# Investigating white matter microstructural changes during demyelination using GRE phase and R2*

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**Introduction** Myelin has been proposed as a key factor driving the signal behaviour of susceptibility-weighted MRI signals in white matter (WM), such as signal phase and magnitude measurements used to estimate frequency and R2*, respectively [1-3]. However, it is unclear how frequency and $R_2$* changes can reflect characteristics of demyelination or remyelination in disease process. In this study, a cuprizone mouse model of demyelination is used to investigate the link between gradient echo (GRE) signal with histological determined amount of myelin. Importantly, geometric WM modelling [4] suggests that GRE signal is sensitive not only to the bulk amount of myelin but also to the spatial distribution of myelin within the WM.

**Methods** MRI GRE phase and magnitude images were obtained at 7T (Bruker Clinscan) with a multi-echo GRE sequence with TE at 3,7,11,...55ms, TR=1500ms, flip angle = 70°, FOV = 10x10mm, matrix size = 128x128, 3 axial slices, slice thickness = 0.3mm, 10 averages. R2* maps were obtained by an exponential fitting of the magnitude images across the different TEs. Frequency maps were obtained by linear fitting the phase evolution in each voxel. Frequency maps were then high-pass filtered by the subtraction of a 5x5 mean filtered image from the original image to remove large scale background field inhomogeneity. *Ex vivo* study 9 eight-weeks old C57BL/6 mice were fed a 0.2% cuprizone diet, each for a different period of time to induce different degrees of demyelination [5]. Animals were then sacrificed and their brains harvested for MR imaging. After imaging, brains were sectioned and stained for myelin using a standard Luxol fast blue (LFB) protocol [6]. LFB stain intensity of each brain slice was normalized by the cortical GM for quantification. Mean R2* and frequency maps were then correlated with the mean normalized LFB stain across ROIs drawn on the corpus callosum (CC) region. Simulation A geometric WM model [4] was used to predict GRE signal changes with a hypothetical case of demyelination in which the thickness of the myelin layer was reduced sequentially with corresponding contraction of the inter-axonal distance (Fig 3a). This scenario represents the removal of myelin from the outside-in followed by rapid clearance of myelin debris from the extra-axonal space and the axons moving closer to one another to fill the gap left by the removed myelin. Magnetic susceptibility $\chi$ and $T_2$ of the extra-axonal, axonal and myelin compartments were modelled as: $\chi_{ea} = \chi_{ax} = 0$ppm, $\chi_{my} = -0.08$ppm, $T_{2ea} = T_{2ax} = 50$ms, $T_{2my} = 25$ms. The WM fiber volume fraction and g-ratio for the fully myelinated scenario were 0.7 and 0.65.

**Results and discussion** LFB stains showed progressive demyelination with increased durations of cuprizone diet, and GRE frequency and R2* also displayed a similar reduction of GM-WM contrast in CC (Fig 1). ROI analysis showed high correlation between normalized LFB intensity and R2* ($r=0.930, p<0.01$) and slightly lower correlation with frequency ($r=-0.656, p<0.01$) (Fig 2a,b), which was likely due to non-local effects of GRE phase. Voxel-wise analysis showed high correlations for both R2* ($r=0.862, p<0.01$) and frequency ($r=-0.828, p<0.01$) (Fig 2c,d). Simulation predicted a non-linear dependence of R2* and frequency on myelination, particularly between R2* and myelin volume fraction (Fig 3b,c). The excellent agreement between the signal model and data in terms of both the trend and range of measured values is particularly compelling as this represents a forward simulation using literature values, *not* a model fit to the data. Our simulations predict that R2* is not only dependent on bulk myelin content, but also its spatial distribution.

**Conclusion** We demonstrated that R2* and frequency are strongly correlated to the amount of myelin during the demyelination process *ex vivo*. Furthermore, our simulation suggested that this relationship deviate from linearity, at least for R2*. Agreement between the forward model predictions and measured results suggests that the spatial distribution of myelin, which is the central feature of the signal model, plays an important role in GRE signal behaviour. In a related *in-vivo* experiment, not presented here for brevity, we studied the cuprizone model under *re*-myelination (following cessation of cuprizone treatment), finding that the trends in frequency and R2* under demyelination were partially reversed.

**References** (1) Lee *et al.* NeuroImage 2012. (2) Liu *et al.* NeuroImage 2011. (3) Lodygensky *et al.* NeuroImage 2012. (4) Chen *et al.* NeuroImage 2013. (5) Matsushima *et al.* Brain Path 2001. (6) Kluver *et al.* J Neuropath 1953.

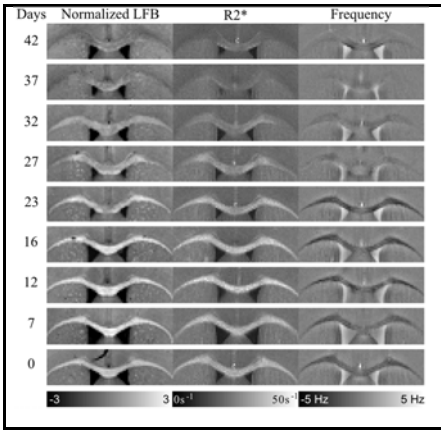


**Fig 1**. *Ex vivo* results showing change in myelination with duration of cuprizone diet. Column 1 to 3 show the normalized LFB images, R2* and frequency maps respectively. Each row represents each time point.

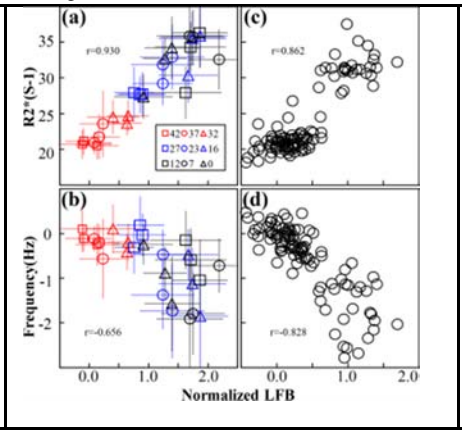


**Fig 2**. *Ex vivo* correlation results. (a) and (b) ROI-based correlation of R2* and frequency with normalized LFB. Error bars represent standard error within each brain slice.Color and shapes of markers represent different time points. (c) and (d) Voxel-based correlation.

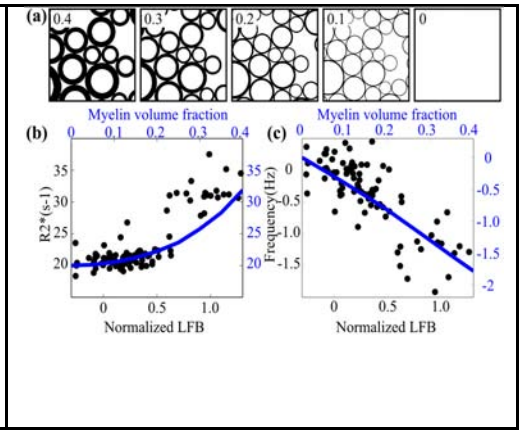


**Fig 3**. Demyelination simulation. (a) Schematic of demyelination scenario with different myelin volume fraction showing reduction of myelin thickness with corresponding contraction of inter-axonal distance. Overlay of simulated change in (b) R2* with myelin volume fraction and change in (c) frequency.