Viable and Fixed White Matter: DTI and Microstructural Comparisons at Physiological Temperature

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Target Audience: Researchers in diffusion weighted MRI. Specifically: microstructural modelling, tractography, and sequence development.

Abstract: *We compare fixed and viable isolated tissue (VIT) in identical conditions at physiological temperature. We acquired DTI data sets with a range of acquisition parameters and a rich multi-b-value diffusion weighted MR (DW-MR) dataset for microstructural tissue model fitting. DTI data demonstrated a significant increase in radial diffusivity (RD) in fixed samples in comparison to VIT. Model fitting demonstrated that similar models best explain data from both samples and differences in parameter estimates reflect DTI measured RD and MD differences. The stability of the model ranking allows us to conclude that fixed tissue is a reasonable model for in-vivo, although significant differences in the fitted model parameters (and DTI measured FA and RD) suggest that water in individual compartments within the tissue behaves quite differently.*

Purpose\Background: Fixed tissue samples are used for testing and validation of DW-MR methods e.g. (1,2). Fixation affects measured diffusivity in neuronal tissue (3,4). VIT mitigates several confounders of in vivo MR acquisitions e.g. movement, vascular and susceptibility effects (5). Comparing VIT with fixed tissue in identical conditions provides information on the efficacy of fixed tissue as a model for viable tissue. In this work we used a VIT maintenance system (6) (Figure 1) to investigate DW-MR detectable differences between VIT and fixed tissue at physiological temperature. DW-MR is highly sensitive to temperature differences (7), previous comparisons between viable and fixed tissue have been conducted at lower temperatures than in this work (3), where we use physiologically realistic temperatures. We acquired: (A) various DTI datasets designed to probe the dependence of DTI indices on acquisition parameters, both within and between VIT and fixed tissue; (B) a rich multi-b-value DW-MR dataset with which we fit a large range of multi-compartment tissue models to see which best explain data from both fixed and VIT samples (see (2) for full model list).

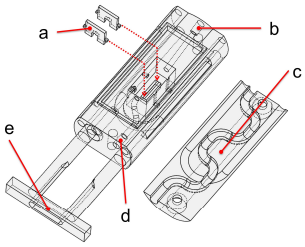

Figure 1: MRI compatible incubation chamber (exploded). Diameter 26mm. a) inserts & bench support the optic nerve, b) inflow, c) preheating system in lower section, d) outflow and e) isocenter positioning bar.

Methods: VIT: rat optic nerves (♂ Sprague Dawley) were maintained at 36.5°C and perfused with oxygenated artificial cerebral spinal fluid (aCSF). Fixed nerves were prepared by immersion in 4% formaldehyde solution (24 h) then washed in phosphate buffered saline (10 h). (A) Diffusion tensor imaging (DTI) experiments (n = 2 & 2): 30 direction pulsed-gradient spin-echo (PGSE) experiments, diffusion times (Δ): 10, 20 & 30ms, gradient durations (δ): 4, 6 & 8ms and gradient strengths (G): 32 – 71 G cm^{-2}. [Exp 1: fix Δ & δ, alter G | Exp 2: fix G, alter Δ & δ | Exp 3: fix G, maintain b-value, alter Δ] TR: 1.5s, TE: minimised. (B) Multi-b-value DW-MR dataset from VIT and fixed nerves (n = 1& 1): [Δ: 10 - 50ms (sets of 10ms) | δ: 1.5 & 3ms | G: 0.04 – 40 G cm^{-2}], 3 perpendicular & 1 parallel direction, TR: 2.0s, TE: minimised. All MR experiments were performed with a 9.4T Agilent VNMRS system. Scan durations were: 6 h (A) and 7h (B), matrix: 48 x 48, FOV: 6 x 6 x 2mm [x, y & z]. The open source Camino diffusion MRI tool kit (8) was used to fit DTI and the set of multi-compartment signal models from (2) to the DW-MR data.

Table 1: Average Fixed and VIT DTI indices

	FA				RD (cm^2/s)			
	VIT	Trend	Fixed	Trend	VIT	Trend	Fixed	Trend
Exp 1: fix Δ & δ, increase G	0.83	+ ve	0.77	+ ve	1.91E-06	- ve	2.38E-06	-ve
Exp 2: fix G, increase Δ & δ	0.87	+ ve	0.77	+ ve	1.63E-06	- ve	2.38E-06	- ve
Exp 3: fix G, increase Δ, decrease δ	0.88	none	0.79	none	1.63E-06	none	2.34E-06	none

Results: Table 1 shows DTI calculated average FA and RD in VIT and Fixed samples. RD in fixed samples was found to be significantly higher than that of VIT for all measurements ($P<0.01$, t-test). In both VIT and Fixed samples, Bayesian information criterion (BIC) ranking of fitted models was similar and selected three compartment models as the best performing (data not shown). In the terminology of (2), tensor and zeppelin models outranked balls (hindered compartment) while cylinder models outperformed sticks (restricted compartment). FA and RD from the best fitting compartment models coincide with DTI detected differences between fixed and VIT samples. Volume fractions estimated by the best fitting models were comparable.

Discussion: A temperature difference of 17ºC has been shown to reduce extracellular ADC by 25% (9), comparing samples at physiological temperature provides more accurate information on differences than previous work e.g. (4). These clear variations and differences in DW-MR parameters should be taken into account when comparing tissue states and studies using different acquisition parameters. Differences in extracellular ADC between VIT and fixed tissue have been previously demonstrated in brain slice preparations at room temperature (3), however this is the first demonstration of increased in RD in fixed white matter in comparison to VIT in identical conditions at physiological temperature. Fixation produces similar model rankings but alters their parameters, suggesting that the broad tissue structure is maintained but individual water populations behave differently.

Conclusion: When measured in identical conditions, fixed tissues DW-MR properties depart significantly from those of viable tissue; however similarity of model rankings suggests that DW-MR detectable tissue features are maintained. We conclude that while VIT has clear advantages (see (6) for discussion), fixed tissue is suitable, when used cautiously, as a basic test-bed for DW-MR development. Further work will increase DTI repeats to elucidate acquisition parameter dependant patterns in experiments 1-3 in both VIT and fixed tissue.

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