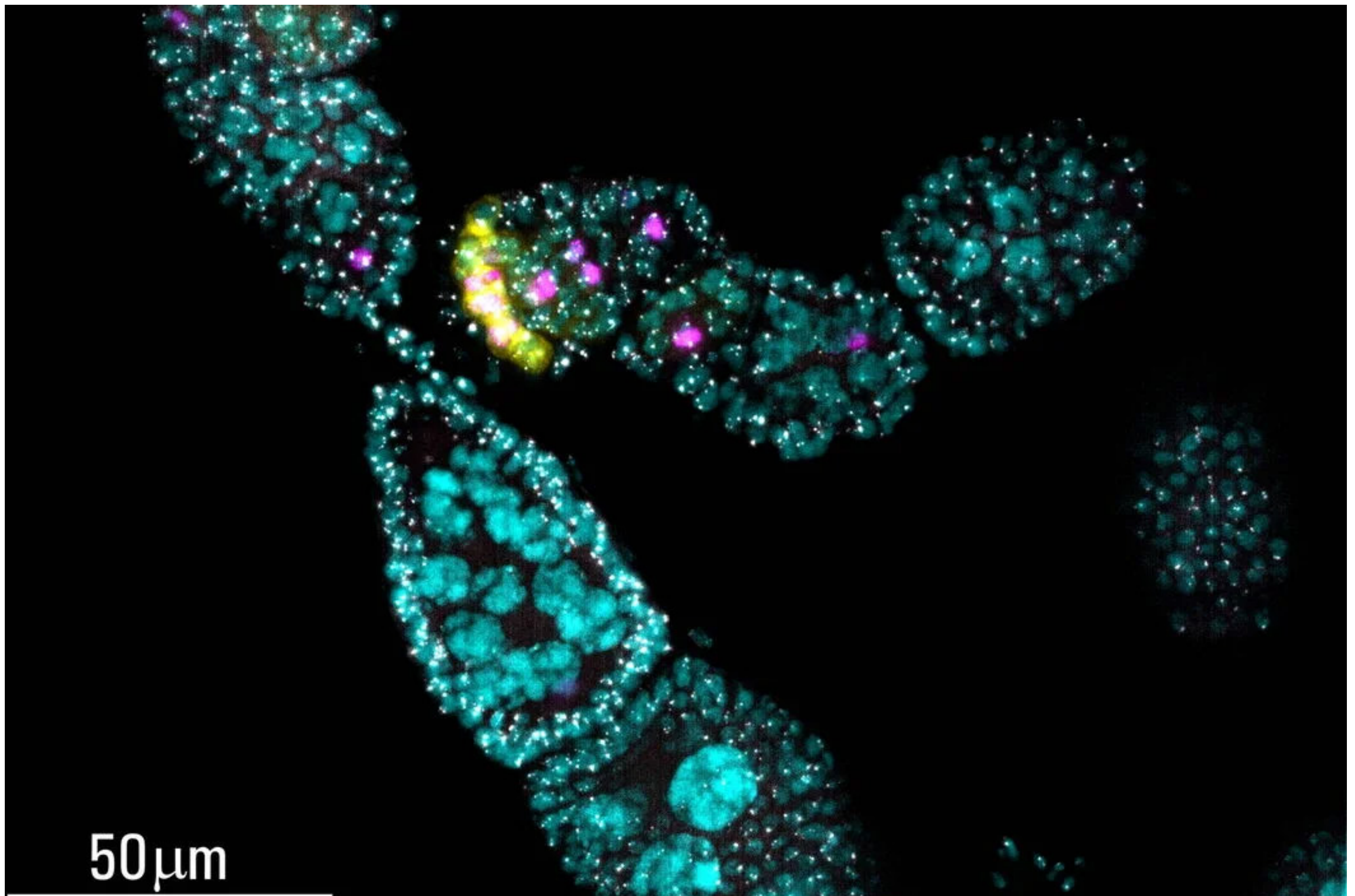
From Eye to Insight

Leica MICROSYSTEMS

HEALTHY OOCYTE DEVELOPMENT

Studying mechanisms behind reproductive fitness, like meiosis and other processes, with haze-free imaging of Drosophila oocytes in the germarium



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Abstract

This article discusses how the study of mechanisms involved in the generation of healthy oocytes in Drosophila fruit flies can be helped with sharp, haze-free images acquired with a THUNDER Imager Live Cell. An example of a poorly understood mechanism is synapsis which is crucial for chromosome segregation. Fine structures of oocytes in the germarium can be revealed in THUNDER images, making them useful for a better understanding of meiosis and other processes leading to healthy oocyte generation.

Background: Research on oocyte development

The fruit-fly model organism Drosophila melanogaster is used in genetics and developmental biology to study the fundamental mechanisms of heredity. An important focus of developmental biology research is the identification and characterization of genes that are important for reproductive fitness, including the segregation of chromosomes during meiosis and other processes that lead to the generation of a healthy oocyte [1-3].

Formation of the synaptonemal complex (SC), or synapsis, between homologs in meiosis is essential for crossing over and chromosome segregation [3]. How the SC assembly initiates is poorly understood, but may have a critical role in ensuring synapsis between homologs and regulating double-strand break and crossover formation. Investigations of the genetic requirements for synapsis in Drosophila have shown that there are 3 temporally and genetically distinct stages of synapsis initiation. These are early zygotene oocytes where the synapsis is only observed at the centromeres, mid-zygotene oocytes where the SC initiates at several euchromatic sites, and late zygotene oocytes where the SC initiates at many more sites that depend on the Kleisin-like protein C(2)M [3].

Challenges when studying oocytes

Thicker specimens like oocytes can be challenging to image with widefield fluorescence microscopy, due to the haze or out-of-focus blur produced by light scattering. Structures of interest deep inside the specimen can be obscured by the haze.

Methods used

Oocytes of Drosophila melanogaster in the germarium in early prophase stage were used for imaging. DNA is labelled and shown as cyan, oocytes express a green-orb fluorescent protein, centromeres were immunolabelled with anti-CENP-A antibodies and appear white, and the C(2)M cohesion protein were labelled showing as magenta. Imaging of the germarium through approximately a 13 µm depth was done utilizing a THUNDER Imager Live Cell with a 63x, 1.4 numerical aperture (NA) objective. A maximum projection was acquired and Instant Computational Clearing (ICC) applied.

Results obtained

An image of the oocytes acquired with the THUNDER Imager Live Cell is shown below in figure 1.

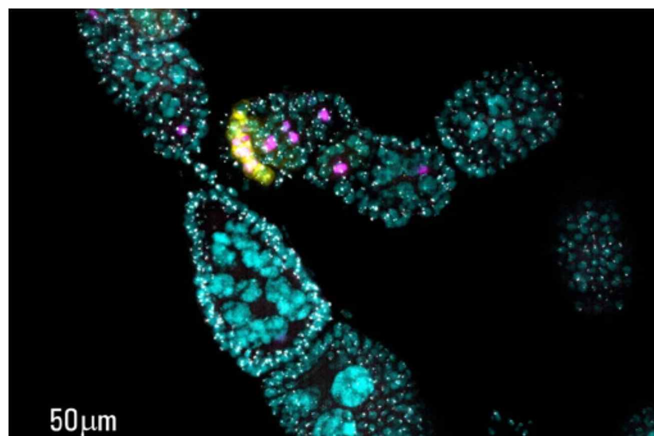

Fig. 1: Maximum projection image of early prophase stage Drosophila germarium after ICC. Cyan indicates DNA, green oocytes, white centromeres, and magenta cohesion proteins. Image courtesy of Dr. Jessica Fellmeth, INSPIRE Postdoctoral Fellow, Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ, USA.

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Conclusions

The results presented here show that oocyte imaging with a THUNDER Imager Live Cell can help reveal details that are useful for studying mechanisms concerning the generation and development of healthy oocytes.

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