In Vivo 4D Magnetic Resonance Microscopy of Neurulation and Somitogenesis in Amphibian Embryos

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Synopsis Magnetic resonance microscopy offers a unique window on deep tissue movements in optically opaque embryos. The aim of this project is to develop MR microscopy for single and multiple embryo, high throughput studies with sufficient spatiotemporal resolution to explore key developmental processes during neurulation and somitogenesis in established amphibian embryonic models.

Introduction The *Xenopus laevis* (African Clawed Frog) embryo is a classical vertebrate model of early embryonic development. Recent studies of vertebrate embryos have demonstrated the potential of MR microscopy in developmental biology, particularly in gastrula stage frog embryos (1,2). Optical imaging techniques such as confocal laser-scanning microscopy are essential in the biological imaging field but are limited by the optical opacity of the organism. MRM may have a valuable role to play in this area since it is unhindered by optical opacity. We describe here initial experiences with high field MR microscopy studying neurulation and somitogenesis in single and multiple frog embryos.

Methods Wild-type *Xenopus laevis* oocytes were harvested and fertilized according to an IACUC approved protocol and incubated at room temperature (18-20°C) prior to imaging. A T1 contrast agent (5-10nl of 100mM gadoteridol, Prohance®, Bracco Diagnostics) was microinjected into either (a) both cells of the two-cell stage embryo or (b) the blastocoel fluid space of the blastula (stage 8-9) reducing T1 to less than 200ms. Volumetric spin echo MRM data were acquired using (1) an 11.7T 89mm vertical bore microscope and (2) a 9.4T 30cm horizontal bore microscope (Bruker Biospin, Billerica MA). At 11.7T, single embryos were mounted within a 1.8mm id water-susceptibility matched tube which was subsequently sealed with dental wax to minimize buffer evaporation. At 9.4T, multiple embryos were imaged *in situ* in a 35mm diameter Petri dish over a 5mm surface coil. Spatiotemporal resolution for a nominal SNR of 5-10 was 10 to 20 minutes/volume with 19um isotropic sampling for single embryos at 11.7T and 30 minutes/volume with 80um isotropic sampling for embryo groups at 9.4T.

Results and Discussion Volumetric time-courses of normally developing *Xenopus laevis* embryos were acquired over the complete course of neurulation and early tailbud stages (Figure 1). Gadoteridol did not cross the embryonic cell membranes, remaining in the labeled compartment following injection. Single embryo imaging allowed tissue-level visualization of key anatomy in neurulation. The successful acquisition of MRM time-courses from multiple embryos suggests that high-throughput, controlled imaging studies of opaque embryos are also feasible provided that spatial resolution can approach that of the single embryo time-series.

Conclusions These proof-of-concept experiments demonstrate that contrast-enhanced 4D MR microscopy of single and multiple embryos can effectively visualize key tissue structures and movements during frog neurulation and somitogenesis, complementing cellular level light microscopy of superficial cell layers.

References 1. Jacobs RE, Papan C, Ruffins S, Tyszka JM, Fraser SE. MRI: volumetric imaging for vital imaging and atlas construction. Nature Cell Biology 2003:Ss10-Ss16. 2. Papan C, Velan SS, Fraser SE, Jacobs RE. 3D time-lapse analysis of Xenopus gastrulation movements using mu MRI. Developmental Biology 2001;235(1):189-189.

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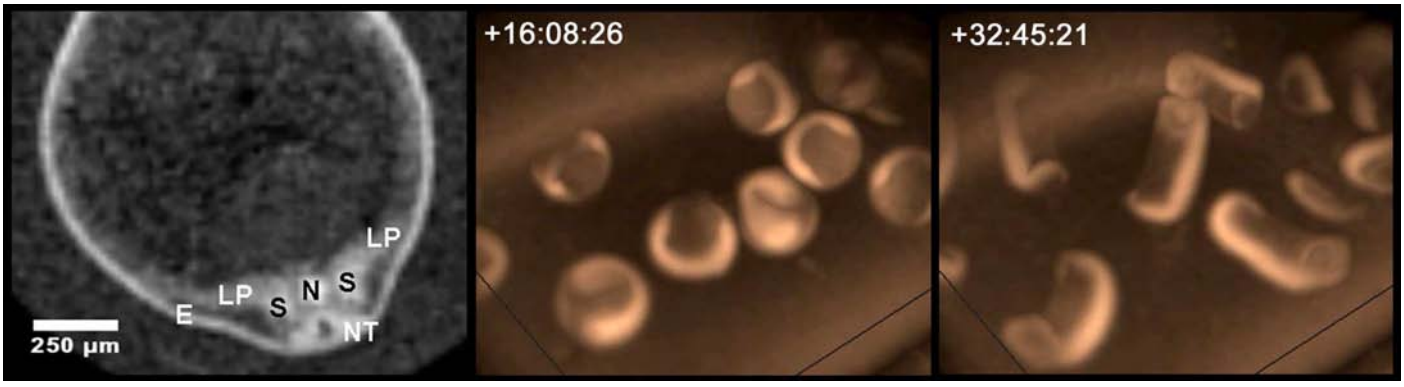

Figure 1: (Left) Transverse section from a single neurula stage embryo demonstrating details of the neural tube and surrounding anatomy (NT=neural tube, N=notochord, S=somite, LP=lateral plate, E=ectoderm) (Middle,Right) Renderings of contrast enhanced embryos during neurula (16h8m) and tailbud stages (32h45m) from a 4D dataset spanning 36 hours of development.