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Title: Nuclear Migration in the *Drosophila* Oocyte

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera? **No**

   If your protocol involves microscopy, but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Zeiss Stemi 2000C Zoom 6,5 à 50X

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 08
Number of Shots: 25

Introduction

Note: VO talent please record all the interview statement

1. Introductory Interview Statements

1.1. This technique makes it possible to observe long biological processes in dissected Drosophila egg chambers such as oocyte nuclear migration using live imaging microscopy.

1.1.1. *6.2.3*

1.2. This protocol describes how to maintain the dissected egg chambers alive for 12 hours to perform live imaging microscopy.

1.2.1. *6.1.2*

Protocol

2. Imaging Medium Preparation

2.1. To begin, pipette 200 microliters of Schneider medium **[1]** and supplement with 30 microliters of 10 milligrams per milliliter insulin **[2]**. Then, add 4 microliters of heat-inactivated fetal calf serum **[3]**.

2.1.1. Talent pipetting 200 microliters of Schneider medium.

2.1.2. Talent adding 30 microliters of insulin.

2.1.3. Talent adding 4 microliters of heat-inactivated fetal calf serum. NOTE: take 2

3. Observation-Chamber Preparation

3.1. Use a pipette tip to apply a small amount of silicone grease all around the hole on the underside of the punctured slide **[1]**. Position a coverslip **[2-TXT]** and use the wide end of a pipette tip to apply pressure on the coverslip to flatten the silicone grease for creating a silicone ring interior to the slide **[3]**.

3.1.1. Talent applying silicone grease. *Videographer: This shot is important!*

3.1.2. Talent placing the coverslip. **TEXT: Coverslip: Dimension-24 x 50 mm; Thickness-0.13–0.16 mm** NOTE: take 2

3.1.3. Talent applying pressure with wide end of tip on the silicone grease. *Videographer: This shot is important!* NOTE: CU of the sealing at the end

4. Ovary Dissection

4.1. Transfer the anesthetized female flies to the dissecting well containing 150 microliters of the imaging medium **[1]**. For dissecting the female, use the forceps to grab the thorax **[2]** and pinch the dorsal abdomen cuticle with a second pair of forceps **[3]**. Isolate and detach the pair of ovaries visible upon the cuticle opening **[4]**.

4.1.1. Talent transferring the female flies in the dissecting well.

4.1.2. Talent grabbing the thorax. NOTE: take 3

4.1.3. Talent pinching the dorsal abdomen cuticle with forceps. NOTE: with 4.1.2

4.1.4. Talent detaching the ovaries. NOTE: with 4.1.2

4.2. Carefully remove the uterus, oviduct, and muscle sheath **[1]**. Place a drop of 10 to 15 microliters of the imaging medium on the ovaries NOTE: goes before step 4.1.4 **[2]** and transfer one ovary to the imaging chamber **[3]**.

4.2.1. Talent removing the uterus, oviduct, and muscle sheath. NOTE: with 4.1.2, goes before step 4.1.4

4.2.2. Talent placing drop of the medium in the imaging chamber.

4.2.3. Talent transferring one ovary in the imaging chamber. NOTE: with 4.2.2, use second part

5. Egg Chamber Isolation

5.1. For separating the ovarioles, use the needle to hold the posterior end of the ovary **[1]**. Use another needle to carefully pull the germarium for teasing the ovarioles apart **[2]**. Hold the sheath with one needle **[3]** and pull the ovariole through the larger chambers with another needle to remove the remaining muscle sheath on the egg chambers **[4]**.

5.1.1. Talent holding the posterior end of the ovary with the needle. *Videographer: This shot is important!* NOTE: take 1 : 5.1.1 to 5.1.4, take 2 : 5.1.1 to 5.1.2, take 3 : 5.1.3 to 5.1.4

5.1.2. Talent pulling the germarium.

5.1.3. Talent holding the sheath with the needle. *Videographer: This shot is important!* NOTE: use 5.1.1 take 3

5.1.4. Talent pulling the ovariole through the larger chambers. *Videographer: This shot is important!*

5.2. Allow the unsheathed ovariole to sink and contact the coverslip **[1]**. Remove the late stages and rest of the ovaries from the micro-chamber using forceps **[2]**. Use needles to carefully distance the ovarioles to facilitate the acquisition **[3]**.

5.2.1. Ovariole sinking and touching the coverslip.

5.2.2. Talent removing the late stages and rest of the ovaries from the micro-chamber. NOTE: with 5.2.1

5.2.3. Talent separating the ovarioles with needles. *Videographer: This shot is important!* NOTE: with 5.2.1

6. Observation Chamber Closing

6.1. Cut a small 10 by 10-millimeter square of the permeable membrane **[1]**. Carefully apply the membrane on the top of the imaging medium to expel any air bubbles **[2]**, then hermetically seal the chamber with a thin layer of halocarbon oil around the well on the contour of the membrane **[3]**.

6.1.1. Talent cutting a square piece of the permeable membrane.

6.1.2. Talent applying the membrane on the top of the imaging medium.

6.1.3. Talent sealing the chamber with halocarbon oil.

6.2. Place the imaging set-up on the slide holder of the inverted microscope and use a 40x objective **[1]**.

6.2.1. Talent placing the imaging set-up on the slide holder of the inverted microscope using the 40x objective lens.

Results

Note: VO talent please record all the interview statement

7. Results: Nuclear Migration

7.1. The migration of the oocyte nucleus of a wild-type egg chamber was captured using live-imaging microscopy **[1]**. The nucleus reaches either the anterior plasma membrane or the lateral plasma membrane before sliding along the trajectories to reach its final position at the intersection of the two plasma membranes **[2]**.

7.1.1. LAB MEDIA: Figure 5

7.1.2. LAB MEDIA: Figure 5B and Movie_2 Loh et al: 00:00 to 00:03

7.2. Membrane deformities, disorganized follicular cells, stunted cells, and shrunken nuclei are the first signs of dying egg chambers **[1]**. The degenerated oocyte before 8 hours of imaging is not usually detected **[2]**. However, rare cases of early degeneration are due to problems during the dissection or mounting steps **[3]**.

7.2.1. LAB MEDIA: Figure 6

7.2.2. LAB MEDIA: Figure 6A

7.2.3. LAB MEDIA: Figure 6B

Conclusion

8. Conclusion Interview Statements

8.1. Damaging the egg chambers compromise their survival, so delicate and precise dissection and preparation of the micro-chamber are paramount.

8.1.1. *5.1.4*

8.2. This procedure allowed us to visualize that the oocyte nucleus can take three different trajectories during its migration and different organelles regulate these trajectories within the oocyte.

8.2.1. *6.2.1*