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Light-Sheet Microscopy: studying zebrafish microtubules during embryonic development

The microtubule network is an essential part of the cell, providing structure and shape. It is also important for intracellular transport of cargos, which is crucial for correct embryo morphogenesis. Microtubules are dynamic structures that undergo continual assembly and disassembly within the cell.
In particular, yolk microtubule organization undergoes several changes over the various developmental stages in zebrafish. Nowadays, its arrangement in the yolk is still poorly understood since the problem has been undertaken only partially, in time and space. The primary approach has been immunostaining on fixed samples, hiding the rich variety of phenotypes over time [1]. Recently, the use of transgenic lines and multiphoton and confocal microscopy allowed the dynamical study of those processes, although only in restricted areas and with several side effects [2].
Light-Sheet Fluorescence Microscopy (LSFM) offers uniques capabilities for the live imaging, such as low phototodamage, fast acquisition rate, and the possibility to reconstruct high quality images of whole organisms. The aim of our project is to obtain a mesoscopic 3D view of the microtubule skeleton dynamics of the zebrafish yolk throughout all epiboly stages using a home-made implementation of LSFM, so called Flexi-SPIM. Our system allows, on a single microscope, to operate in different modalities. Samples can be either embedded in agarose blocks to perform multi-view time-lapse movies of the whole embryo, or be transported through FEP tubes [3], increasing the imaging throughput.
Different zebrafish transgenic lines are investigated in order to unveil the microtubule dynamics. We observed a high variability between embryos, showing various phenotypes during epiboly stages. In order to establish statistical value to our findings, we exploited the unique high-throughput capabilities of our system by imaging tens of samples, in a straightforward manner, through a semi-automated fluidic sample loading system.

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