

The cell lineage in the zebrafish brain from progenitors to stem cells

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Laboratory

Multiscale dynamics in animal morphogenesis
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Subjects / Tools-Methodologies

- 1 : Morphogenesis/4D imaging
- 2 : Multiscale reconstruction/Image processing and analysis
- 3 : Biomechanics/Tensors calculation

Summary of lab's interests

We work to establish strategies for achieving the reconstruction of living systems multiscale dynamics. To this aim, we first provided a framework for the cellular level of organisation and we now tackle the phenomenological reconstruction of micro (molecular and genetic) and macro levels (biomechanical constraints) of organisation. We work in an interdisciplinary context to define the appropriate theoretical framework for the multiscale modelling, representation and visualisation of the system dynamics. Such a systematic and systemic approach has not been achieved so far and is a challenge both at the experimental and theoretical level. Our operational goals include: - Reconstructing molecular and genetic network architecture with special attention to connecting cell cycle regulation, adhesion regulation, cytoskeleton regulation and gene expression regulation. This is achieved through combining available data, functional genetics, in vivo imaging of transgenic fish lines reporting biological activities, double fluorescent in situ hybridization, QRT-PCR and RNA profiling. This combination of strategies should allow gathering quantitative data with the appropriate spatial and temporal resolution to constrain the theoretical models parameters range. Testing the dynamical models will be achieved through gain and loss of function experiments (genetics and experimental embryology). - Improving and extending our reconstruction strategies of the cell lineage from in vivo and in toto 4D data. This includes the exploration of i) novel microscope prototypes (modified SPIM with double illumination and detection and improved detection means), ii) new staining strategies (collaboration with chemists tailoring probes for in vivo imaging of kinases activity and RNA dynamics) and iii) improved algorithmic pipelines (ongoing collaboration with CREA, UPM and STUBA our EC projects partners). - Achieving the reconstruction of tissues biomechanics by combining in vivo imaging of transgenic fish lines with either stained microfilaments, or microtubules or extracellular matrix and direct mechanical perturbation of embryogenesis.

Summary of project

The automated reconstruction of the cell lineage from in toto 4D imaging provides adequate measurements for the spatio-temporal correlation of cell proliferation regulation with specific morphogenetic events. From early embryogenesis to adulthood, the proliferation rate is thought to slow down until post mitotic differentiation and be kept at slow rates for stem cells involved in tissues homeostasis.

We aim at following this process to reconstruct the clonal history of adult tissues stem cells through 4D in toto imaging of cell clones fluorescently labelled with a random strategy: nuclear staining with an H2B/eGFP (or H2B/cerulean) fusion protein expressed early during development (pre-gastrulation stages) under the control of ubiquitous regulatory sequences after intragenic recombination by a tamoxifen activated Cre/ERT recombinase, in the context of the

expression of a red membrane staining under the control of regulatory sequences expected to target neural stem cells (GFAP:mcherry). In toto 4D imaging focusing on a morphological compartment such as the forebrain allows the phenomenological reconstruction cell clonal history and characterize the cell proliferation modes.

We validated this approach in the context of the EC projects Embryomics et BioEmergences by reconstructing the complete cell lineage 4D branching process during early steps of the zebrafish brain morphogenesis. Changes in the proliferation rate or cell division characteristics correlation with cell neighbourhood characteristic features will be assessed through:

- Cell neighbourhood persistence - Visco-elastic properties of the tissue estimated through the measurement of cell neighbourhood deformation around dividing cells - Cell shape changes measured by using membrane staining segmentation
- Cell division characteristics: equal, unequal, symmetric or asymmetric
- Modulation in intracellular calcium concentration observed through 4D imaging of a calcium sensor (G-CAMP or Pericam) expressed under the control of ubiquitous regulatory sequences (green cytoplasmic staining combined with blue nuclei and red membranes)
- Redox metabolism measured through two-photon (2P) ratiometric redox fluorometry and microscopy of pyridine nucleotide (NAD(P)H) and flavoprotein (FP)

We previously demonstrated our 4D imaging strategies either by multiphoton laser scanning microscopy or by SPIM/DSLIM and of the algorithmic chain of the BioEmergences platform for 4D image processing and cell morphodynamics phenomenological reconstruction throughout early embryogenesis of chosen animal models including *Danio rerio* (Embryomics and BioEmergences EC projects). We now aim at using the same strategies to follow the lineage of neural progenitor during late developmental stages and possibly until early adulthood by in toto 4D imaging through extended periods of times in different animals to insure animal and cell survival, in order to produce partially overlapping 4D sequences allowing to reconstruct at least in probability the cell lineage within a morphogenetic compartment. To this aim, imaging will be performed in a non-pigmented fish line.

This strategy should allow characterizing the features correlating with the maintenance of cell proliferation and identifying a hypothetical stem cells niche and the cell morphodynamics within this niche.

This phenomenological reconstruction of cell behaviours will serve as the basis for the integration of processes at other levels of observation including the molecular and genetic level. Models to begin with at least qualitative will be provided as an attempt to reconstruct the causal chain of events underlying cell behaviours. Models predictions will be tested through the transplantation at early developmental stages of mutant cells affected in signalling pathways potentially involved in the segregation and maintenance of neural progenitors lineages.