Optimizing OoCount: a machine-learning based approach to oocyte counting

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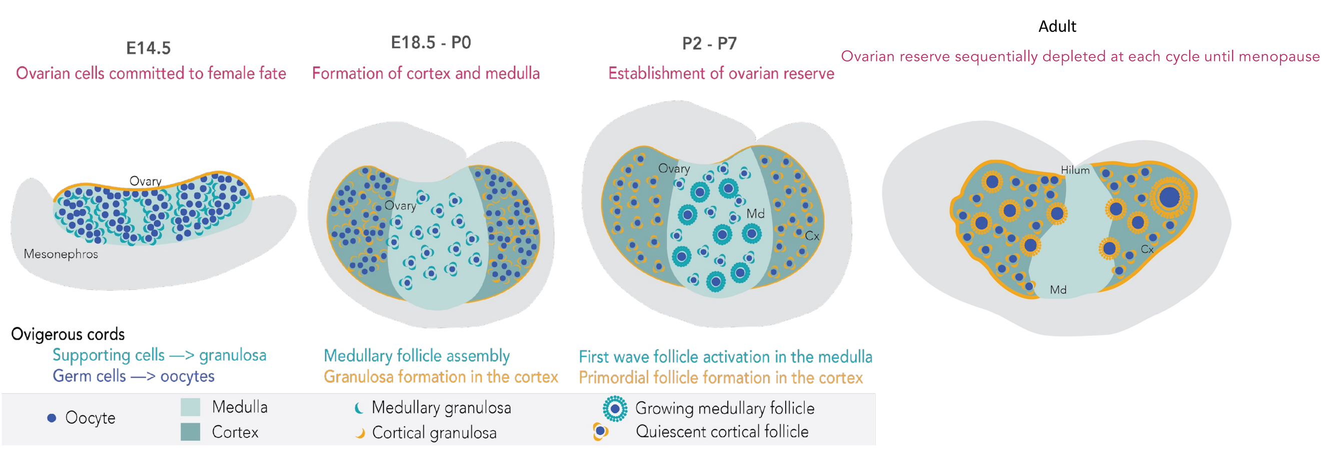
Goal: Create an open-source, high-throughput method for automatic oocyte segmentation and classification from fluorescent microscopy images using a machine-learning based approach.

Lifelong fertility is established during fetal and perinatal ovarian development



There is currently no standardized method for follicle counting in the field

The total reported number of follicles present in the ovaries at any given time in life can vary by 10 - fold or more, depending on the study[2,3]

While our methods for 3D imaging have improved the study of ovarian morphogenesis and female reproductive success, we lack accessible tools to analyze and extract data from these images

Analysis of bio-images using deep learning algorithms is efficient and in recent years has become more user-friendly and accessible to non-specialists

Methods

Tissue collection and processing: Ovaries were collected from postnatal day (P) 3 and 7 CD-1 mouse pups and prepped for whole mount using a methanol gradient.

Immunostaining for confocal imaging: Samples were rehydrated in PBS. IHC for Vasa, an oocyte marker, was performed. Samples were embedded in tissue clearing gel and staged in reversible 3D printed slides.[1]

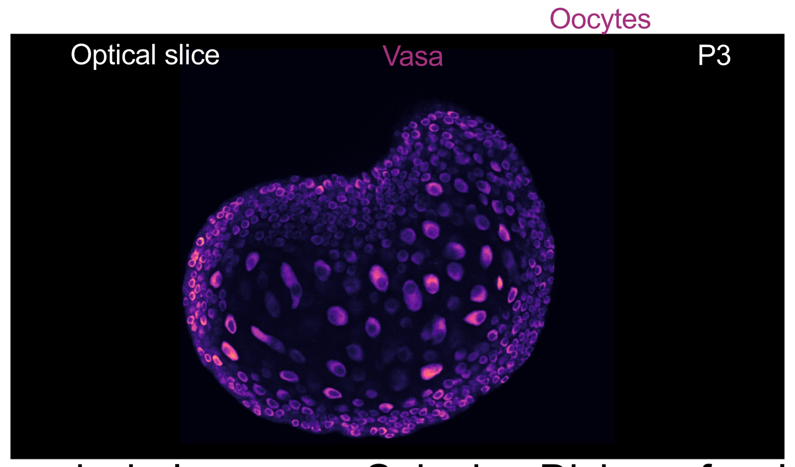
Image acquisition and processing: Images were acquired using the Andor Dragonfly Spinning Disk confocal and confocal Z-stacks converted from .ims to .tif to be compatible with our open-source pipeline. Images were viewed and annotated in Napari.[4]

Machine learning: Manually annotated training dataset was used to augment StarDist segmentation model using ZeroCostDL4Mic, a collection of self-explanatory Jupyter notebooks for Google CoLab for deep-learning applications for microscopy.[5,6]

Workflow

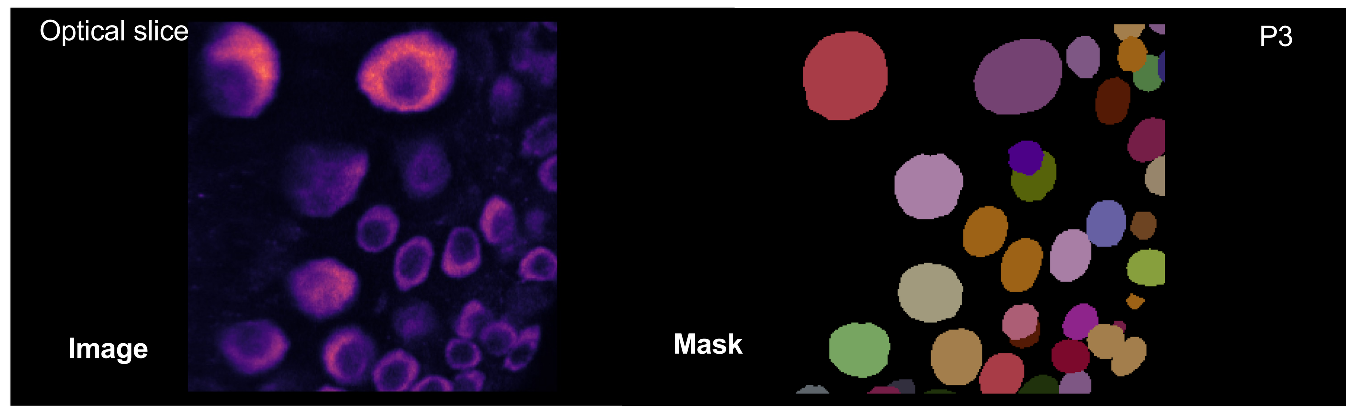
napari

Create training dataset with manually annotated images

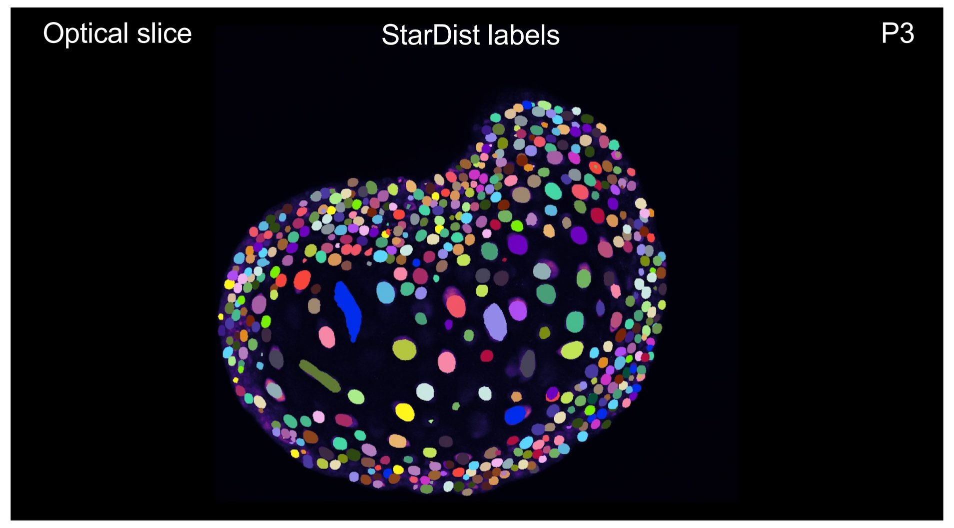


Cleared whole ovary – Spinning Disk confocal

Retrain existing StarDist model with manually annotated oocyte dataset



Automatic segmentation of oocytes with retrained model

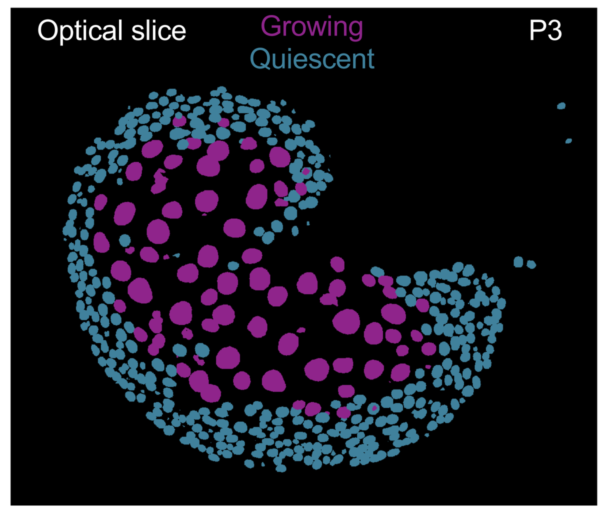


Cleared whole ovary – Spinning Disk confocal

APOC

Accelerated Pixel and Object Classification

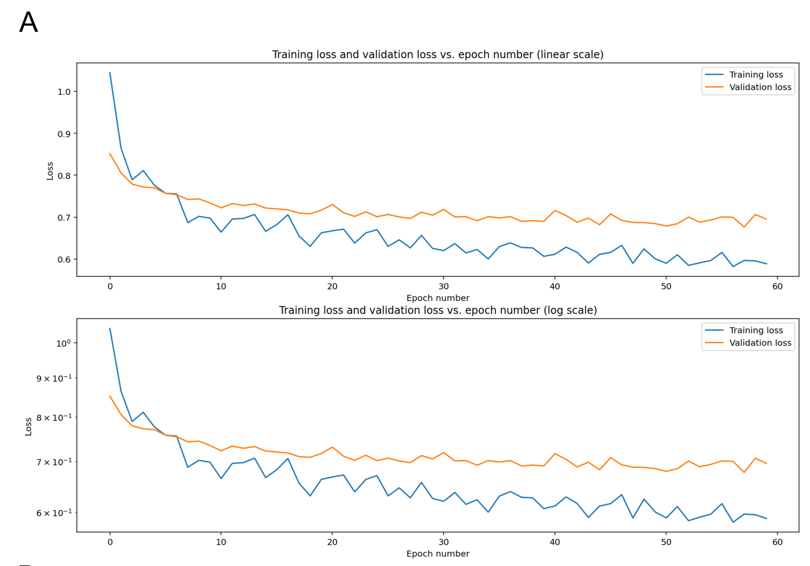
Train classifier to recognize growing vs quiescent follicles



Count growing vs quiescent follicles

Cleared whole ovary – Spinning Disk confocal

OoCount identifies oocytes in whole mount ovaries with 73% accuracy



A: Representative learning curve from machine learning to determine performance. Plots shown are from training dataset applied to existing StarDist model with 60 epochs

B: Intersection over Union (IoU): 0.733

Conclusions and Implications

- Currently OoCount identifies oocytes in whole mount ovaries with ~73% accuracy
- An open-source image analysis pipeline is a much-needed resource in the field

Future work

Increase versatility of OoCount to:

- Stage follicles in adult ovaries
- Count oocytes in cryosections of ovaries

Create a python package to make OoCount an accessible ready-to-use tool

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References: 1. McKey, et al. 2022. 2. Tilly. 2003. 3. Meyers, et al. 2004 4. napari contributors, 2019. 5. Schmidt, et al., 2018 6. Chamier, et al. 2021

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