MRI and OPT Comparison for Mouse Embryonic Development

M. D. Wong[1], J. Ellegood[1], J. Dazai[1], X. J. Chen[1], and R. M. Henkelman[1]
[1]Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, Canada

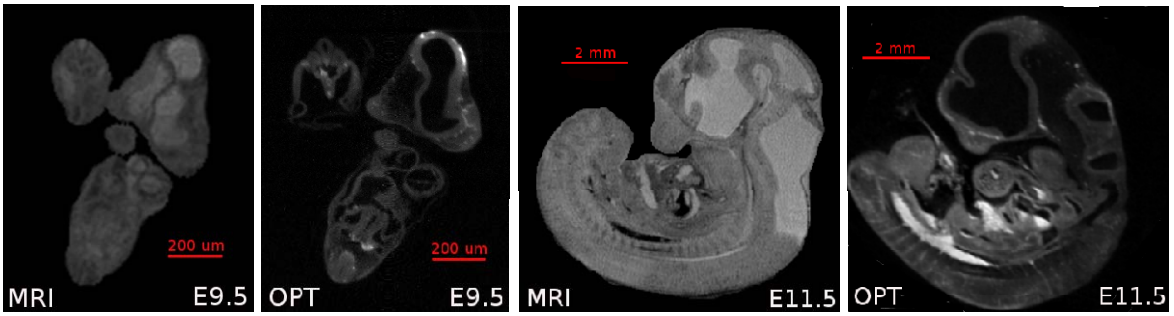
Introduction – The role of MRI in developmental biology, specifically in mouse embryo organogenesis and phenotyping, is vastly increasing due to technologies that allow for high image resolution and throughput [1]. The soft tissue contrast in MRI makes it a candidate for observing the normal development of the mouse embryo along with genetic abnormalities that may affect organogenesis. Several studies, such as developing a mouse embryo atlas [2] and the analysis of genetic mutations in the heart during mouse embryo development [3] have been accomplished through the use of MR images. However, the studies to date do not utilize MRI for meaningful analysis of embryo development before 11.5 dpc (days post coitum) because the resolution needed to resolve structures of younger embryos demands a very high SNR. The imaging modality that has become the standard for extracting 3D isotropic data sets of fixed mouse embryos is Optical Projection Tomography (OPT). OPT is an optical analogue of X-ray CT developed by Sharpe [4]. It is capable of generating high-resolution three-dimensional images of mouse embryos, ex-vivo, from the beginning of organogenesis (E9) to the approximate point of organ maturity (E15.5). These high-quality images are acquired from UV induced emission of endogenous flourophores within the embryo and is possible due to the tissue clarification with BABB (2:1 benzyl benzoate/benzyl alcohol), which allows for minimal scattering. This study specifies the role of MRI in developmental biology by comparing its ability to resolve anatomical structures throughout organogenesis against OPT.

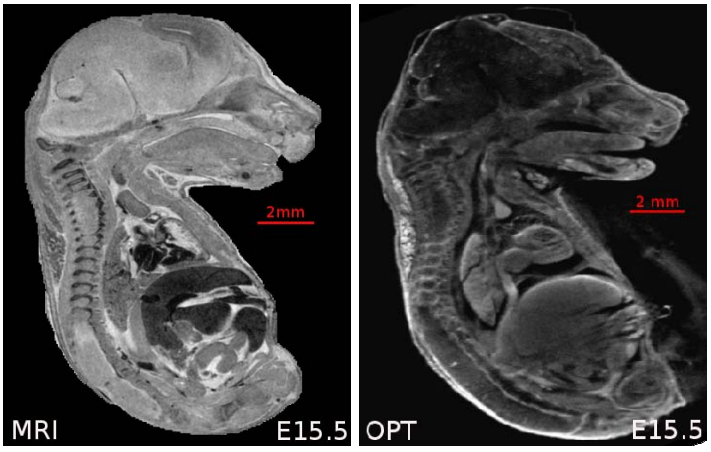
Sample Preparation – C57BL/6 mice were mated and the detection of a vaginal plug the following morning was considered 0.5 dpc. Pregnant mice were sacrificed by cervical dislocation. The embryos were then dissected and fixed in 4% paraformaldehyde (PFA) for ~ 1 week. In preparation of auto-fluorescence OPT imaging, the embryos were then washed with phosphate buffered saline (PBS) and embedded in 1% agar. Finally, these were then dehydrated with methanol for ~ 2 days, cleared with BABB in ~ 3 days and then imaged with OPT. For preparation for MR imaging, the embryos, after the 1 week of fixation, were embedded in a mixture of 1% agar and 2 mM of a MR contrast agent [gadoteridol] [ProHance, Bracco Diagnostics, Princeton, NJ] to allow for a hyper-intense background of the resulting images and the introduction of the contrast agent into the embryo.

MRI Imaging - MR images of mouse embryos from E9.5 – E15.5 were acquired with a multi-channel 7.0-T MRI scanner (Varian Inc., Palo Alto, CA) with a 6cm diameter insert gradient set. Embryos larger than $(7\ mm)^3$ were imaged in parallel with a custom built array of three 14 mm solenoid coils with overwound ends. Those smaller than $(7\ mm)^3$ were imaged in a custom built 9 mm diameter solenoid coil. A T2-weighted, 3D fast spin-echo sequence (TR = 325 ms) was used with six echos, where the first TE = 50 ms and subsequent echos with a TE = 10 ms. The center of k-space was acquired on the second echo and TE_{eff} = 60 ms. Embryos in the 14 mm solenoid coils were imaged with a FOV = 14 mm × 14 mm × 25 mm, acquisition matrix of 444 × 444 × 780, and 4 averages. The total imaging time was 11.8 hours and images with 32 µm isotropic voxels were obtained. Embryos in the 9 mm solenoid coils were imaged with a FOV = 8 mm × 8 mm × 14 mm, acquisition matrix of 324 × 324 × 560, and 10 averages. The total imaging time was 15.8 hours and images with 25 µm isotropic voxels were obtained without zero filling or extrapolation.

Methods – E9.5, E11.5, and E15.5 C57Bl/6 mouse embryos were imaged with both MRI and OPT. The same embryo was not imaged with both MRI and OPT, however, but equivalent embryos at the same developmental time point. The 9.5 dpc and 11.5 dpc mouse embryos were imaged with the 9 mm solenoid coil and the 15.5 dpc embryo was imaged in the 14 mm solenoid coil array. The OPT volumetric data produced pixel sizes of $(3.17\ \mu m)^3$, $(6.58\ \mu m)^3$, and $(15.18\ \mu m)^3$ for the 9.5 dpc, 11.5 dpc, and 15.5 dpc embryos respectively. In the 3D MRI data sets, the embryos were manually segmented from the hyper-intense background of the gadolinium doped agar using the appropriate software [Amira version 4.1]. OPT and MR data sets of embryos of the same developmental stage were then manually registered in order to view appropriate slices detailing similar anatomical structures.

Results – These figures (right) display a qualitative comparison of OPT and MRI images of embryos at similar stages of growth and development. At early stages of organogenesis, the higher resolution that OPT employs noticeably resolves anatomical structures better than MRI. However, imaging embryos at E15.5 demonstrates the depth of field limitation of OPT as the relatively large size of the embryo causes anatomy to be out of focus. Therefore, the image quality of E15.5 data sets is far superior with MRI.





Conclusions – MRI indeed has a role in developmental biology as an imaging technique. It is evident from this study that analysis of embryos at developmental stages including and past E11.5 will benefit with the use of MRI. The soft tissue contrast and consistency of the images does prove to be of, at least, comparable quality to OPT images. However, imaging embryos from E9.5 to E11 is still a challenge for MR imaging due to the resolution needed to resolve individual anatomical structures. With future developments in MR systems technology, contrast agent protocols, and MR pulse sequences, one can believe that MRI will be sufficient to monitor all stages of organogenesis in the near future.

References

1. D. Turnbull. NMR Biomed. 2007; 20: 265-274.
2. M. Dhenain. Dev Biol. 2001 Apr 15; 232(2):458-70.
3. J. E. Schneider. BMC Dev. Biol. 2004 Dec 22; 4:16.
4. J. Sharpe. J Anat. 2003 Feb; 202(2):175-81.