1024
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# Establishing Three Dimensional High Throughput Imaging Pipeline for Deep Phenotyping Mouse Embryonic Development

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The mission of the International Mouse Phenotyping Consortium (IMPC) is to systematically phenotype mice resulting from null alleles in the approximately 20,000 known or predicted protein encoded genes [1] to determine the function of each gene and the relationship to human disease. With a prediction of more than 30% of the mouse lines will either be lethal (no homozygous pups were recovered at postnatal day 14) or sub-viable (less then 12.5% of homozygous pups at postnatal day 14), an embryonic lethal pipeline including an assessment of the approximate stage of lethality, followed by 3D imaging to further analyze and archive the phenotype has been established [2]. The goal of this work is to establish a high throughput screening pipeline utilizing optical projection tomography (OPT) and microCT for studying embryo gross morphology and deep phenotyping digitally to determine the developmental defects that cause embryonic lethality.

For mouse embryos at stage E9.5 and E12.5, an in-house build OPT system (BCM-Build) modified from previous published version [3] was built and used to acquire 3D information of the embryos [4]. Samples were imbedded in 1% agarose and underwent serial dehydration in ethanol, which then cleared in BABB solution (one part benzyl alcohol and 2 parts benzyl benzoate) before imaging. Autofluorescence images of the embryos were generated with an X-Cite illumination light source and with a 425/26 nm BrightLine bandpass excitation filter and a 520/20 nm emission filter. The embryos were rotated 360 degrees through the anterior-posterior (AP) axis, with each projection view taken with a 0.3-degree step size. For embryos at stage E15.5 and E18.5, STABILITY protocol was implemented to generate a tissue-hydrogel complex to prevent sample deformation and organ shrinkage [5]. To generate contrast in soft tissue of the embryos, samples were immersed in 0.1N iodine solution before imaging. Each data set was acquired via SKYSCAN 1272 micro-CT scanner (Bruker) with the X-ray source at 70 kV and 142 uA with a 0.5 mm aluminum filter. Each sample was rotated 180 degrees through the AP axis with each view taken with a 0.3-degree step size at an average of 3 images. Acquired projection images from both OPT and microCT were then reconstructed by using Fledkamp Algorithum [6] for cone-beam CT data through NRecon Reconstruction (Version: 1.6.9.8; Bruker) software.

By implementing both imaging modalities, we have successfully established the imaging pipeline for screening gross morphology and deep phenotyping of mouse embryonic development. By acquiring the 3D information from the embryos, we can easily render the surface of the embryo to screen its morphology as well as navigate through the 2D virtual sections digitally. Figure 1 shows the 3D surface rendering of the OPT acquired E9.5/E12.5 samples and microCT acquired E15.5/E18.5 samples for gross morphology analysis. Figure 2 shows the 2D virtual sections at different body axes of the E15.5 and E18.5 embryos to as detail to 11 um/pixel. By applying 3D imaging techniques, images that are comparable to histological sections at high resolution can be easily generated and navigated as virtual sections through different body axes to identify potential developmental defects.

Reference:
[1] W. C. Skarnes, B. Rosen, A. P. West, et al., Nature **474** (2011), p. 337–342.
[2] D. Adams, R. Baldock, S. Bhattacharya, et al., Disease Models and Mechanisms **6** (2013), p. 571–579.
[3] M. D. Wong, J. Dazai, J. R. Walls, et al., PloS one **8** (2013)**,** p. e73491.
[4] M. Singh, A. Nair, T. Vadakkan, et al., SPIE **9334** (2015), pp. 93340J–93340J–8.
[5] M. D. Wong, S. Spring, and R. M. Henkelman, PloS one **8** (2013), p. e84321.
[6] L. A. Feldkamp, L. C. Davis, and J. W. Kress, *JOSAA* **1** (1984), p. 612–619.

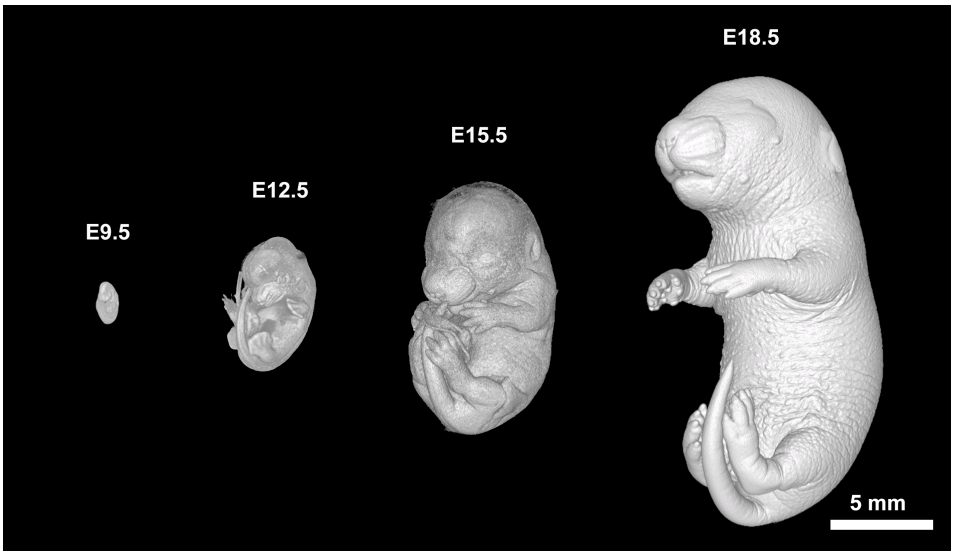


**Figure 1.** Surface rendering of embryos imaged on OPT (E9.5 and E12.5) and microCT (E15.5 and E18.5) for gross morphology analysis.

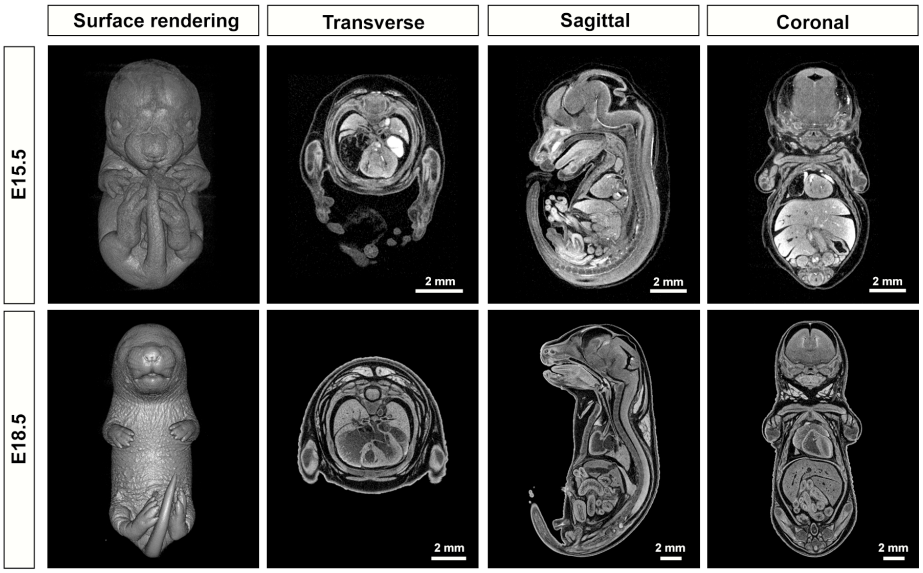


**Figure 2.** 3D surface rendering and 2D virtual sectioning for mouse embryos at stage E15.5 and E18.5 for deep phenotyping.

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