Mouse Embryo Phenotyping with Contrast-enhanced micro-Diffusion Tensor Imaging

B. M. Siow*[1,2], J. O. Cleary*[1,3], N. D. Greene[4], P. Daga[3], M. Modat[2], R. J. Ordidge[3], S. Ourselin[2], D. Alexander[2], and M. F. Lythgoe[1]
[1]Centre for Advanced Biomedical Imaging, Department of Medicine and Institute of Child Health, UCL, London, United Kingdom, [2]Centre for Medical Image Computing, UCL, London, United Kingdom, [3]Department of Medical Physics and Bioengineering, UCL, London, United Kingdom, [4]Neural Development Unit, Institute of Child Health, UCL, London, * Equal Contribution

Introduction Mouse embryos are widely used as models for the study of human congenital abnormalities such as spina bifida, which is one of the most common birth defects. Morphological phenotyping of these embryos is still heavily reliant on histological sectioning: a destructive process that is time-consuming and operator dependent. Diffusion-tensor imaging (DTI) is a powerful technique that can explore tissue structure non-invasively, providing microstructural information such as the direction of tissue fibres. DTI techniques are particularly useful where traditional anatomical MR contrast is limited, such as in the developing foetal brain[1]. In the mouse embryo, DTI can characterise brain development[1] as well as examine phenotype in mutant animals[2]. However, these studies have imaged only the brain in mid-gestation embryos, with a modest number of diffusion directions (typically 6) and long acquisition times (~10 hours). Gadolinium chelates, such as Gd-DTPA, are commonly used in the fixation of embryos for MR microscopy**,** to reduce T_1 and boost SNR[3]. While the use of Gd-DTPA is common in anatomical embryo imaging, its use has been limited in embryo DTI studies[4]. We have developed a contrast-enhanced technique for whole-body DTI of mid-gestation embryos, comparing embryo preparation, pulse sequences and resolution. Subsequently, we have applied our methodology to investigate the phenotype of the *splotch* mouse model of spina bifida, which carries a mutation in the gene *Pax3*[5] a transcription factor implicated in the condition.

Methods 15.5 dpc embryos (C57Bl/6 background), were dissected from the mother and fixed for 2 weeks in a solution of 4% formal-PBS doped with 2mM Gd-DTPA. Later they were moved to a solution of PBS+2mM Gd-DTPA for at least 2 weeks. Imaging was performed on a Varian VNMRS system using a 26mm volume coil (RAPID Biomedical GmbH) and 3D spin-echo (3D-SE) and fast-spin-echo (3D-FSE) diffusion sequences (diffusion parameters: G=50G/cm, ∂=3.5ms, Δ=8ms, 42 directions+6 b0 images), with parameters chosen to maintain sample temperature during the acquisition: SE: TR=500ms, TE=15.3ms, b≈1550s/mm^2; FSE: TR=500ms, TE_1=14.75ms, ESP=5.75ms, ETL=4, b≈1660s/mm^2. Voxel size: 100x100x100μm^3 and 75x75x75μm^3 isotropic. 3D-SE T_1 and T_2 map data were also acquired. Sample temperature was 19±1°C. Images were corrected for movement by global registration[6]. Direction encoded colour (DEC) maps were visualised in MedINRIA (Asclepios Project); FA value calculation and tractography was performed in MRtrix[7].

Results After rehydration in PBS+Gd-DTPA, mean whole-body T_2 increased from 14 to 25ms, giving improved SNR in the subsequent diffusion images (typically 39 vs. 22, 100μm SE data). DEC maps and values were similar between 3D-SE and FSE datasets (resulting 75μm DEC maps from FSE data are shown in Fig.1). Subsequently we imaged wild-type and *splotch* mutant mice, identifying differences in brain structure, in addition to spinal cord phenotype (Fig.2).

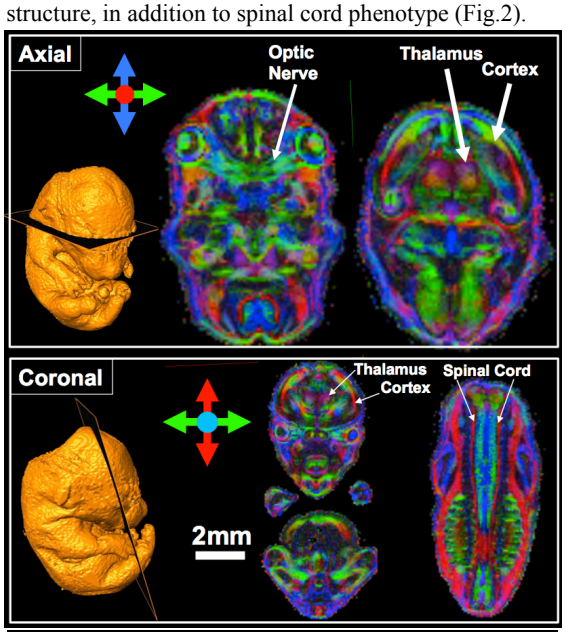

Figure 1: Axial and coronal DEC maps of a C57Bl/6 wild-type embryo, imaged using our 75μm-FSE protocol, showing well-defined CNS anatomy and corresponding tissue directionality. (Arrows indicate principal eigenvector direction)

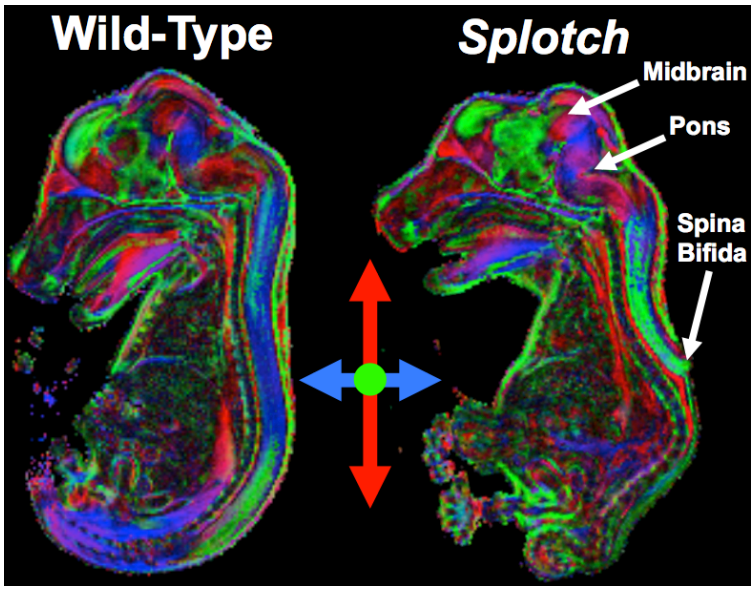

Figure 2: Similar mid-sagittal DEC maps through wild-type and *splotch* homozygous embryos. Although the primary defect is the spina bifida indicated, clear differences may also be seen in overall brain size and regions demarcated by principal eigenvector, such as in the pons and midbrain. (Arrows indicate direction of principal eigenvector)

Conclusion In this study, we have shown that whole-body μDTI is able to delineate a number of anatomical brain regions according to diffusion directionality. We have used 42 directions in this embryo study, achieving high-resolution (75μm) and a moderately high b-value (1660s/mm^2) in a 19h scan. Although we have concentrated on achieving high quality diffusion data for tractography through the acquisition of 48 images per scan, resorting to the minimum of 7 would give a total scan time of ~2.75 hours per embryo, improving throughput. As the final part of this study, we have described the phenotype of the *splotch* model of spina bifida for the first time with MR, characterising changes in brain structure and their relation to spinal cord phenotype. We hope our methodology may enable wider adoption of DTI to embryo studies, which may offer new insights into new and existing congenital disease models.

Acknowledgements We are grateful to Prof. Andy Copp for useful comments and support of EPSRC, BHF and BBSRC.

References 1. Mori S *et al.* MRM 46:18–23 2001; 2. Zhang J *et al.* NeuroImage 20:1639–1648 2003; 3. Cleary JO *et al.* NMR Biomed. 22: 857–866 2009; 4. Aggarwal M et al. MRM 64: 249-261 2010; 5. Greene NDE, *et al.* Birth Defects Res A 2009 85:322-330; 6. Ourselin S *et al.* Image and Vision Computing 19:25–31 2000; 7. Tournier JD *et al.* NeuroImage 35:1459-72 2007