APPLICATION NOTE

High-resolution, live-imaging of *C. elegans* germline using *vivo*Chip™

Purpose

The study of chromatin organization and defects in embryonic development of *C. elegans* using the *vivo*Chip, which allows for high-resolution imaging of the entire 3-dimensional (3D) structures of the germline in *C. elegans.*

*vivo*Chip™-2x

Methods

- Up to 40 adult animals are immobilized and oriented in the *vivo*Chip-2x within 3 min.
- High-resolution images of the germlines are captured in 3D.
- The entire germline is visualized using two-color fluorescence reporters (see Fig 1).
- Chromatin organization/defects are identified and dead/viable embryo populations are quantified from the live time-lapse imaging of immobilized animals.

100μm
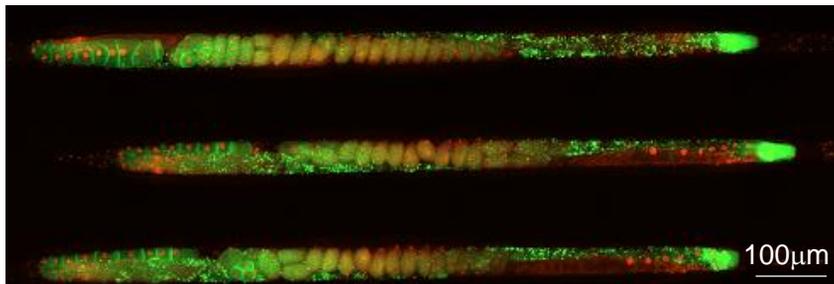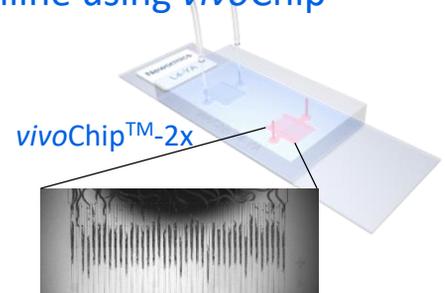
Figure 1. Images of AUM1039 animals using 20×, 0.75NA objective in the Lionheart BioTek. The animals express a membrane bound GFP (pie-1::gfp::PH) and a nuclear mCherry (pie-1::mCherry::his-58)[1].

Oocytes

Worm #1

Spermatheca

Pachytene

Worm #2

Z-stack #1   Z-stack #2
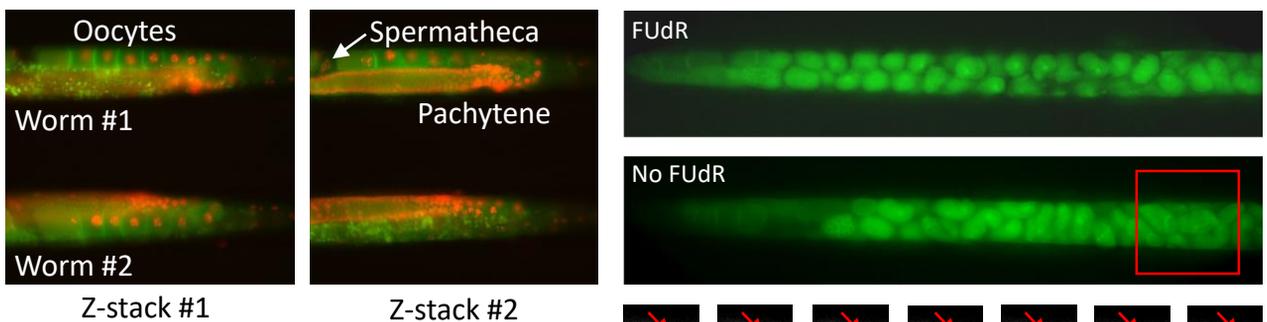
Figure 2. AUM1039 germlines from two animals immobilized inside the parallel channels showing oocytes, spermatheca, and pachytene structures in two z-stack images.

FUdR

No FUdR

0s   2.1s   4.2s   6.3s   8.4s   10.5s   12.6s

Figure 3. Adult *C. elegans* in presence and absence of FUdR, an inhibitor for DNA synthesis. Bottom panel: Time-lapse images of the ROI (red box) collected at 2.1 s intervals show movement of viable embryos.

Conclusions

➢ The *vivo*Chip enables uniform orientation of animals during immobilization for high-resolution imaging of entire germline organization.

➢ Anesthetic-free, time-lapse imaging provides a unique method to identify viable embryos.

➢ The *vivo*Chip facilitates statistical evaluation of worm size, total number of embryos, and embryo viability for the development and reproductive toxicity (DART) assessment.

➢ Live imaging using *vivo*Chip also allows researchers to investigate mechanisms of toxicity by identifying defects across different stages of meiosis and embryonic development.

[1]AUM1039 ItIs37 [P-pie-1::mCherry::his-58 (pAA64) + unc-19(+)]; ItIs38 [P-pie-1::GFP::PH (PLC1delta1) + unc-19(+)] was gifted by Prof. Swathi Arur, MD Anderson.