The effect of formaldehyde fixation over time: DTI parameters of brain tissue

C. A. Wheeler-Kingshott[1], K. Schmierer[1], P. A. Boulby[1], D. H. Miller[1]

[1]Department of Neuroinflammation, Institute of Neurology, London, United Kingdom

Introduction: Diffusion tensor imaging (DTI) of post mortem (PM) brain offers the potential to study the pathological correlates of DTI indices. Access to fresh PM brain is limited due to the fast decay of the tissue. Hence, the use of brain tissue after fixation in formaldehyde is desirable. However, few data are available on DTI changes due to fixation, compared to the fresh condition (1). The aim of this study was to evaluate the changes of DTI indices in PM brain tissue due to (i) fixation and (ii) time of fixation. Results of weekly scans of a brain slice from a patient with Multiple Sclerosis (MS) are presented.

Methods: One coronal PM brain slice, 10mm thick, from a MS patient was obtained from the UK MS Tissue Bank. The sample was kept at 2-8ºC before the first scan was performed, 41 hrs post mortem. After formaldehyde fixation, the same sample was scanned 9 times, spread over a two months period. The temperature of the specimen was recorded on every scanning session and was found to vary less than 5% around a mean of 21°C. Data were acquired on a GE 1.5T Signa Horizon whole-body clinical scanner, with maximum gradient strength of 22 mTm^{-1}, using a multi-shot diffusion-weighted spin-echo EPI sequence (2). The b-factor (b =1940smm^{-2}) was optimised for the reduced mean diffusivity (MD) of PM tissue. For each set of diffusion weighted scans, a volume of b_0 images (i.e. with b=0) was also acquired. Other imaging parameters were: sl. thick. = 5 mm; no. slices = 3; FOV =120 mm; matrix = 48x48 (reconstructed 64x64); resolution = 2.5x2.5 mm^2 (reconstructed 1.9x1.9 mm^2); TR = 3s; TE = 86 ms; no. shots = 8; NEX = 4; the total acquisition time was 10 min.

DT parameter maps (fractional anisotropy, FA, and MD) were calculated and the analysis was performed on two regions-of-interest (ROIs): one in the normal appearing white matter (NAWM) and one in a MS lesion, both chosen on the averaged b_0 image of the middle slice. The same areas were chosen at each time point, after visual comparison with the corresponding fresh image (figure 1a and 1b).

Results: MD and FA are shown in table 1 and figure 2. Table 1 compares MD and FA in the two ROIs under fresh conditions with the mean of MD and FA over the 9 separate scans under fixed conditions.

Compared to values regularly obtained *in vivo* the MD of NAWM in our sample was markedly reduced. Fixation led to further reduction of MD in NAWM (~20% of the corresponding fresh PM value) and remained relatively invariant throughout the rest of the study ($(220\pm16)\times10^{-6}mm^2s^{-1}$). The FA of NAWM in the fresh brain was also significantly lower compared to values obtained *in vivo*. After an initial increment following fixation, FA almost returned to the value measured in the fresh specimen (table 1 and figure 2).

The MS lesion also had a reduced MD value compared to *in vivo*; however, after approximately 5 weeks the MD of the MS lesion returned to the value measured under fresh conditions. Notice that FA in the lesion did not follow the same pattern as MD and seemed to be stable over the observation time of this study (0.11±0.02).

Discussion and conclusions: DTI values in human PM brain remain remarkably stable after fixation in formaldehyde. The fixation process appears to have a different effect on DTI indices in MS lesions and NAWM, notably the increment of MD in parallel to stable FA in the lesion. If confirmed in further samples this pattern may lead to enhanced tissue contrast following several weeks of formaldehyde fixation, as the NAWM values appear to remain fairly stable. The different patterns of MD and FA in the MS lesion warrant further exploration using other DTI indices such as the *eigenvalues* which give more information on the directional arrangement of the underlying tissue structures. Our preliminary findings suggest that DTI of fixed PM brain tissue could facilitate the investigation of the pathological correlates of DTI changes as such studies may not require fresh tissue.

References:

1. Sun, S. W et al. Magn Reson.Med. 50:743-748 (2003); 2. C. A. Wheeler-Kingshott et al. Proc. XI ISMRM p. 2137 (2003).

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	FRESH (1st time point)		FIXED (average of 9 time points) mean (standard deviation)	
	NAWM	**MS lesion**	**NAWM**	**MS lesion**
FA	0.19	0.12	0.23 (0.02)	0.11 (0.02)
MD ($10^{-6}mm^2s^{-1}$)	281	458	220 (16)	397 (52)

Table 1: DT-derived parameters in fresh and fixed brain tissue. The values reported for the fresh condition correspond to a single measurement over each ROI. The values reported for the fixed condition are the mean of 9 separate measurements of the same ROIs.

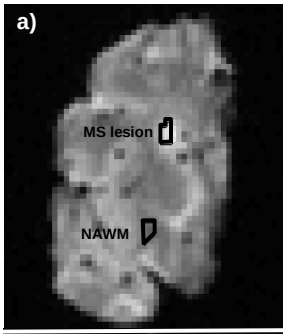
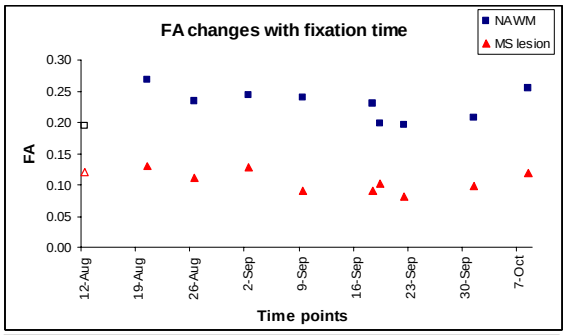


Figure 1 – **a)** Non-diffusion weighted (b_0) image of PM brain slice in fresh condition. **b)** Corresponding non-diffusion weighted (b_0) image of the same slice after one week of fixation. On both images, the ROI used for the analysis are drawn in black.
No registration algorithm was used between time points (slight changes in the ROI shape between time points were allowed).
The top right-hand corner and bottom left-hand corner of the fixed slice were dissected prior starting the longitudinal study of fixed tissue.



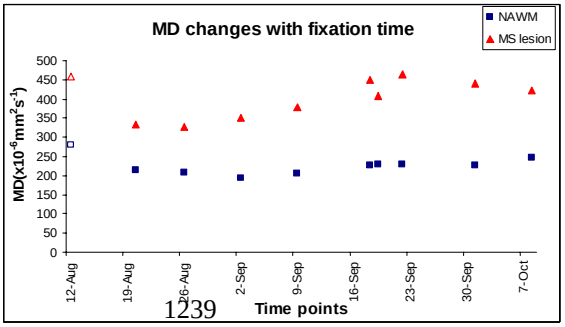


Figure 2 – Plot of MD and FA changes over time on the two ROIs shown in figure 1. The time interval between points is approximately 7 days, apart from a couple of acquisitions on consecutive days. The red triangles show the values of parameters in the MS lesion. The blue squares represent the NAWM regions. The first point of both graphs and of each ROI is shown with white background to be distinguished from all the other points; in fact, it corresponds to FA and MD measurements under fresh condition.

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