Phenotyping in the Mouse Embryo using a µMRI atlas

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Introduction: Voxel and deformation-based morphometry are techniques that can detect subtle differences in anatomy between two populations[1]. Both use similar methods to combine a number of subjects, creating an average atlas. Two such groups may then be compared statistically. This has been successfully used in studies of the adult mouse brain[2], a highly conserved structure, amenable to the co-registration methods employed. This method could also be useful in the embryo to examine phenotypic changes[3], particularly in the CNS. µMRI is an emerging technique for high-throughput imaging of mouse embryos, imaging up to 32 in a single scan[4]. The current method of analysis involves inspection of each embryo by a trained observer – a time consuming and labour-intensive process. Semi-automated morphometric methods of analysis, where large numbers of embryos may be compared at once, show promise in combining high-throughput imaging with high-throughput analysis. We present an initial study to examine the viability of producing an embryo atlas, focusing on the degree of anatomy that is preserved after averaging individual embryos, especially in the CNS.

Methods: *Embryo Preparation:* 6 E15.5 CD-1 mouse embryos were bled out in 37°C Hanks solution and fixed for at least 2 weeks in 4% PFA and 8mM Gd-DTPA. They were then embedded in a centrifuge tube containing 1% agarose gel doped with 8mM Gd-DTPA–identical to initial fixation. *Imaging:* Scans were performed on a Varian 9.4T VMRS system with a 39mm volume coil (RAPID Biomedical GmbH), using a 3D spoiled gradient-echo sequence (TE/TR/FA/NSA=9/20/60/7), matrix-size 512^3, FOV 27x27x27mm. Acquisition time was 10 hours.
Atlas generation: Data was zero-filled to a voxel size of 26x26x26µm. The 6 embryos were extracted and aligned, then rigid, affine and finally non-rigid registration was applied. After each step, the deformed images were averaged to create an atlas and each method was repeated until convergence. Rigid and affine registration were performed using a block-matching technique[5]; non-rigid was based on Free-Form Deformation[6]. Our algorithm[7] was GPU-based, enabling atlas creation in a minimal amount of time, with small control point spacing (65µm) – some 4M DoF. A penalty term and its analytical gradient[8] favoured diffeomorphic transformation. Stopping criteria: no control point displacement larger than 0.01 of a voxel size to produce further improvement. Maps were created to demonstrate the normalised variance in signal intensity between the six embryos after affine and non-rigid stages of the registration. Lower anatomical variation between subjects would be expected to have a lower variance.

Results: Fig. 1 shows representative axial slices through a single embryo and the embryo atlas, demonstrating that although single embryo images show contrast between various brain regions, following registration, signal to noise and anatomical delineation are visibly improved in a number of CNS structures. Fig. 2 shows a sagittal slice though the embryo, demonstrating clear visualisation of structures especially the developing cortex. Fig. 3 indicates a measure of the variability between embryos after affine and non-rigid registration. Embryo anatomy is highly conserved between subjects (Fig 2b) especially in the CNS and areas of heart myocardium (Fig. 3 – blue indicating lower normalised variance values).

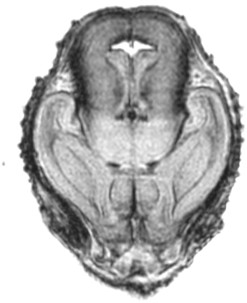


Fig. 1a: *Axial slice through the brain of a single embryo*

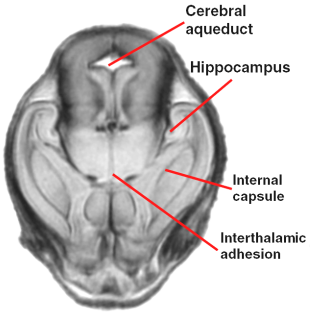


Fig. 1b: *Axial view through composite atlas of 6 CD-1 embryos showing key anatomical features*

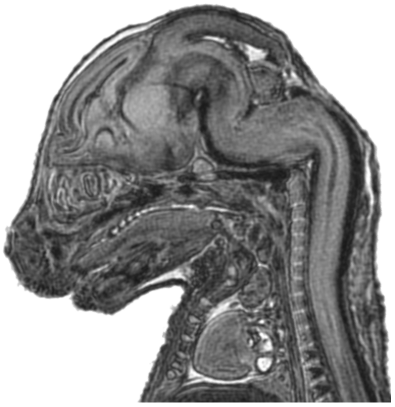


Fig. 2a: *Sagittal slice through a single embryo*

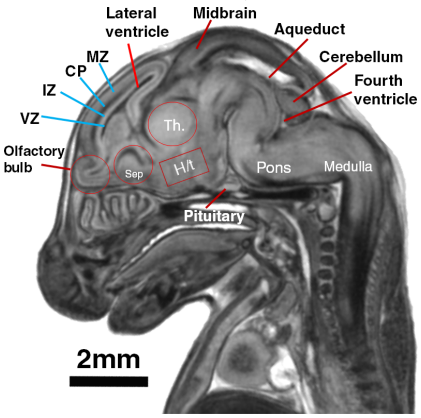
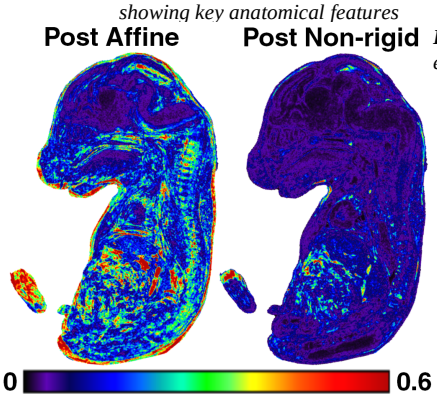


Fig. 2b: *Similar sagittal slice from the 6-embryo atlas. Cortical plate (CP), ventricular (VZ), intermediate (IZ) and marginal zones (MZ) are visible (blue arrows). Also thalamus (Th.) and hypothalamus (H/t)*

Fig. 3: *Sagittal maps of normalised variance in signal intensity between embryos after affine and non-rigid registration. (Zero indicates no variance)*



Conclusion In this study we have been able to combine optimised high resolution µMRI with image processing techniques to maintain CNS and cardiac anatomy, which is essential for investigating phenotypic changes in the mouse. We have produced an embryo atlas in which signal to noise is enhanced over single embryo data and also preserves anatomical features. We note that there is low variation in the CNS after affine registration indicating that anatomy is well conserved between subjects. Low variance after non-rigid registration over the whole embryo indicates good performance of the registration algorithm. We present an initial step in applying statistical morphometry methods to phenotyping the mouse embryo, which may enable subtle anatomical differences between populations to be detected.

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References [1]Ashburner J. & Friston K.J., *NeuroImage* 2000, 11: 805-821; [2]Lau J.C. et al., *NeuroImage* 2008, 42: 19–27; [3]Zamayadi M. et al., *Proc. ISMRM* 2008, 16: #3099, [4]Schneider J.E. et al., *BMC Dev. Bio.* 2004, 4:16; [5]Ourselin S.et al., *Image Vis. Comput.* 1999, 18: 25-31; [6]Rueckert D. et al., *IEEE Trans. Med. Imag.* 1999, 18:8, 712-721; [7]Modat M. et al., *High-Performance MICCAI Workshop* 2008; [8]Modat M. et al., *Proceedings of SPIE* 2009, accepted