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In toto light sheet imaging for the reconstruction of developing vertebrate embryos

Long-term in toto imaging of developing vertebrate embryos to achieve the full reconstruction of their lineage tree is still a major challenge. Based on Single Plane Illumination Microscopy (SPIM by PhaseView http://phaseview.com/alpha3/), we developed a methodology for long-term in toto imaging of zebrafish and rabbit embryos and demonstrate its performance in terms of cell detection and tracking. We achieved an optimal trade off between spatial and temporal resolution, signal to noise ratio and field of view to accurately follow cell displacements and cell divisions in whole embryos, while minimizing phototoxicity. We developed specific molds and protocols for live specimen mounting and the maintenance of homeostatic conditions for up to 4 days in the case of mammalian embryos. Following our main goal of reconstructing cell dynamics in embryonic morphogenesis to reveal pattern formation, 3D+time image data was processed for cell detection and tracking with the BioEmergences workflow (http://bioemergences.eu). Validation and annotation of the reconstructed data was done with Mov-IT, our custom-made visualization tool [1]. Comparing the cell lineage accuracy between our SPIM and 2-photon point laser scanning data demonstrates the advantage of our SPIM based strategy. In addition, we propose that our protocol for in toto imaging and reconstruction of zebrafish early embryogenesis performs far beyond the current state of the art. Our SPIM imaging strategy together with the BioEmergences image processing workflow is meant to build large cohorts of 3D+time datasets of the highest quality to open the way to large scale *in silico* embryology.

[1] E. Faure et al., “A workflow to process 3D+time microscopy images of developing organisms and reconstruct their cell lineage”, *Nat. Commun.* 7, 8674, (2016).

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