Diffusion Tensor Microscopy of Gastrula Stage Xenopus Laevis Embryos

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Introduction The *Xenopus Laevis* (African Clawed Frog) embryo is a classical vertebrate model of early embryonic development, particularly gastrulation and neurulation. The embryo is essentially opaque at optical wavelengths due to intracellular Yolk inclusions, providing an ideal opportunity for non-invasive study with MRI. Previous MR microscopy of *Xenopus* has included anatomic time-lapse *in vivo* imaging of gastrulation (1), water/lipid selective MRI of the oocyte (2) and diffusion spectroscopy of the oocyte (3). We present here the results of a proof-of-concept study for *ex vivo* DTI microscopy in the gastrula stage *X. Laevis* embryo.

Methods All data were acquired using a 12T Bruker Avance MR microscope equipped with 100G/cm imaging gradients. Stage 12 (late gastrula) *X. Laevis* embryos were initially fixed in PFA then saturated with 10mM Prohance® in PBS and 0.01% sodium azide for one week prior to imaging. The embryos were mounted in low-melting point agarose and imaged at 15°C in a 3mm transverse solenoid. Diffusion-weighted MRI data were acquired using an optimized 3D non-selective PGSE sequence (TR/TE = 500/12 voxel size = 36μm isotropic, NEX = 4, 2h17m/DWI). All imaging gradient pulses excepting refocus pulse spoilers were placed after the refocusing pulse to reduce residual diffusion weighting from imaging gradients, which for imaging volumes on the order of 1ml, become highly significant. Slight spatial shifts in the embryo due to diffusion gradient eddy current effects were corrected using a rigid body co-registration prior to tensor calculation. The apparent diffusion tensor field was calculated using standard methods implemented in Matlab. MR diffusion tensor images were compared to optical surface-imaging microscopy (SIM) data from an equivalent stage embryo (4). The latter has a spatial resolution of approximately 1.75μm and serves as a gold standard for cellular morphology in this study.

Results and Discussion The mean diffusivity (Tr[D]/3) in the surrounding agarose was measured at (1.65 ± 0.03) $\mu m^2/ms$ (c.f. 1.777 $\mu m^2/ms$ for pure water at 15°C). The mean diffusivity dropped to 1.0-1.25$\mu m^2/ms$ in the mesendoderm and to 0.5-1.0$\mu m^2/ms$ in the vegetal cell mass. The two most significant obstacles to successful diffusion tensor measurements in the fixed embryo are: (a) the generalized shortening of tissue T_2 which in turn limits the diffusion encoding time and (b) minimization of residual diffusion weighting from imaging gradients. In this experiment, the diffusion delay, Δ, was limited to 10ms to avoid excessive signal loss in the neurectoderm, mesendoderm and vegetal cell mass. The mean 2D diffusion displacement during Δ was approximately 5μm, which is significantly smaller than most cell diameters (10-25μm), but comparable in scale to extra-cellular spaces within the embryo. Initial analysis of tensor invariants suggest that this experiment was only weakly sensitive to restricted diffusion in the extra-cellular spaces, which have length-scales on the order of the mean diffusion displacement of the PGSE sequence. Anisotropy measures were noticeably increased and heterogeneous in the vegetal cell mass, but this most likely arose from the low signal-to-noise ratio in this tissue. Anisotropy of neurectoderm and mesendoderm tissues was only marginally higher than that of the surrounding gel, implying that increasing Δ beyond 100ms (for example by diffusion weighted stimulated echo) will be required to improve detection of *Xenopus* cellular anisotropy with DT microscopy.

Conclusions DTI on the scale of the *Xenopus* embryo is feasible and despite T_2 limitations on diffusion length, anisotropy contrast is observed which may prove useful in following developmental interactions during gastrulation and neurulation. These results suggest that further development of DTI methodology for *in vivo* application in *Xenopus* could be of value to developmental biology.

References 1. Papan, C., Velan, S. S., Fraser, S. E. & Jacobs, R. E. (2001) *Developmental Biology* 235, 189-189.
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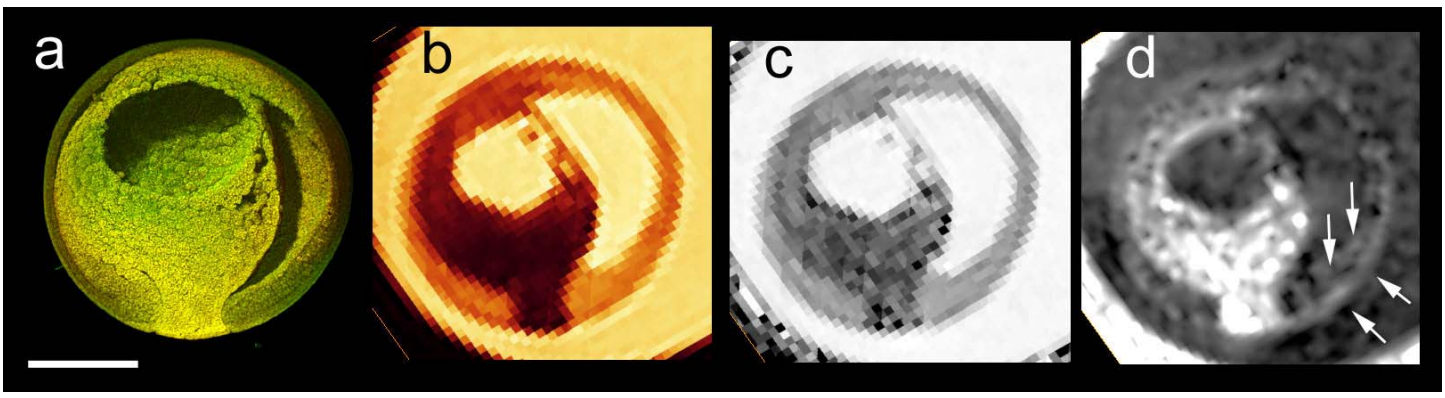

Figure 1: Comparison of mid-sagittal sections through stage 12 *Xenopus* embryos from (a) SIM rendering, (b) iDWI, (c) Tr[D] and (d) FA maps. Note the FA contrast between the ectoderm and mesoderm (arrows). Scale bar: 500μm.