In vivo characterisation of colorectal tumour microstructure with DW-MRI

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Target Audience: Biophysical modellers, diffusion MRI researchers, cancer imaging researchers.

Purpose This work is the first to explore the diffusion weighted (DW) MR signal of cancer cell lines with a selection of multi-compartment diffusion models, adapting a method for diffusion model identification in brain tissue[1], but which is also able to incorporate the complex structure of tumours. Characterisation of colorectal tumour microstructure could be used to assess response to treatment[2]. Most previous cancer cell classification studies involve invasive histological methods that alter the state of the sample. This study uses DW-MRI to quantitatively characterise the microstructure of colorectal tumours non-invasively.

Methods We evaluated two human tumour xenograft models that have previously been used as a system to explore differential tumour pathophysiology[3]. We constructed two- and three-compartment models of the DW signal from combinations of simple models of diffusion inside and outside cells. We defined a DW-MRI protocol that allows evaluation and comparison of all our models using in vivo data from mice bearing the cell lines. We then used the best-ranked model to suggest which features of the cellular architecture cause the differences in the raw signals.

Environment	Model	Form	Deg of Freedom
Non-Restricted	Ball	$\mathbf{D} = d\mathbf{I}$	d
Non-Restricted	zeppelin	$\mathbf{D} = \alpha\mathbf{n}\mathbf{n}^T + \beta\mathbf{I},\ d_\parallel = \alpha + \beta, d_\perp = \beta$	$d_\parallel, d_\perp, \theta, \phi$
Restricted	Sphere	GPD approx. $R > 0$	d, R
Restricted	Stick	$S = \exp(-bd(\mathbf{n}\cdot\hat{\mathbf{G}})^2)$	d, θ, ϕ

Fig.1 Compartment models. The signal for the DT models is $S=\exp(-bG^T DG)$, G=gradient direction, b=weighting factor, I=identity tensor, n=principal direction of the cell (defined by θ, φ), d, d‖=diffusivity

Tissue Models We model tumour microstructure with up to three compartments. Each compartment provides a separate normalized MR signal S_i, i=1, 2, 3. The total diffusion MR signal for a multi-compartment model is the weighted sum of the signals from each compartment, with weights summing to 1. Figure 1 shows the candidate models for each compartment. We investigate the combinations of four basic models. The first two are diffusion tensors (DT)[4] with different constraints: the 'Ball', which is isotropic, and the 'Zeppelin', which is anisotropic but cylindrically symmetric. The other two models are models of restricted diffusion: Behrens' 'Stick' model[5] which describes diffusion in an idealised cylinder with zero radius and 'Sphere' model, which has particles diffusing inside an impermeable sphere of radius R[6] using the Gaussian phase distribution approximation. All models are used in weighted combinations and have different intrinsic diffusivities. In total we consider 18 multi-compartment models.

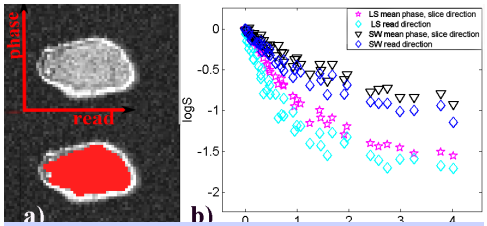

Fig.2 a) Normalised log signal of the two cell lines. b) DW image example of the LS174T with the ROI in red.

Biological Tumour Models Two human colorectal adenocarcinoma cell lines, LS174T (LS, n=3) and SW1222 (SW, n=3)[3], were injected subcutaneously in the flank of 2-month-old nude mice. These two cell lines produce tumours with differing microstructure: SW tumours are highly differentiated with ductal features that closely resemble the tissue of origin, while LS tumour cells are poorly differentiated [3]. Animals were scanned when the tumours were ~15 mm in diameter.

Table 1. BIC of the best models with their number of parameters

Models	SW1	SW2	SW3	LS1	LS2	LS3	N.Par
BallStickSphere	73.80	63.27	104.16	116.32	55.89	70.86	9
ZeppelinSphere	76.01	63.27	105.71	119.83	60.18	71.56	8
BallZeppelinSphere	79.60	68.94	105.23	119.02	64.46	71.89	10
BallSphere	80.28	70.70	119.54	120.34	53.39	64.28	5
ZeppelinStickSphere	84.04	68.72	106.11	119.74	64.28	71.80	10

MRI Acquisition DW-MR images of the six mice were acquired in vivo, using a small bore 9.4T scanner. We use the pulse-gradient spin-echo (PGSE) sequence for 46 diffusion weightings: with diffusion times Δ=10, 20, 30, 40ms, gradient durations δ=3ms for all Δ and δ=10ms for Δ=30, 40ms. Gradient strength G varied from 40 to 400mT/m in ten steps of 40mT/m. Diffusion gradients were placed along the three imaging coordinate axes. We normalise the data for T_2 dependence. We also perform a separate diffusion tensor imaging (DTI) acquisition of 42 directions with *b* value 2.2×10^9s/m² and six *b*=0 measurements. The field of view is 25x25mm, the matrix size is 64x64 and 5x0.5mm slices. The acquisition per animal was 2 hours. We manually segment a tumour region of interest (ROI) that excluded surrounding skin (Fig.2a).

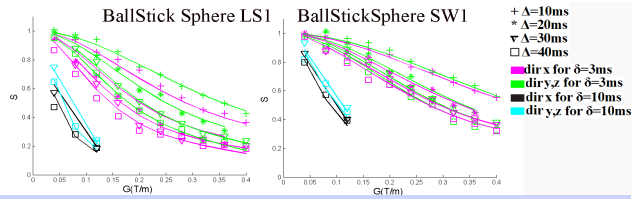

Fig.1 Synthesised data from the best model (lines) for LS, SW with the scan data

Model fitting We fit each model to the combined DW and DTI data by minimising the sum of squared errors using a Levenberg-Marquardt algorithm with the offset Gaussian noise model. We choose the best-fit parameters after 1000 perturbations of the starting point to avoid local minimum.

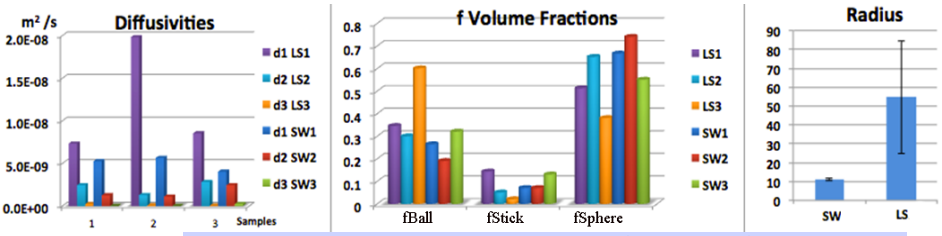

Fig.4. Charts showing the estimated model parameters for all samples from the best model

Results We averaged the data contained from all voxels within each ROI, referring to LS datasets as LS1, LS2, LS3 and SW as SW1, SW2, SW3. The raw DW signals show observable differences between the two cell lines, illustrating the sensitivity of the signal to tumour microstructure (Fig.2b). The Bayesian information criterion (BIC)[7] evaluates the models accounting for varying complexity. Table 1 presents the best-ranked models for all cell lines with their BIC score. The ranking exhibits only small variations between datasets. The model that best describes both cell lines was the three-compartment BallStickSphere model (Fig.3). Figure 4 shows the parameters of the best model for both cell lines. All compartment diffusivities (d1=dBall, d2=dStick, d3=dSphere) are larger in LS than for SW. We see the same observable differences in the estimated radius, where for LS R is much larger and has a higher standard deviation than for SW. We do not see immediate differences in the volume fractions of the two cell lines.

Discussion & Conclusions The key conclusion is that we observe restriction in all tumours as all of the top models contain the Sphere. This is strong evidence for an isotropically restricted signal, most likely inside the tumour cells. Weak anisotropy is also observed, since the Stick is also favoured in the ranking, however with the lowest volume fractions. The parameter estimates appear consistent within tumour types, however we can also identify differences between the types. The parameters that can potentially characterise the two cell lines are the intrinsic diffusivities, and the radius R, that both appear much larger in the LS samples than the SW. Future work will validate these differences with more samples and histology to find a reliable model for designing economical imaging protocols for tumour characterisation.

References & Acknowledgements 1 Panagiotaki et al, NeuroImage (2012) **2** Yang et al Biomicrofluidics (2004) **3** El Emir et al, Cancer Res, (2007) **4** Basser et al, Biophys J, (1994), **5** Behrens et al, MRM, (2003), **6** Murday and Cotts, JChemPhys, (1984), **7** Schwarz, Annals Stat (1978) This work is funded by the EPSRC grant EP/E056938/1. Also thanks to King's College London, UCL Comprehensive Cancer Imaging Centre, CR-UK & EPSRC, in association with the MRC and DoH (England) (C1060/A10334), British Heart Foundation.