/cell geometrical shapes and diameters or intracellular volume fractions differently impact $ADC_{PGSE}$ and $S/V$. Both metrics sense restrictions at different scales and can provide complementary information regarding tumor characteristics and heterogeneity, suggesting that $S/V$ [or restriction scale indicator $L=6/(S/V)$] can be used as a marker to evaluate cellular structural changes associated with treatment efficacy or tumor progression with minimal assumptions on tumor microenvironment. Future investigations is warranted to assess the potential of $S/V$ for assessment of treatment response in tumor as well as histo-pathologic validation for a better understanding of the underlying microscopic mechanisms underlying $S/V$ variation in cancer.

**REFERENCES** – [1] Gore et al., NMR in Biomed (2010). [2] Aggarwal et al., MRM (2012). [3] Xu et al., PlosOne (2012). [4] Mitra et al., Phys Rev B (1993). [5] Novikov and Kiselev, JMR (2011) [6] Sukstanskii, JMR (2013). [7] Novikov et al., PNAS (2014). This work was supported by NIH R01 CA160620.