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

Mammalian females are born with a limited supply of oocytes, thus reproductive longevity is determined by the endowment of oocytes at birth and the length at which that pool lasts. The study of ovarian morphogenesis and female reproductive success has been advanced by the development of techniques for three-dimensional (3D) imaging of ovaries *in toto*. Currently, the field lacks a standardized method to quantify the number and stage of follicles from 3D images of ovaries. Analysis of bio-images using deep learning algorithms is efficient and in recent years has become more user-friendly and accessible to non-specialists. We are creating and optimizing OoCount, a high-throughput method for automatic oocyte segmentation and classification from fluorescent microscopy images using a machine-learning based approach. Fluorescently labeled oocytes from 3D images of whole mount perinatal ovaries are manually annotated in Napari, a python-based image viewer, to develop a machine learning training dataset. Next, a deep-learning Stardist model is trained using the dataset and applied to ovary images, where it now labels all oocytes in the ovary. Then, oocytes are classified as growing or quiescent based on the model in Accelerated Pixel and Object Classification. The finalized version of OoCount will be user-friendly and require minimal computational skill. We expect OoCount to become a major resource for the field.