

## Mn-DPDP: an MRI histological stain for mouse embryo cell density

Francesca C Norris<sup>1,2</sup>, Jack A Wells<sup>1</sup>, Bernard M Siow<sup>1,3</sup>, Simon Walker-Samuel<sup>1</sup>, Peter J Scambler<sup>4</sup>, and Mark F Lythgoe<sup>1</sup>

<sup>1</sup>UCL Centre for Advanced Biomedical Imaging, Division of Medicine and Institute of Child Health, University College London, London, United Kingdom, <sup>2</sup>Centre for Mathematics and Physics in the Life Sciences and Experimental Biology, University College London, London, United Kingdom, <sup>3</sup>Centre for Medical Image Computing, University College London, London, United Kingdom, <sup>4</sup>Molecular Medicine Unit, UCL Institute of Child Health, London, United Kingdom

**Introduction:** Disruption of specific, individual genes during embryonic development can result in death. In the mouse, one third of genes are essential for development [1] and disruption of these genes results in embryonic lethality. Worldwide efforts to understand developmental processes demand new high-resolution 3D methods to detect the consequences of gene function in embryo development and diseases. Encouragingly, MRI contrast agents can highlight specific tissue structures in *ex vivo* adult mouse brain studies [2, 3]. MR imaging of mouse embryos is currently limited by a lack of tissue differentiation staining capabilities that provide the flexibility and target specificity offered by histological stains conventionally used for embryo phenotyping. In this study, we have investigated the biodistribution and MR relaxation mechanism of Mn-DPDP, a clinically approved contrast agent, in *ex vivo* mouse embryos. We identify Mn-DPDP as a potential MRI 'histological stain' for embryonic cellular density.

**Methods:** E15.5 C57BL/6 embryos were bled out and fixed in 4% PFA doped with 4 concentrations of Mn-DPDP (VWR International) (4 weeks fixation, n=3 per concentration, 0–10mM). T<sub>1</sub> and T<sub>2</sub>\* mapping data was acquired for a single slice (0.5mm slice, matrix 256x256, FOV 27x27mm<sup>2</sup>) using an inversion recovery spin echo and gradient echo sequence (Varian 9.4T, 33mm volume coil). Maps were created using an in-house MATLAB program. Relaxivity was determined for doped agarose and tissue regions (cerebellar primordium and cerebellar ventricular zone [VZ]) by plotting mean R<sub>1</sub> and R<sub>2</sub>\* at each concentration and performing a linear regression. 3D images were acquired for embryos stained with 0 and 10mM Mn-DPDP using a 3D gradient echo sequence (FOV 27x27x27mm<sup>3</sup>, matrix 512x512x512, 7 averages) with scanning parameters (0mM: TR=46ms, TE=10ms, FA=10°; 10mM: TR=20ms, TE=5ms, FA=37°) simulated for maximum brain contrast using a MATLAB program. The above was adapted from [4]. Embryos stained with 10mM Mn-DPDP were placed in 30ml phosphate buffered solution (PBS) for 5 weeks. T<sub>1</sub> and T<sub>2</sub>\* maps were acquired at 1 week intervals. R<sub>1</sub> and R<sub>2</sub>\* was measured in the cerebellar primordium and cerebellar VZ at each time point. Embryos were sectioned and stained with haematoxylin and eosin (H&E) to compare microstructure with contrast-enhanced regions.

**Results:** Very limited contrast was observed in the unstained brain, despite using optimised scanning parameters for brain contrast (Figure 1i). Mn-DPDP markedly improved tissue contrast and SNR (SNR=33) compared with unstained embryos (SNR=17) (Figure 1ii), and enabled visualisation of anatomical structures, such as the posterior region of the midbrain, external granular layer (EGL) of the cerebellum and cerebellar VZ (red, blue and green arrows in Figure 1iii, respectively). These regions are densely packed with cells as shown by the H&E-stained section (Figure 1iv). There was no protein or macromolecular binding of Mn-DPDP as indicated by the r<sub>1</sub> values (Table 1) and PBS washout data (Figure 2). Also, no correlation was observed between the observed tissue contrast and r<sub>1</sub>/r<sub>2</sub>\*.

**Discussion and Conclusion:** We have identified Mn-DPDP as a promising MRI histological stain for delineation of densely packed tissue sub-structures in *ex vivo* embryos. Our findings indicate that the observed contrast enhancement is due to local cytoarchitecture leading to concentration differences of Mn-DPDP, rather than intrinsic tissue contrast, preferential contrast agent binding or tissue relaxivity differences. This suggests that other contrast agents will differentially enhance contrast, providing a host of MRI histological stains. Further investigation will determine how the tissue cytoarchitecture influences the site-specific distribution of Mn-DPDP and if this varies at other developmental stages. In conclusion, selective Mn-DPDP MRI staining will enable phenotypic characterisation of mutant mouse embryo models and the generation of novel, high-resolution embryo atlases for morphometric analyses.

**References:** [1] Brown S.D. *et al. Annu Rev Genet* 2009, 43:305-33; [2] Huang S. *et al. Neuroimage* 2009, 46:589-99; [3] Cleary J.O. *et al. Neuroimage* 2011, 56:974-83; [4] Cleary J.O. *et al. NMR Biomed* 2009, 22:857-66.

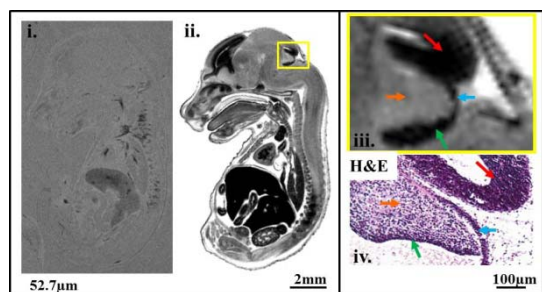


Figure 1. MR image of i) unstained embryo, ii) 10mM Mn-DPDP. Arrows highlight posterior region of the midbrain (red), cerebellar primordium (orange), cerebellar VZ (green) and EGL of cerebellum (blue) in iii) MR image of embryo stained with 10mM Mn-DPDP and iv) histological section stained with H&E.

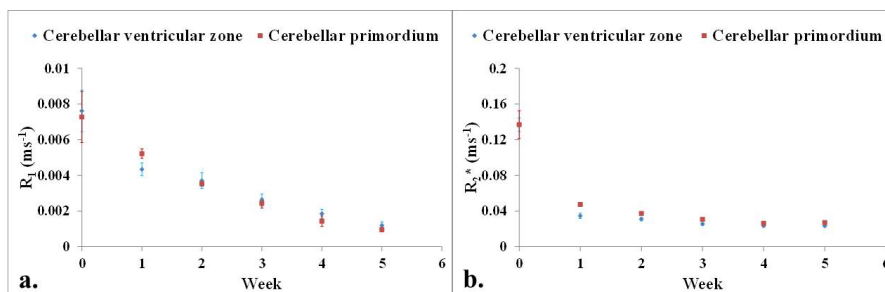


Figure 2. Change in a) R<sub>1</sub> and b) R<sub>2</sub>\* of cerebellar VZ and primordium for embryos stained with 10mM Mn-DPDP and immersed in PBS for 5 weeks at 1 week intervals.

	Agarose	Cerebellar primordium	Cerebellar VZ
r <sub>1</sub> (mM <sup>-1</sup> s <sup>-1</sup> )	2.01 ± 0.1	1.84 ± 0.3	1.65 ± 0.3
r <sub>2</sub> * (mM <sup>-1</sup> s <sup>-1</sup> )	5.63 ± 1.4	31.68 ± 6.4	32.43 ± 3.4

Table 1. Mn-DPDP relaxivity in agarose and *ex vivo* embryo ROIs.