Propagation-based Morphometry in an ex vivo mouse embryo atlas – assessment and validation

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Introduction: There is a worldwide initiative to create mutant mouse models for all ~21,000 mouse genes [1]. This increasing number of knockouts has highlighted the need for effective methods of phenotyping these mice. Conventionally, embryo phenotyping is performed *ex-vivo* by microscopic examination with histology, which results in tissue distortions making it unsuitable for volumetric measurements. Propagation-based morphometry (PBM) is an emerging technique that enables non-invasive and rapid acquisition of volumetric data using an average population atlas for morphometric analysis [2]. The current method of analysis involves manually segmenting volumes of interest (VOIs) on MR images, which is time-consuming and labour-intensive. Thus, PBM shows promise for combining high-throughput µMR imaging of late-gestation mouse embryos with high-throughput analysis. We present the first study to assess and validate the accuracy of volumes generated via PBM in an *ex vivo* mouse embryo atlas comprising three different groups against manual segmentation. Additionally, the intra- and inter-observer variability of manual segmentation was assessed to validate its repeatability and reproducibility, respectively.

Methods: 19 E15.5 embryos (5 *Chd7*$^{+/+}$ and 8 *Chd7*$^{+/-}$, where *Chd7* knockout mice are models for human CHARGE syndrome, and 6 CD-1) were used in this study. Imaging was performed on a Varian 9.4T VNMRS 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.

Intra- and inter-observer variability study: The whole brains and hearts of two randomly chosen CD-1 embryos were manually segmented using Amira (Visage Imaging, Inc. CA, USA). Successive segmentations were performed at a time interval of one week. For the inter-observer variability study, the whole hearts of three randomly chosen CD-1 embryos were manually segmented by two observers. The similarity between segmentations was assessed using the Dice Score Coefficient (DSC), where 0 corresponds to no overlap and 1 a perfect overlap, and absolute percentage difference.

Propagation-based morphometry: The 19 embryos were spatially normalised by performing a groupwise registration. A block-matching approach was used to complete the affine registration step and further local alignments were performed using a fast Free-Form Deformation implementation [3]. This resulted in an average image, or atlas, on which the whole body, brain and heart were manually segmented. The deformation obtained while spatially normalising the data enabled us to propagate the manual segmentations in the original space of each subject.

Validation study: The whole brains, hearts and bodies of the CD-1 embryos were manually segmented in a random order. The corresponding propagated volumes were compared with these using the DSC and absolute percentage difference.

Results and discussion: The repeated and reproduced segmentations for the brain and heart have high DSC and low absolute percentage difference values (Table 1), indicating excellent intra- and inter-variability, respectively, using manual segmentation. The mean DSC values and absolute percentage difference values for the whole body, brain and heart (Table 2) are comparable with those in another study, based on adult mice rather than embryos [4], and indicate good correlation between the propagated and manually segmented volumes. However, the absolute percentage difference values for the hearts of embryos 4 and 6 are biased as shown in Figure 1, which may be due to poor volume propagation. For example, the blue arrows in Figure 2c indicate regions where the propagated heart volume (green) has extended over or not reached the boundary of the manually segmented heart volume (red) for embryo 4, which may be contrasted with the relatively accurate heart propagation (green) for embryo 1 in Figure 2b. Poor volume propagation may be due to the fact that the atlas comprises three different groups of embryos, of which the CD-1 group is a minority, such that the atlas and atlas segmentations, as shown in Figure 2a, are more representative of the two Chd7 groups. Also, the varying systolic phases of the hearts may have resulted in low contrast boundaries on the atlas while the relatively small size of the heart may mean that errors are proportionately larger.

	Intra-observer (n=2)		*Inter-observer (n=3)*	
	Mean DSC	**Mean abs % diff**	**Mean DSC**	**Mean abs % diff**
Heart	0.97	1.98 ± 2.33	0.95	2.70 ± 1.99
Brain	0.98	0.43 ± 0.51	No data	No data

***Table 1**. Mean DSC and absolute percentage difference values for the heart and brain for the intra- and inter-observer studies.*

	Mean propagated volume (mm^3)	**Mean DSC**	**Mean abs % diff**
Whole body	387.93 ± 26.86	No data	2.76 ± 0.98
Brain	45.66 ± 1.19	0.95	3.08 ± 2.29
Heart	2.42 ± 0.31	0.87	8.25 ± 3.72

***Table 2**. Mean propagated volume, DSC and absolute percentage difference values for the whole body, brain and heart.*

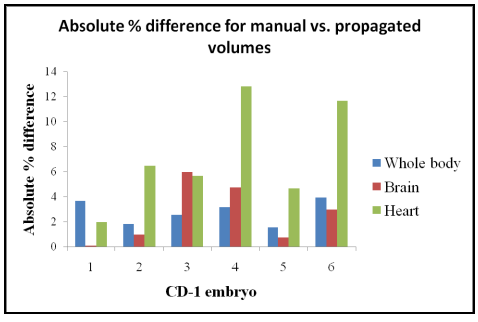

***Figure 1**. Absolute % difference between manually segmented and propagated volumes for whole body, brain and hearts of CD-1 embryos.*

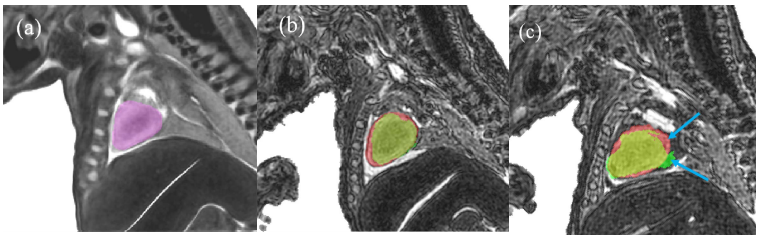

***Figure 2**. **(a)** Atlas heart segmentation (purple) overlaid on atlas;**(b)**best case – manual (red) and propagated (green) heart segmentations overlaid on MR image of embryo 1; **(c)** worst case – manual (red) and propagated (green) heart segmentations overlaid on MR image on embryo 4 and blue arrows indicating regions of poor propagation.*

Conclusion: Assessment of tissue volumes on a large scale is very time consuming with conventional analysis techniques. We have demonstrated that calculation of the heart, brain and whole body volumes of six mice, based on volumes from a single atlas, is possible using PBM, even if the atlas is calculated from three different groups of mice. The validation of propagation-based morphometry for this mouse embryo atlas shows promising results towards the broad applicability of this technique for phenotyping mutant mouse models. Future work will include generation of a C57Bl/6N (the standard background for UK large-scale mutagenesis programs) atlas, investigating more complex structures, and using the current propagated volumes to make morphometric comparisons between the different strains. In conclusion, we present the first validation step towards high-throughput volumetric assessment of multiple mouse lines from a single atlas and suggest that PBM is a strong candidate for embryo phenotyping.

References: [1] *Cell* 2007, 128: 9-13; [2] Kovacevic N. *et al. Cerebral Cortex* 2005, 15: 639-645 [3] Modat M. *et al. Computer Methods and Programs in Biomedicine* 2009 (in press); [4] Chen X. *et al*. ISMRM 2009 #2911

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