3D imaging-based analysis of the germline in teleost

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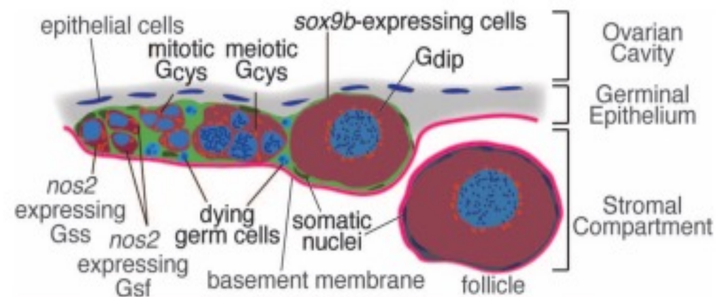
Context

- In mammals, the stock of oocytes is predetermined at birth, whereas in teleosts, the oocyte reserve is renewed throughout life
- Use of the reserve in adults to produce mature oocytes and reconstitution of the reserve from germline stem cells
- Germline stem cells organized in germline cradles surrounded by somatic cells expressing sox9 (1)



How do germline stem cells contribute to the renewal of the oocyte reserve and what are the regulatory mechanisms involved in this renewal?

Objectives

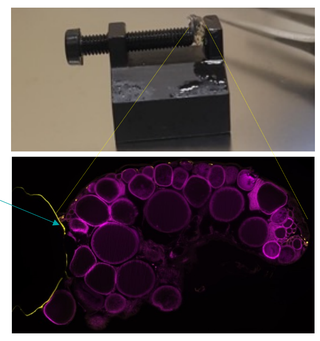
To explore the oogenesis renewal process and its regulation, we have chosen the medaka as a model organism and developed a **3D imaging strategy for the whole ovary**. This approach aims to provide us with **quantitative data to study the cellular dynamics of germinal cradles**.

- Study oocyte reserve formation and renewal
- Identify the molecular regulators of oocyte reserve

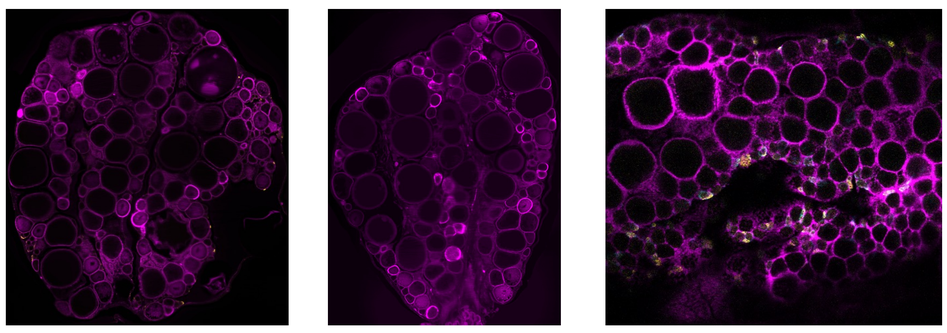
Imaging and image analysis

In order to image the germinal cradles and follicles, we have developed a whole ovary immunolabeling protocol combined with the C-ECi clearing protocol (2). Once the protocol is completed, we must transfer our samples to agarose for **3D imaging using Light Sheet Blaze imaging** (APEX platform, ONIRIS, Nantes). Without this agarose mounting, the screw used during acquisition was visible and distracting, and the pressure exerted by the screw could damage the sample.

Without agarose :



With agarose :

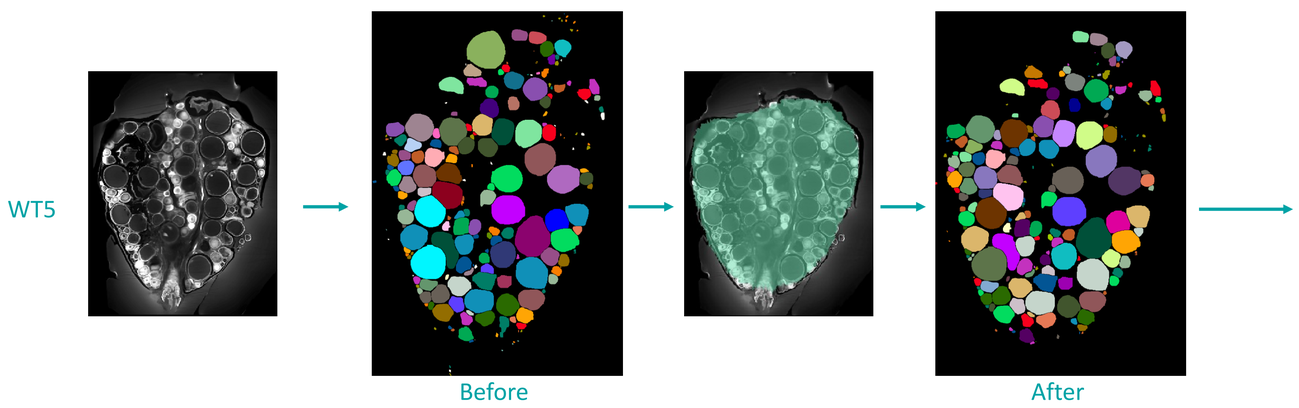


Denoising and segmentation of images using the N2V module and Cellpose to study follicle distribution, followed by error correction and diameter measurement with AMIRA software (3).
Adaptation of these analysis steps for the quantitative study of the germinal cradles.

<u>Emergence of several difficulties with Light Sheet acquisitions</u>

- 1st difficulty

Cellpose segmentation detecting many false positives in agarose.
Development of a custom Cellpose model to segment the whole ovary and create an ovarian mask, avoiding false-positive labels in agarose.

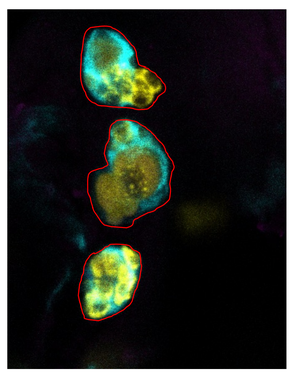


- 2nd difficulty

Very large Light Sheet microscopy images, over 100GB each after stitching.
Pre-processed images between 800KB and 2.5GB, causing challenges in opening and segmenting them with Cellpose (potential use of CellposeDask extension).

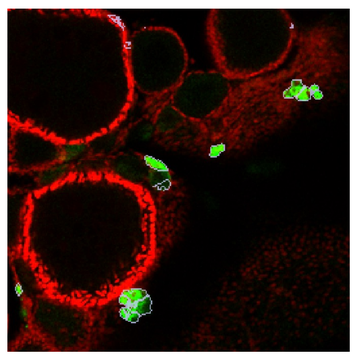
Prospects and difficulties for image analysis

1- Study the distribution and number of cradles in medaka ovaries



- Denoising with N2V
- Segmentation of vasa-positive germline stem cell clusters surrounded by somatic cells expressing sox9 with Cellpose_Napari

2- Study the composition of germinal cradles



- Difficulty : vasa-labelling is cytoplasmic so these cells are difficult to segment individually
- We want to use MG core labelling to separate vasa-positive cells individually with Cellpose Napari

References

1- Nakamura, Shuhei, et al. "Identification of germline stem cells in the ovary of the teleost medaka." *Science* 328.5985 (2010): 1561-1563.

2- Lesage, Manon, et al. "C-ECi: a CUBIC-ECi combined clearing method for three-dimensional follicular content analysis in the fish ovary." *Biology of Reproduction* 103.5 (2020): 1099-1109.

3- Lesage, Manon, et al. "An end-to-end pipeline based on open source deep learning tools for reliable analysis of complex 3D images of ovaries." *Development* 150.7 (2023): dev201185.