Optimisation of *ex vivo* diffusion imaging: the effects of tissue preparation and imaging parameters on data quality

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Purpose: Diffusion-weighted magnetic resonance imaging of fixed human brain tissue has the potential to reveal neuroanatomical details at a scale that remains largely unexplored and to provide a clearer understanding of diffusion indices. As opposed to *in vivo* imaging, *ex vivo* imaging has the advantage of allowing substantially longer acquisition times using MR hardware that can easily outperform clinical MR systems. An additional advantage is that with *ex vivo* imaging it is possible to control sample's characteristics and optimise relaxation and diffusion properties to best suit the MR scanner/sequence. In this study we present preliminary optimisation results for human *ex vivo* diffusion imaging by controlling tissue sample preparation and imaging parameters. Tissue relaxivity properties and diffusion indices were measured before and after soaking in phosphate-buffered saline (PBS) and increasing concentrations of the contrast agent Gd-DTPA. Signal-to-noise ratio (SNR) efficiency was optimised according to different diffusion times using a model of signal dependency. The final aim of this study is to offer clear guidelines for optimising *ex vivo* diffusion imaging methods, providing maximal data quality in terms of SNR and diffusion contrast.

Methods: A post-mortem human brain, formalin-fixed for 4 weeks, from a 61 yr old male was made available from the Clinical Neuropathology Department at King's College Hospital under the hospital post-mortem consent for medical and genetic research. The brain was sectioned into 7mm thick coronal slices around the central sulcus from which 12 smaller samples (approx. 7x30x30mm) were obtained. Samples were soaked in PBS for up to 66 days. T1/T2 relaxivity and DTI indices (fractional anisotropy, FA; mean diffusivity, MD) were measured at 5 time points. T1/T2 relaxivity changes were also measured with increasing Gd-DTPA concentrations (0,1,2,3,6 mM). Data was acquired on a 7T Varian/Agilent MRI system equipped with a 250/120HD gradient coil system and maximum gradient strength of 400 mT/m. T1 was estimated using an inversion recovery spin-echo sequence. T2 was estimated using a multi-echo spin-echo sequence. DTI data was acquired using a Pulse Gradient Spin-Echo (PGSE) sequence (voxel size=0.47x0.47x1mm, b=4000 s/mm^2, 4 b0s, 30 DWI-directions). Additional diffusion data was acquired with increasing diffusion times (12 to 50ms). Images were processed to generate T1, T2, FA and MD maps. Relaxation rate changes with Gd concentration were fitted by a linear function, allowing T1 and T2 to be expressed as a function of Gd concentration. These results were used to model signal dependency on Gd concentration and imaging parameters (TR, TE) using the following equation for spin-echo signal-efficency[1]:

$$S \propto \exp(-TE/T2)[1-\exp(-TR/T1)\,(2\exp(TE/2T1)+1)](1/\sqrt{TR})$$

Results and Discussion: After 26 days in PBS the tissue samples showed 87% and 58% increases in GM and WM T2 respectively (Figure 1). No significant changes were observed for T1 over this time (GM T1≈600ms; WM T1≈430ms). After 66 days in PBS there was a 26% and 9% increase in GM and WM MD respectively (GM: 0.25±0.02 to 0.31±0.03μm^2/ms; WM: 0.14±0.01 to 0.15±0.01μm^2/ms). Mean WM FA decreased by 15% (0.25±0.06 to 0.21±0.05) whilst there were no significant changes to GM FA (0.11±0.03). These findings are consistent with those of previous studies[2,3]. The model of signal dependency showed that high concentrations of Gd-DTPA maximise SNR-efficiency for shorter values of TE and TR (Figure 2) and, as expected, shorter TE and TR values also provide the highest overall signal-efficiency (Figure 3). Table 1 shows the optimum Gd concentration and TR value for a given TE, along with the potential increase in signal-efficiency. Figure 4 shows a trend of increasing FA values for increasing diffusion times from an average of 6 regions of interests. This trend suggests that as diffusivity is greatly reduced after fixation[4], diffusion times in *ex vivo* experiment may need to be significantly longer than conventional *in vivo* DTI experiments in order to maximize diffusion contrast and anisotropy.

Conclusion: Optimising *ex vivo* fixed tissue sample preparation for diffusion MR experiments using PBS and Gd-DTPA has the potential to greatly improve signal-efficiency, providing improved data quality, enhanced resolution and/or faster acquisitions[2]. Shorter values of TE and TR provide the greatest potential increase in signal-efficiency, however, at such TE values, the allowed diffusion times in a PGSE pulse sequence may not be optimal to probe tissue-microstructure and provide the best diffusion contrast for DTI experiments. We are now investigating stimulated-echo pulse sequences to access longer diffusion times while keeping short TE values and the use of 3D pulse sequences, instead of 2D, to better take advantage of the Gd-DTPA improvements in signal efficiency at short TR. Future works will also investigate the consistency of these results across multiple samples with varying post-mortem intervals and fixation times.

References: 1. M.A. Bernstein *et al.*, *Academic Press* (2004), 579-647; 2. H.E. D'Arceuil *et al.*, *Neuroimage* (2007), 553-565; 3. T.M. Shepherd *et al*., *Neuroimage* (2009), 820-826; 4. S.W. Sun *et al.*, *MRM* (2005), 1447-451.

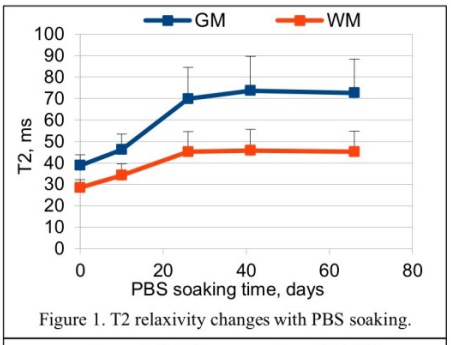

Figure 1. T2 relaxivity changes with PBS soaking.

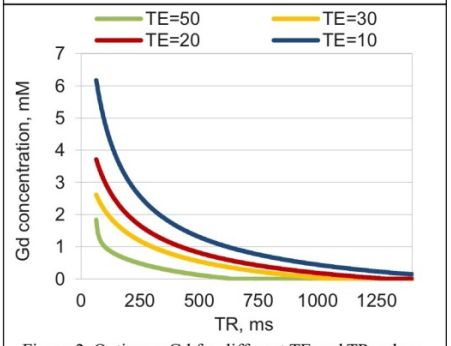

Figure 2. Optimum Gd for different TE and TR values.

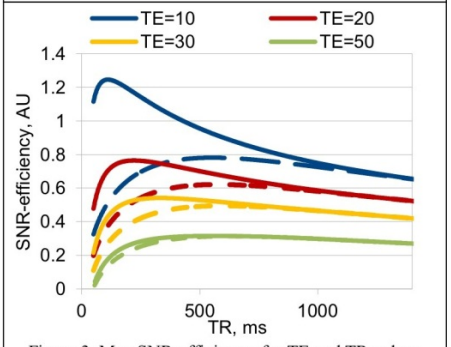

Figure 3. Max SNR-efficiency for TE and TR values. Dashed lines show SNR-efficiency curves without Gd.

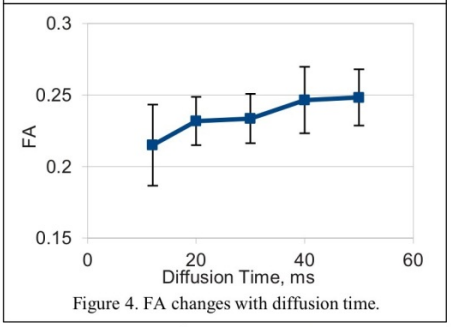

Figure 4. FA changes with diffusion time.

	TE, ms			
	10	20	30	50
Opt Gd, [mM]	4.6	1.8	0.9	0.2
Opt TR, [ms]	111	223	333	539
SNR-eff increase	59.5%	23.0%	9.4%	0.6%

Table 1. Optimum Gd/TR and increased SNR-efficiency for values of TE.