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Nuclear migration in the Drosophila oocyte

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TITLE:

Nuclear Migration in the *Drosophila* Oocyte

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KEYWORDS:

nuclear migration, nucleus positioning, *Drosophila*, oocyte, microtubules, live imaging

SUMMARY:

In *Drosophila*, the oocyte nucleus undergoes microtubule-dependent migration during oogenesis. Here, we describe a protocol that was developed to follow the migration by performing live imaging on egg chambers *ex-vivo*. Our procedure maintains egg chambers alive for 12 h to acquire multi-position 3D time-lapse movies using spinning-disk confocal microscopy.

ABSTRACT:

Live cell imaging is particularly necessary to understand the cellular and molecular mechanisms that regulate organelle movements, cytoskeleton rearrangements, or polarity patterning within the cells. When studying oocyte nucleus positioning, live-imaging techniques are essential to capture the dynamic events of this process. The *Drosophila* egg chamber is a multicellular structure and an excellent model system to study this phenomenon because of its large size and availability of numerous genetic tools for this model system. During *Drosophila* mid-oogenesis, the nucleus migrates from a central position within the oocyte to adopt an asymmetric position mediated by microtubule-generated forces. This migration and positioning of the nucleus are necessary to determine the polarity axes of the embryo and the subsequent adult fly. One characteristic of this migration is that it occurs in three dimensions (3D), creating a necessity for live imaging. Thus, to study the mechanisms that regulate nuclear migration, we have developed a protocol to culture the dissected egg chambers and perform live imaging for 12 h by time-lapse acquisitions using spinning-disk confocal microscopy. Overall, our conditions allow us to preserve *Drosophila* egg chambers alive for a long period of time, thereby enabling the completion of nuclear migration to be visualized in a large number of samples in 3D.

INTRODUCTION:

For several years, the *Drosophila* oocyte has emerged as a model system to study nuclear migration. The *Drosophila* oocyte develops in a multicellular structure called the egg chamber. Egg chambers encompass 16 germ cells (15 nurse cells and the oocyte) surrounded by an epithelial layer of follicular somatic cells. Egg chamber development has been subdivided into 14 stages (**Figure 1A**), during which the oocyte will grow and accumulate reserves necessary for the early development of the embryo. During the development, upon microtubule reorganization and asymmetric transport of maternal determinants, the oocyte polarizes along the antero-dorsal and dorso-ventral axes. These axes determine the subsequent polarity axes of the embryo and the adult arising from the fertilization of this oocyte[1]. During oogenesis, the nucleus adopts an asymmetric position in the oocyte. In stage 6, the nucleus is centered in the cell. Upon a yet to be identified signal emitted by the posterior follicular cells which is received by the oocyte, the nucleus migrates toward the intersection between the anterior and lateral plasma membranes in stage 7 (**Figure 1B**)[2,3]. This asymmetric position is required to induce the determination of the dorso-ventral axis.

[Place **Figure 1** here]

For many decades, this nuclear migration has been studied on fixed tissues by immunostaining. This approach has notably made it possible to demonstrate that this process depends on a dense network of microtubules[4,5]. More recently, we developed a protocol offering conditions compatible with live imaging of the oocyte during several hours making it possible to study this process dynamically[6].

Hence, for the first time, we have been able to describe that the nucleus has preferential and characteristic trajectories during its migration, one along the anterior plasma membrane (APM) and another along the lateral plasma membrane (LPM) of the oocyte (**Figure 2**). These latest results underline the importance of live-imaging protocols when studying dynamic processes such as nuclear migration.

[Place **Figure 2** here]

The oocyte nucleus migration is a phenomenon of about 3 h[6], and so far, the event triggering the start of the actual migration is unknown. The start of the migration can also be delayed by protein mutants used to study this mechanism. These unknown variables motivated us to acquire images over long time periods (10–12 h). It is, therefore, important to ensure that the oocytes remain alive. As the egg chamber develops, it elongates along the antero-posterior axis from a spherical to an elliptical shape. This elongation is driven by the rotation of follicular cells, which occurs from stage 1 to stage 8, perpendicular to the antero-posterior axis[7]. In addition, a tubular sheath of muscle with pulsatile property surrounds the egg chambers. Its physiological function is to push the developing follicles toward the oviduct continuously[8]. In order to limit the movements that induce oscillations of the egg chambers after their dissection, we designed an observation micro-chamber measuring 150 µm in height (**Figure 3A**). This height is marginally higher than the size of a follicle at stages 10 and 11. It considerably limits the vertical movements of the sample while preserving the rotation of the egg chamber, thereby resulting in limited defects in follicle

development. We then perform live imaging for 12 h on dissected egg chambers by multi-position time-lapse acquisitions using a spinning-disk confocal microscope. Here we describe our protocol for studying the oocyte nuclear migration between stages 6 and 7.

[Place **Figure 3** here]

In order to follow the nuclear migration and precisely assess trajectories in the oocyte, markers for both the nuclear envelope and plasma membrane are needed. With this aim, two transgenes that have a high signal/noise ratio and do not fade over the course of live imaging have been selected. To label the plasma membrane, the use of a *P[ubi-PH-RFP]* that encodes the Pleckstrin Homology (PH) domain of the Human Phospholipase C ð1 (PLCð1) fused to RFP is recommended. This PH domain binds to the phosphoinositide PI(4,5)P2 distributed along the plasma membrane of the oocyte[9]. For the nuclear envelope, the *P[PPT-un1]Fs(2)Ket-GFP* protein-trap strain where GFP is inserted within the gene encoding the *Drosophila* ß-importin displays a homogeneous and an intense signal[10]. Young flies (1–2 days old) are placed in fresh vials containing dry yeast 24–48 h prior to ovary dissection.

For this live-imaging assay, a 1 mm thick piece of aluminum, which is nonreactive for the sample, has been cut into the dimensions of a microscopy slide. It has a 16 mm diameter hole in the center of the slide that has been counterbored to 0.85 mm. This counterbore has an additional 6 mm diameter hole with a depth of 150 µm (**Figure 3A**). A coverslip is glued with silicone grease (inert for the sample) at the bottom of the aluminum chamber (**Figure 3B**). After placing the samples in the medium-filled well, a membrane permeable to O_2/CO_2 exchange is placed over the medium and surrounded by halocarbon oil (**Figure 3C**).

For the dissection, it is recommended to use stainless steel forceps with a tip dimension of 0.05 x 0.02 mm, and 0.20 mm diameter needles for the separation of the ovarioles (**Figure 4B,C**). The migrating nuclei are imaged on a spinning-disk confocal inverted microscope CSU-X1 equipped with a camera. Multi-position images were acquired by time-lapse every 15 min at 24 °C. A 15 min interval allows performing multi-position acquisitions with limited photobleaching of the fluorescent proteins and phototoxicity for the samples. Furthermore, a shorter interval would not provide much more informative data to follow the nuclear trajectories. The movies are processed and analyzed *via* Fiji software[11].

PROTOCOL:

1. Imaging medium preparation

1.1 Prepare fresh media on the day of use. Pipette 200 µL of Schneider medium (containing L-Glutamine and 0.40 g/L of $NaHCO_3$ complemented with 10% heat-inactivated fetal calf serum, 100 U/mL of penicillin, and 100 mg/mL of streptomycin).

1.2 Supplement with 30 µL of insulin 10 mg/mL.

1.3 Add 4 µL of heat-inactivated fetal calf serum.

2. Observation-chamber preparation

2.1 With a pipette tip, apply a small amount of silicone grease all around the hole on the underside of the punctured slide (**Figure 4D**).

2.2 Position a 24 x 50 mm coverslip of 0.13–0.16 mm thickness.

2.3 With the wide end of a pipette tip, apply pressure on the coverslip to flatten the silicone in order to seal the coverslip and create a silicone ring interior to the slide (**Figure 4F,G**).

3. Ovary dissection

3.1 Anesthetize a female fly of the desired phenotype on a CO_2 pad.

3.2 Transfer the female in 150 µL of the imaging medium in a dissecting well (**Figure 4H**).

3.3 Open one female by grabbing its thorax with forceps and pinching the dorsal abdomen cuticle with a second pair of forceps.

3.4 Isolate and detach the pair of ovaries, which should be readily visible upon cuticle opening.

3.5 Carefully remove the uterus, oviduct, and muscle sheath (**Figure 4I**).

3.6 Place a drop of 10–15 µL of the imaging medium and transfer one ovary in the imaging chamber (**Figure 4J,K**).

4. Egg chamber isolation

4.1 To separate the ovarioles, hold the posterior end of the ovary (toward the older stages) with the needle. Tease apart the ovarioles by carefully pulling on the germarium with another needle.

4.2 Remove the remaining muscle sheath on the egg chambers; one needle holding the sheath and the other pulling on the ovariole through the larger chambers (stage 9 or older).

4.3 Allow the unsheathed ovariole to sink and contact the coverslip.

4.4 Remove late stages and the rest of the ovaries from the micro-chamber with the help of forceps. Carefully distance the ovarioles from the others with needles to facilitate the acquisition (**Figure 4L**).

5. Observation chamber closing

5.1 Cut a small square (10 x 10 mm) of permeable membrane (**Figure 4M,N**).

5.2 Carefully apply the membrane on top of the imaging medium to expel any air bubbles (**Figure 4O**).

5.3 Hermetically seal the chamber with a thin layer of halocarbon oil around the well on the contour of the membrane (**Figure 4P,Q**).

6. Imaging

6.1 Place the imaging set-up on the slide holder of the inverted microscope using a 40x objective (HCX PL Apo, 1.25NA, oil immersion).

6.2 Locate and save positions of different stage 6 oocytes in which the nucleus is ready to migrate.

6.3 Set-up the 488 and 561 nm lasers. With a measured output laser power of 150 mW, use 30% of the laser power and 300 ms and 500 ms exposure, respectively.

6.4 Set-up the experiment. Take a time-lapse of 12 h with the interval of 15 min—41 sections with an interval of 1 µm centered on the nucleus.

NOTE: According to the exposure time described above, this setting allows the acquisition of one position in around 45 s. Since there is a delay due to position changing, it is recommended to set a maximum of 12 positions in these conditions.

7. Image analysis

7.1 Process the movies on the software Fiji, using the plug-in Orthogonal view and manually track the nuclei.

[Place **Figure 4** here]

REPRESENTATIVE RESULTS:

Before migration, the nucleus is dynamic and oscillates around a central position during a period defined as pre-migration. These small movements reflect a balance of pushing and pulling forces that maintain equilibrium in the middle of the oocyte. By quantifying the trajectories of the nuclei, we have shown that the APM and LPM trajectories had similar proportions. We define the nature of the trajectory by the first contact between the nucleus and the plasma membrane[6]. Thus, the nucleus reaches either the APM or the LPM before sliding along it, to reach its final position at the intersection of the two plasma membranes **(Figure 5, Movies 1–2**). Furthermore, we have shown that distinct cues favor each of the trajectories. For example, the centrosomes

clustered between the nucleus and the posterior membrane of the oocyte enable the APM trajectory. Conversely, the microtubule-associated-protein (MAP) Mushroom Body Defect (Mud), which is located asymmetrically to the nuclear envelope, supports a trajectory along the LPM[6]. Moreover, the depletion of either centrosomes or Mud affects the migration speed of the nucleus compared to the wild type context[6].

[Place **Figure 5** here]

The oocyte and egg chamber growths are signs of correct development, which are easily visible in time-lapse recordings (**Figure 5**). On the contrary, membrane deformities disorganized follicular cells, stunted cells, and shrunken nuclei are the first signs of dying egg chambers (**Figure 6**, **Movie 3–4**). Upon the observation of these degenerative egg chambers, the nuclei movements are no longer exploitable for analysis. Usually, we do not observe any degenerated oocyte before 8 h of imaging (**Figure 6A**) and the rare cases of early degeneration are due to problems during the dissection or mounting steps (**Figure 6B**). We consider that 50% of oocytes are still alive after 10 h of imaging.

[Place **Figure 6** here]

Excessive movements could be an additional issue resulting in unusable data and further analysis, as the egg chambers will not remain in the imaged frame. To circumvent this problem, we use a 40x objective that provides an adequate frame of observation and allows movements of the egg chambers along the x-y plane while providing enough resolution for a qualitative assessment of migratory path taken by the oocyte nucleus. In addition, to limit the effects of excessive movements in the z-axis and in order to keep the oocyte within the range of the z-stack, we perform z-sections over 40 µm stack (41 sections 1 µm apart), while stage-6 oocyte has a size of 20 µm.

FIGURE AND TABLE LEGENDS:

Figure 1: ***Drosophila melanogaster* egg chambers**. (**A**) Fixed ovariole from transgenic flies expressing *Fs(2)Ket-GFP* that labels the nuclear envelopes and *ubi-PH-RFP* that labels the plasma membranes. The ovariole is composed of developing egg chambers at different stages. Maturation increases along the antero-posterior axis with the germarium at the anterior tip (left) where the germ stem cell resides and the older stage at the posterior tip (right). (**B**) Z-projection of living egg chamber by spinning disk confocal microscopy at stage 6 of oogenesis (left), in which the nucleus is centered in the oocyte. The nucleus will migrate to adopt an asymmetrical position at stage 7 (right) in contact with the anterior plasma membrane (between the oocyte and the nurse cell) and the lateral plasma membrane (between the oocyte and the follicular cells). This position will induce the determination of the dorsal side and, thus, the dorso-ventral axis of the egg chamber.

Figure 2: Schematic representation of the different migration paths of the nucleus. At stage 6 of the oogenesis, the oocyte is a large cell with a central nucleus. At this stage, the antero-

posterior polarity axis is set with a posterior/lateral plasma membrane of the oocyte in contact with the follicular cells and the anterior plasma membrane (in yellow) is in contact with the nurse cells[2]. We have previously reported that the nucleus could migrate either along the anterior plasma membrane (APM), along the lateral plasma membrane (LPM), or through the cytoplasm (STAD, straight to the antero-dorsal cortex)[6].

Figure 3: Schematic representation of the observation chamber. (**A**) (Top view) Precise dimensions of the aluminum slide with the heights (A') and circumferences (A'') of the well drilled in the middle of the slide. (**B**) (Bottom view) A coverslip blocking the well is sealed to the slide with silicon grease. (**C**) (Top view) Dissected ovarioles develop in an imaging medium that is covered by a gas permeable membrane. Halocarbon oil is used to stabilize the membrane.

Figure 4: Step by step micro-chamber mounting pictures. (**A,B,C**) Preparation of the needed tools: dissecting well plate, forceps, needles, imaging media, silicon grease, permeable membrane, and the aluminum slide. (**D**) Application of the silicon grease at the back of the aluminum slide with a pipette tip. (**E**) A glass coverslip is glued on the silicon grease to create the bottom of the chamber. (**F,G**) Pressure application on the coverslip with the wider extremity of a pipette tip to create a joint inside the chamber. (**H,I**) Dissection of the fly ovaries in the imaging media. (**J**) Pipetting of a drop of imaging media in the micro-chamber. (**K,L**) Separation of the ovarioles in the micro-chamber using needles. (**M,N,O**) Permeable membrane cut into a 10 x 10 mm square and placing over the drop of the medium in the micro-chamber. (**P,Q**) Sealing of the micro-chamber with halocarbon oil. The samples are ready to be imaged.

Figure 5: Representative images of the migration of the oocyte nucleus by live-imaging microscopy. (**A**) Selected frames extracted from time-lapse **Movie 1** showing the nuclear migration of the oocyte nucleus of a wild-type egg chamber expressing the nuclear envelope marker (*Fs(2)KetGFP*) and the plasma membrane marker (*ubi-PH-RFP*). (**B**) Selected frames from **Movie 2** (the cropped version of **Movie 1**) focusing on the oocyte. Time (in h:min) relative to the beginning of the time-lapse is indicated on the top of each selected frame.

Figure 6: Representative images of the degeneration of an egg chamber during live imaging. (**A**) Selected frames extracted from time-lapse **Movie 3** showing the degeneration of a wild type egg chamber after 8 h, expressing the nuclear envelope marker (*Fs(2)KetGFP*) and the plasma membrane marker (*ubi-PH-RFP*). (**B**) Selected frames extracted from **Movie 4** showing the rapid degeneration of a wild type egg chamber. Such early degeneration is characteristic of a problem during the dissection or mounting steps. Time (in h:min) relative to the beginning of the time-lapse is indicated on the top of each selected frame.

Movie 1: **Representative movie of a wild-type egg chamber expressing both the nuclear envelope marker (*Fs(2)Ket-GFP*) and the plasma membrane marker (*ubi-PH-RFP*)**. In this example, the oocyte nucleus contacts the anterior membrane first and slides along it to reach the antero-dorsal corner. The elapsed time of the time-lapse is indicated in h:min in the upper-left corner of the video.

Movie 2: **Cropped version of Movie 1, focusing on the oocyte**. The elapsed time of the time-lapse is indicated in h:min in the upper-left corner of the video.

Movie 3: **Representative movie of a wild-type egg chamber expressing both the nuclear envelope marker (*Fs(2)Ket-GFP*) and the plasma membrane marker (*ubi-PH-RFP*)**. In this example, the egg chamber starts to degenerate at 8:45 h before completion of nucleus migration. The elapsed time of the time-lapse is indicated in h:min in the upper-left corner of the video.

Movie 4: **Representative movie of early degeneration of a wild-type egg chamber expressing both the nuclear envelope marker (*Fs(2)Ket-GFP*) and the plasma membrane marker (*ubi-PH-RFP*)**. The elapsed time of the time-lapse is indicated in h:min in the upper-left corner of the video.

DISCUSSION:

Other protocols describe how to prepare and culture Drosophila egg chambers *ex vivo* for live-imaging assay[12,13]. The novelty of this protocol is the use of an imaging chamber constructed using a hollowed aluminum slide, a coverslip, and an O_2/CO_2 permeable membrane. The main advantage of this set-up is to limit the movement in Z without exerting pressure on the sample. Thus, the oocyte can still move freely, and this is why first, the 40x objective is used and second, z-stacks are acquired along 40 µm, while the oocyte is around 20 µm height at stage 6.

Although this protocol is relatively simple, each step is critical for the assay. The preparation of the micro-chamber, the permeable membrane, and the sealing of the chamber are essential to allow gas exchange and prevent drying of the imaging medium and, therefore, the samples. Damaging the egg chamber compromises its survival, so delicate and precise dissection techniques are paramount. Additionally, the muscular sheath must be completely removed as remaining pieces of this structure will result in unwanted egg chamber movement.

Aluminum, in addition to its safety for the sample, has been chosen for its strength, durability, and the ease of cleaning. Other materials have not been yet investigated under these conditions. The use of plastic with the development of 3D printer is tempting; however, one must be very precise with creating a hole with a height of 150 µm.

Recently, Huynh and colleagues have developed an imaging technique adapted to *Drosophila* germarium based on hydrogel[14]. Whether or not this can be adapted to stage 6 in oogenesis remains to be tested. Specifically, it is not known whether movements such as rotation of the follicle can occur in the hydrogel system. These rotation movements are essential for the elongation of the egg chamber and hence, normal development.

Other protocols have used halocarbon oil to follow oocyte nucleus migration[15,16]. The optical properties of oil and the imaging medium used in our protocol are very different. Especially, the Schneider medium has a maximum of light absorption for wavelengths around 450 nm. Therefore, we observe a higher signal/noise ratio with oil. However, the halocarbon oil greatly reduces the oocyte survival to 1–2 h, which limits the ability to follow the entire migration of the

nucleus, particularly in genetic contexts where this migration is disturbed. With this present protocol, the imaging medium allows a long survival of egg chamber allowing us to perform time-lapse recording up to 12 h. Thus, we maximize our chance to capture the entire migration process.

Using our current parameters, we can only perform a maximum of 12 positions for the time-lapse. On average, one third of the imaged nuclei are viable and can be analyzed. To improve the efficiency, a spinning-disk microscope equipped with a dual camera system, which would allow acquisitions of both wavelengths at the same time, can be used to speed up the acquisition time and thus, increase the number of positions.

Furthermore, as this micro-chamber allows to culture the dissected egg chambers for a relatively long period of time, its use can be extended to the study of other dynamic mechanisms of drosophila oogenesis that need to be assessed *ex vivo* (e.g., cytoplasmic streaming in the oocyte, follicle rotation, follicle cells morphogenesis, etc.).

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DISCLOSURES:

The authors declare no competing interests.

REFERENCES:

1. Merkle, J. A., Wittes, J., Schüpbach, T. Signaling between somatic follicle cells and the germline patterns the egg and embryo of Drosophila. *Current Topics in Developmental Biology*. **140**, 55–86 (2020).
2. Roth, S., Lynch, J. A. Symmetry breaking during drosophila oogenesis. *Cold Spring Harbor Perspectives in Biology*. **1** (2), a001891–a001891 (2009).
3. Bernard, F., Lepesant, J.-A., Guichet, A. Nucleus positioning within Drosophila egg chamber. *Seminars in Cell and Developmental Biology*. **82**, 25–33 (2017).
4. Koch, E. A., Spitzer, R. H. Multiple effects of colchicine on oogenesis in Drosophila: Induced sterility and switch of potential oocyte to nurse-cell developmental pathway. *Cell and Tissue Research*. **228** (1), 21–32 (1983).
5. Januschke, J. et al. The centrosome-nucleus complex and microtubule organization in the Drosophila oocyte. *Development (Cambridge, England)*. **133**, 129–139 (2006).

6. Tissot, N. et al. Distinct molecular cues ensure a robust microtubule-dependent nuclear positioning in the Drosophila oocyte. *Nature Communications*. **8**, 15168 (2017).
7. Cetera, M., Horne-Badovinac, S. Round and round gets you somewhere: collective cell migration and planar polarity in elongating Drosophila egg chambers. *Current Opinion in Genetics & Development*. **32**, 10–15 (2015).
8. Hudson, A. M., Petrella, L. N., Tanaka, A. J., Cooley, L. Mononuclear muscle cells in Drosophila ovaries revealed by GFP protein traps. *Developmental Biology*. **314**, 329–340 (2008).
9. Gervais, L., Claret, S., Januschke, J., Roth, S., Guichet, A. PIP5K-dependent production of PIP2 sustains microtubule organization to establish polarized transport in the Drosophila oocyte. *Development*. **135** (23), 3829–3838 (2008).
10. Villányi, Z., Debec, A., Timinszky, G., Tirián, L., Szabad, J. Long persistence of importin- b explains extended survival of cells and zygotes that lack the encoding gene ´ n Villa. *Mechanisms of Development*. **3–4** (125), 196–206 (2008).
11. Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. *Nature Methods*. **9** (7), 676–682 (2012).
12. Prasad, M., Jang, A. C. C., Starz-Gaiano, M., Melani, M., Montell, D. J. A protocol for culturing drosophila melanogaster stage 9 egg chambers for live imaging. *Nature Protocols*. **2** (10), 2467–2473 (2007).
13. Weil, T. T., Parton, R. M., Davis, I. Preparing individual Drosophila egg chambers for live imaging. *Journal of Visualized Experiments: JoVE*. (60) (2012).
14. Chanet, S., Huynh, J. R. Collective cell sorting requires contractile cortical waves in germline cells. *Current Biology*. **30** (21), 4213–4226.e4 (2020).
15. Zhao, T., Graham, O. S., Raposo, A., St Johnston, D. Growing microtubules push the oocyte nucleus to polarize the drosophila dorsal-ventral axis. *Science (New York, N.Y.)*. **336** (6084), 999–1003 (2012).
16. Legent, K., Tissot, N., Guichet, A. Chapter 7 Oogenesis using fixed and live imaging. *Drosophila Oogenesis: Methods and Protocols*. **1328**, 99–112 (2015).

Figure 1

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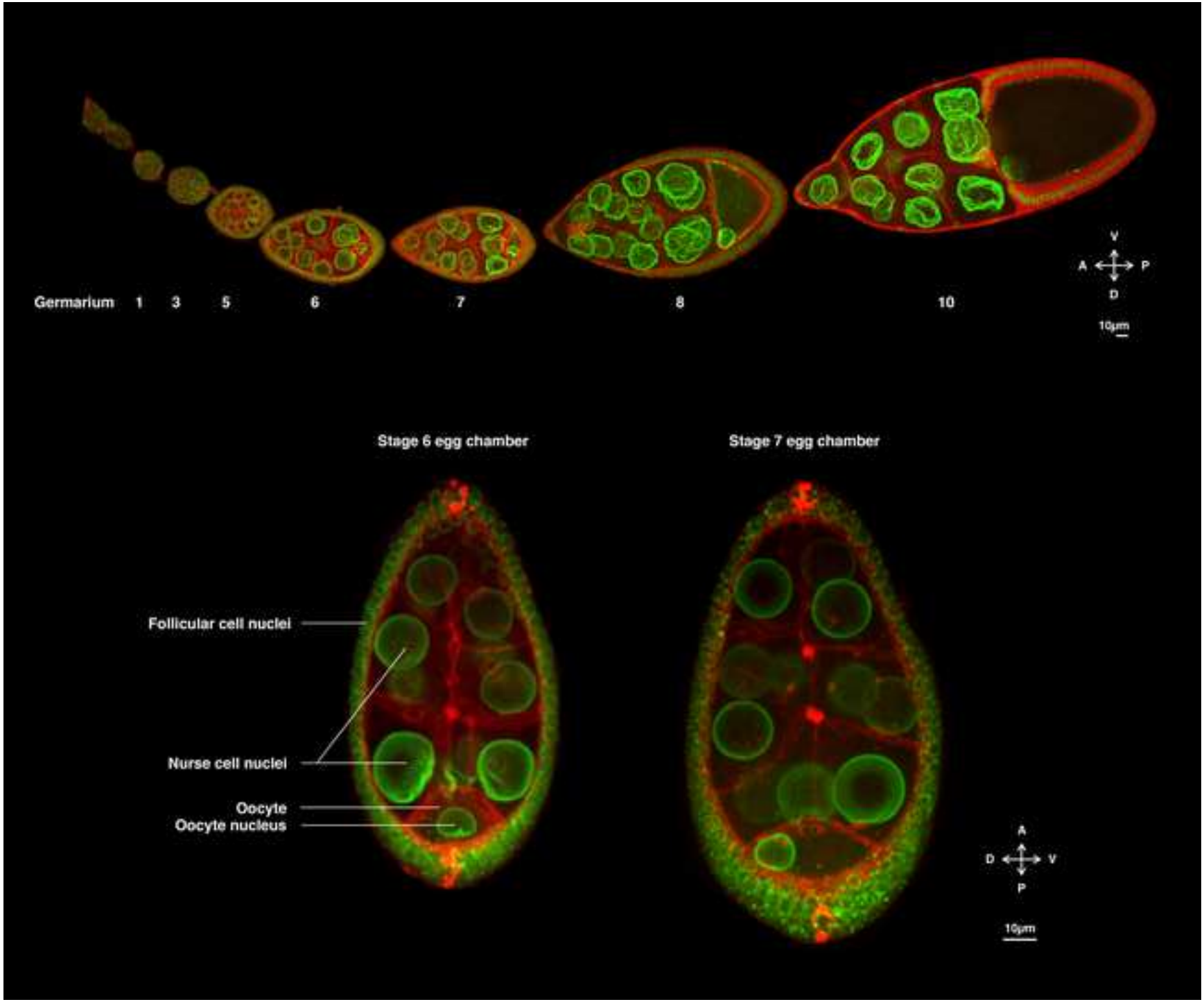

Figure 2

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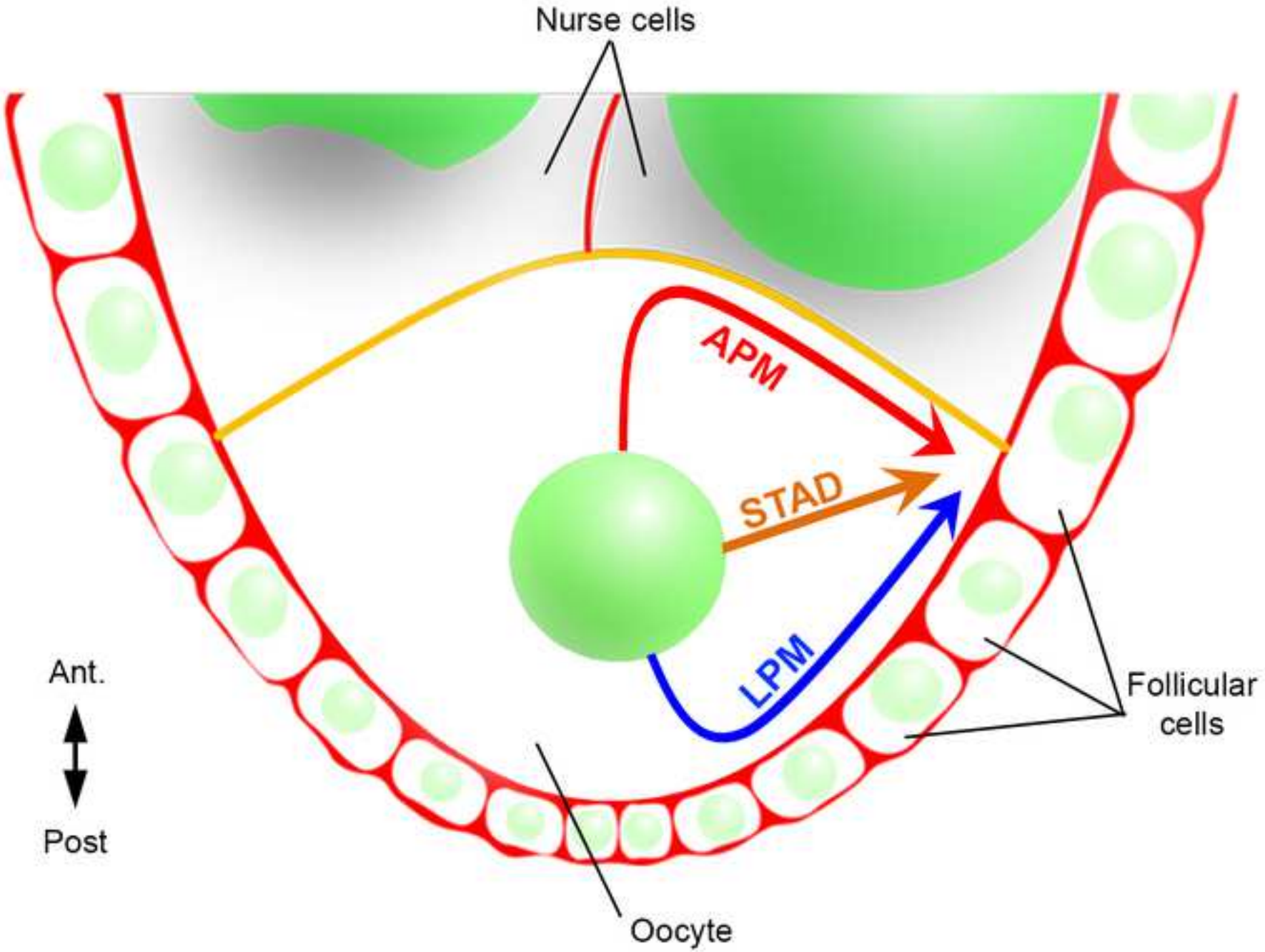

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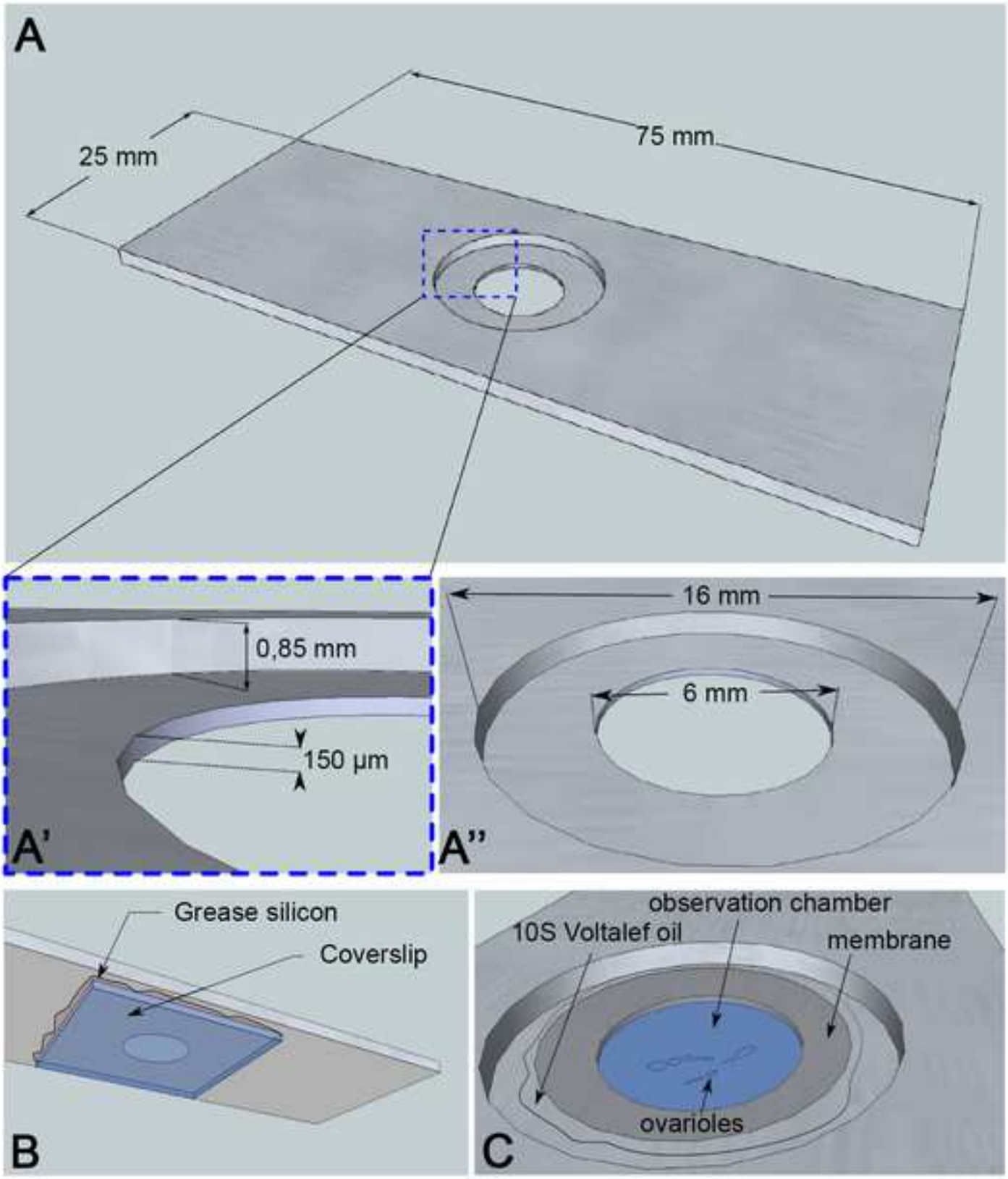

Figure 4

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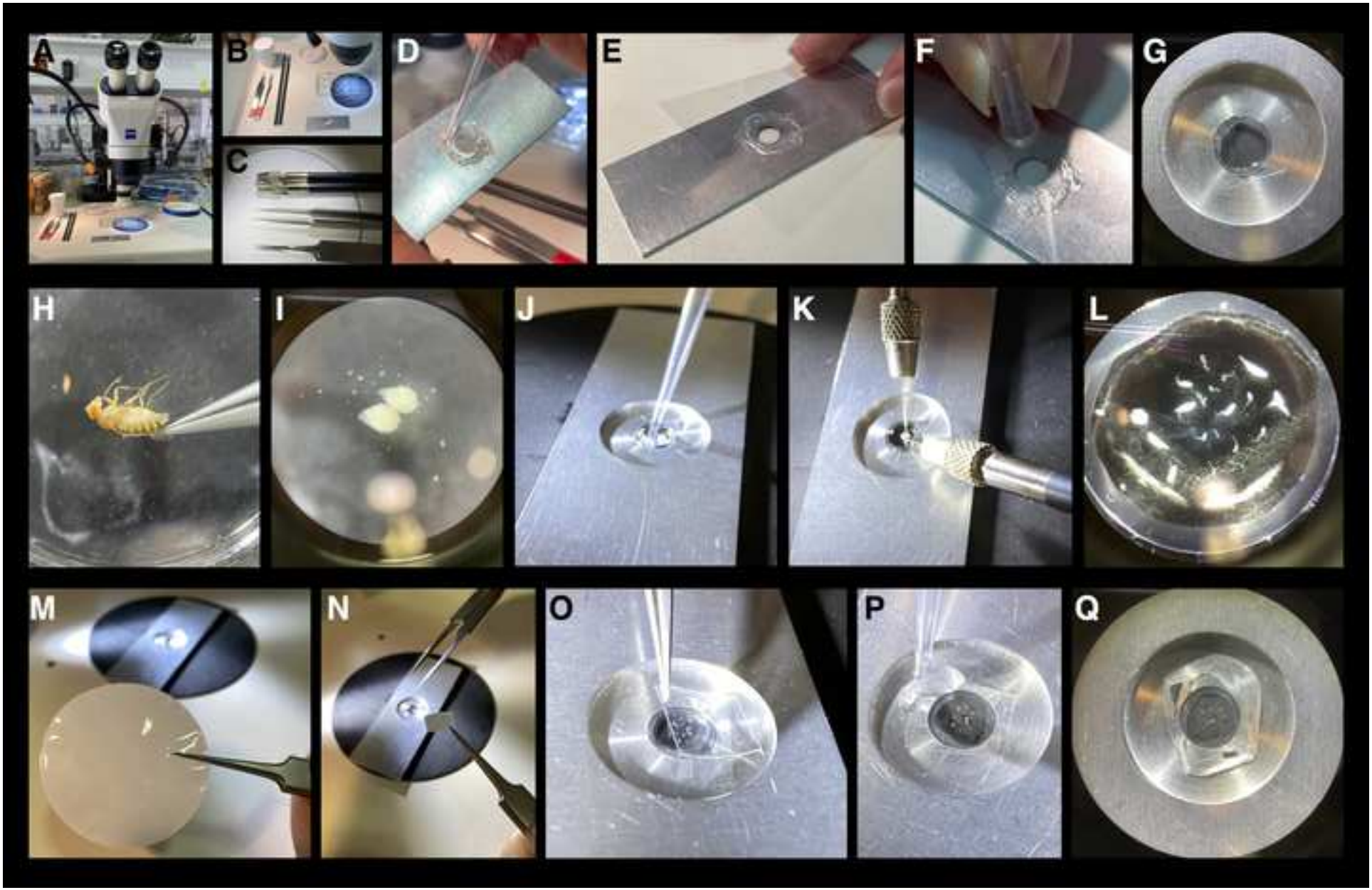

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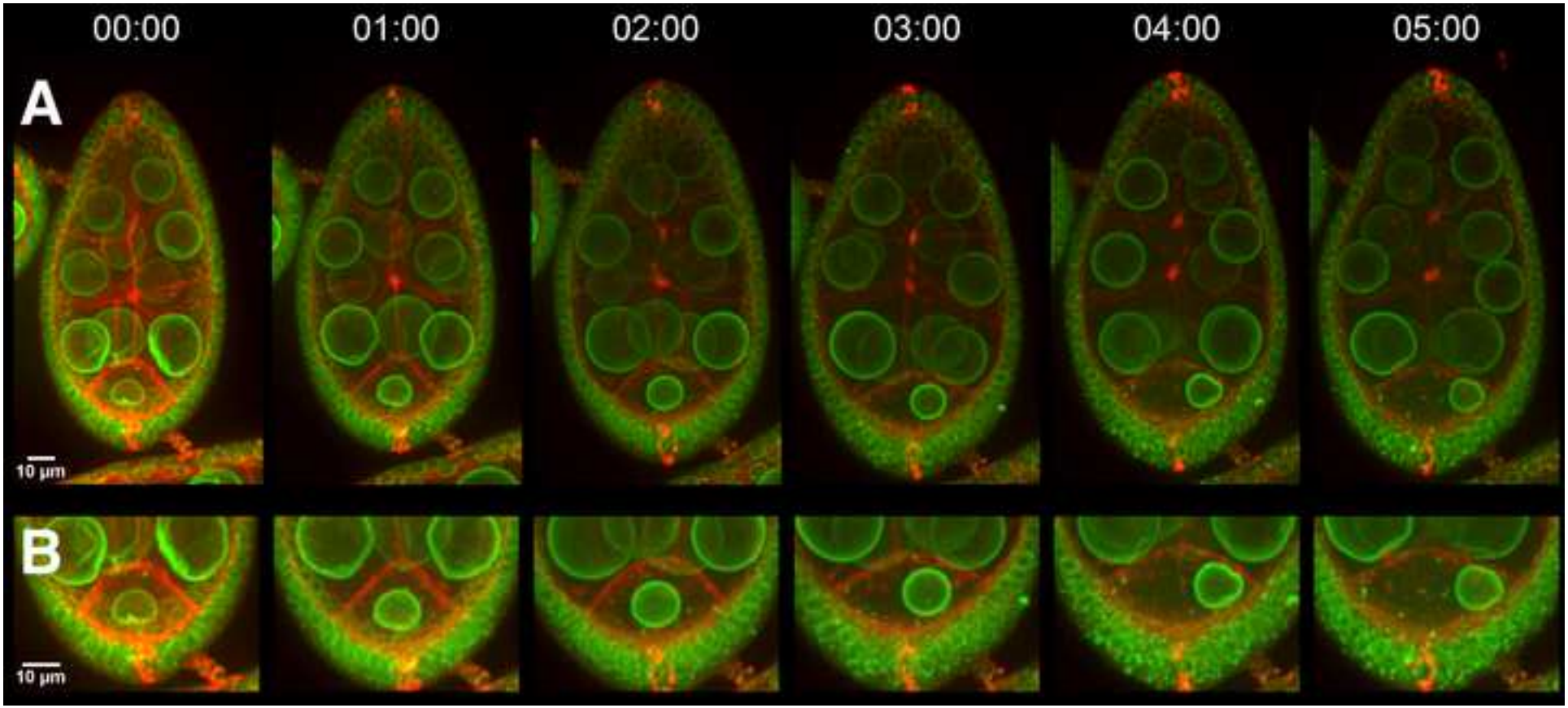

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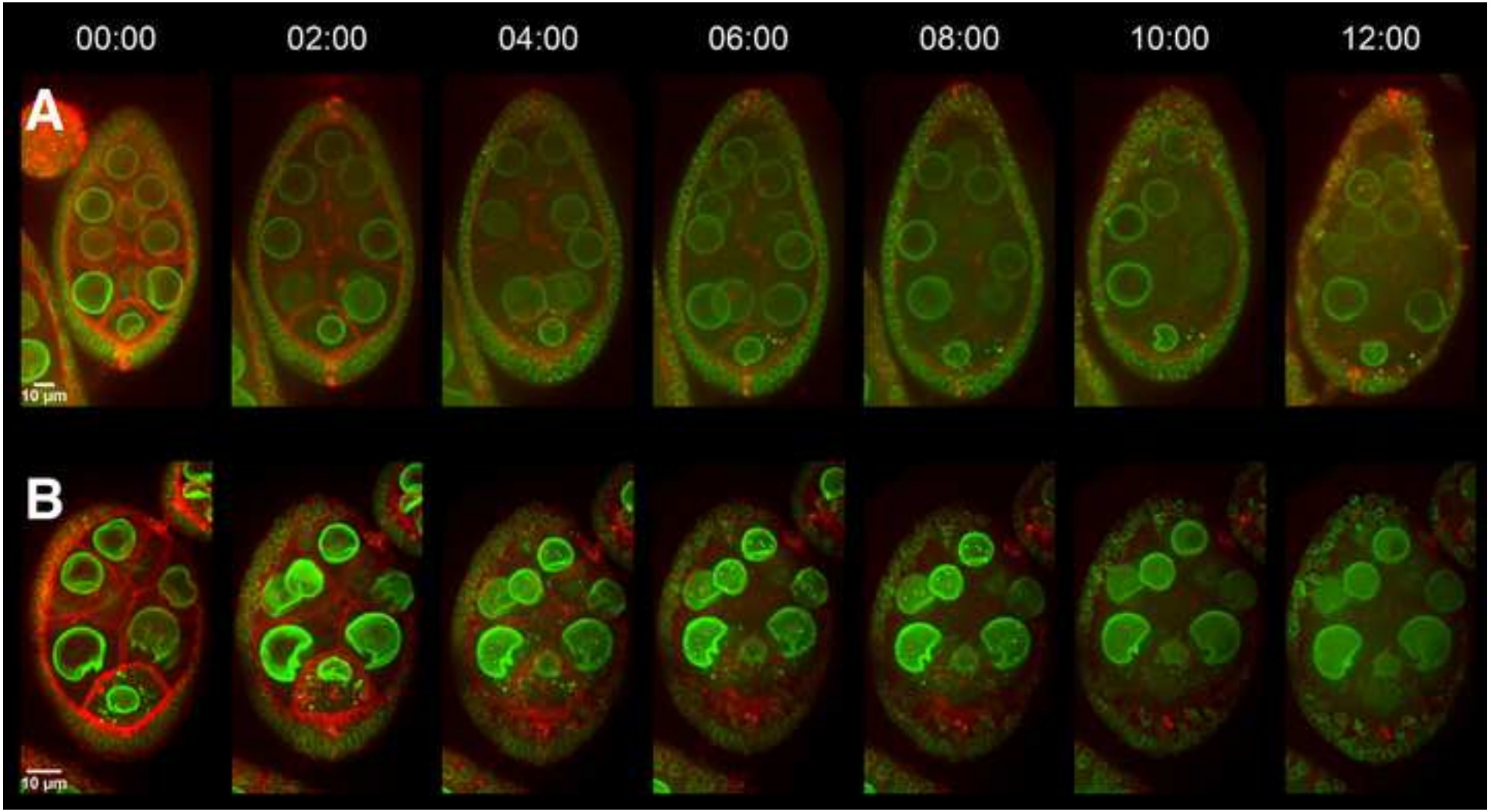

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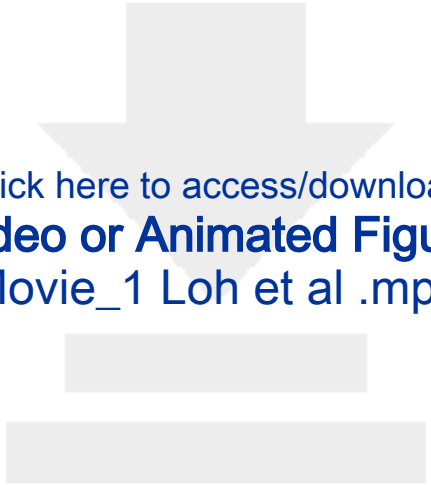

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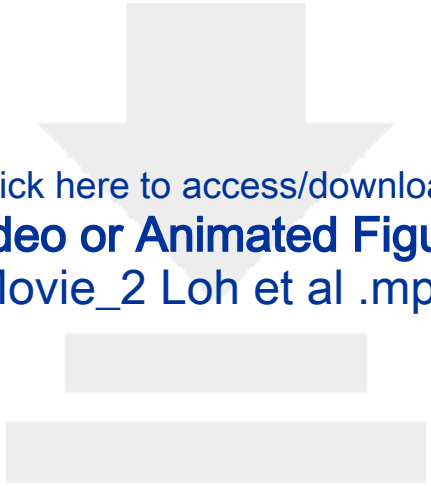

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Movie 3

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Movie 4

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Table of Materials

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Name of Material/Equipment	Company	Catalog Number
Anesthetize CO2 pad	Dutscher	789060
Coverslip (24x50 mm)	Knittel Glass	VD12450Y100A
Forceps Dumont #5	Carl Roth	K342.1
Stainless steel needles	Entosphinx	20
Heat-inactivated fetal calf serum	SIGMA-ALDRICH	F7524
Insulin solution bovine pancreas	SIGMA-ALDRICH	10516 - 5ml
Penicilin/Streptomycin solution	SIGMA-ALDRICH	P0781
Permeable membrane	Leica	11521746
Schneider Medium	Pan Biotech	P04-91500
Silicon grease	BECKMAN	335148
Spinning disk confocal	Zeiss	CSU-X1
Voltalef oil 10S	VWR	24627 - 188

Comments/Description

Anesthetize flies
Observation-chamber preparation
Dissection
Dissection
Imaging medium

Imaging medium
Imaging medium
Observation-chamber preparation
Imaging medium
Observation-chamber preparation
Nuclear migration observation
Observation-chamber preparation

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Paris, April 19, 2021

To Vidhya Iyer
To the Editorial Board of JoVE

Dear Vidhya Iyer

Thank you very much for your interest in our manuscript. We are now submitting a revised version of our previous manuscript JoVE62688 entitled "Nuclear migration in the Drosophila oocyte".
We have addressed the editorial comments and all the points raised by the referees and modified the present manuscript according to their recommendations. Furthermore, according to your recommendation, all changes are highlighted in the text. As you recommend, we have also highlighted in yellow the protocol text that will correspond to the protocol section of the video.

We hope that you will find that we have met satisfactorily the suggestions and comments made by the reviewers and we wish to thank you for your help in the edition of our manuscript.

Yours Sincerely,

Antoine Guichet

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Reply to reviewer's comments

First of all, we would like to thank all three reviewers for their comments and suggestions. We believe that addressing the points brought up by the reviewers have helped us to further clarify the results and improve the manuscript.

Reviewer 1

We agree with the different points raised. We modified the manuscript taking into account all the suggestions of the referee.

Point 1:

More details in the protocol section are needed, i.e microscopy section..

We have modified accordingly the protocol section (lines 105-106): “The migrating nuclei are imaged on a spinning-disk confocal inverted microscope CSU-X1 equiped with a sCMOS PRIME 95 camera.” In addition, we expanded the Imaging section (lines 157-167)

I also recommend citing previously published detailed protocols in egg chamber dissection (i.e Preparing Individual Drosophila Egg Chambers for Live Imaging doi: 10.3791/3679)

We have added the following references (lines 263)

Point 2:

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Quantification of time-lapses is required for most publications. Did the authors analyze nucleus migration with any tracking software? If so, please describe it in the main text.
We have modified accordingly the protocol section (lines 169-171):

Step 7: Image analysis

7.1 The movies are processed on the software Fiji, using the plug-in Orthogonal view, and the nuclei are tracked manually.

Point 3:

One of the main challenges of imaging egg chambers in suspension is that they are floating, making it difficult to keep them in frame for long periods of time. What are the authors' thoughts regarding this issue and how did they solved it?

We have modified accordingly the introduction section (lines 75-79): "In order to limit the movements that induce oscillations of the egg chambers after their dissection, we designed an observation micro-chamber measuring 150 µm in height (**Figure 3A**). This is marginally higher than the size of a follicle at stages 10 and 11. It considerably limits the vertical movements of the sample while preserving the rotation of the egg-chamber, thus resulting in limited defects on follicle development."

and in the discussion section (lines 265-268): "The main advantage of this setup is to limit the movement in Z without exerting pressure on the sample. Thus, the oocyte can still move freely and this is the reason why first, the 40X objective is used and second, z-stacks are acquired along 40 µm, while the oocyte is around 20 µm height at stage 6."

Point 4

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Is there a specific reason why the chamber is made of aluminum? Can it be made with other materials such as plastic?

We have modified accordingly the discussion section (lines 275-278): "Aluminum, in addition to its safety for the sample, has been chosen for its strength, durability, and ease of cleaning. Other materials have not been yet investigated under these conditions. Use of plastic with the development of 3D printer is tempting, however it has to be very precise in order to form a hole of 150 µm height."

Point 5

I recommend the authors add a comment about additional applications of this chamber
We have modified accordingly the discussion section (lines 298-301): "Furthermore, as this micro-chamber allows to culture the dissected egg chambers for a relatively long period of time, its use can be extended to the study of other dynamic mechanisms of drosophila oogenesis that need to be assessed *ex vivo* (e.g : cytoplasmic streaming in the oocyte, follicle rotation, follicle cells morphogenesis,…)".

Reviewer 2

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We agree with the different points raised. We modified the manuscript taking into account all the suggestions of the referee.

Point1

The writing is at times very hard to understand, both because of English grammar/usage issues and because some explanations are not clear enough. The text will require careful copy-editing.

We apologize for this and we have modified the text accordingly.

Point 2

Line 145: what is a 15 min delta t timepoint of 15 min?

We have modified accordingly the protocol section (lines 164-166): Set-up the experiment: take a time-lapse of 12 h with interval of 15 min. 41 sections with an interval of 1µm centered on the nucleus. It is recommended to set a maximum of 12 positions in these conditions.

It would be useful to comment on how long it takes to acquire the 41 z sections, so that the reader has an idea how the image collection time compares to the time interval.

We have modified accordingly the protocol section (lines 165-168): "According to the exposure time described above, this setting allows acquisition of one position in around 45 seconds. Therefore, with the delay due to position changing, it is recommended to set a maximum of 12 positions in these conditions."

Finally, to allow readers to adjust the protocol to their own needs, it might be useful to know how the 15 min interval was chosen. Does more frequent imaging cause phototoxicity or bleaching?

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We have modified accordingly the protocol section (lines 106-110): "Multi-position images were acquired by time-lapse every 15 min at 24 °C. 15 min interval allows to perform multi-position acquisitions with limited photobleaching the fluorescent proteins and phototoxicity for the samples. Furthermore, a shorter interval would not provide much more informative data for the following of the nuclear trajectories."

Point 3

Line 162: It is crucial for the reader to be able to determine if their samples develop normally or are dying. This requires a bit more detail than what is provided in the text.

We have modified accordingly the section representative results (lines 191-198): "On the contrary, membrane deformities disorganized follicular cells, stunted cells and shrunken nuclei are the first signs of dying egg chambers (**Movie 3-4**). Upon the observation of these degenerative egg chambers, the nuclei movements are no longer exploitable for analysis. Usually we do not observe any degenerated oocyte before 8 h of imaging and the rare cases of early degeneration are due to problems during the dissection or mounting steps. We consider that 50% of oocytes are still alive after 10h of imaging."

Moreover, we added some arrows on Movie 3 and provide another example of degeneration with the Movie4 to show a case of early degeneration due to incorrect mounting or egg chamber deterioration during dissection

Point 4

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Lines 164-166: I do not understand how using a 40x objective guards against excessive movement. Is the idea that at higher magnification movement of the egg chamber might be so severe that the sample would no longer be in the field of view? This needs to be clarified.
We have modified accordingly the section representative results (lines 199-206): Excessive movements could be an additional issue resulting in unusable data and further analysis, as the egg chambers will not remain in the imaged frame. To circumvent this problem, we use a 40X objective that gives a sufficient frame of observation and allow movements of the egg chambers along the x-y plan while providing enough resolution for a qualitative assessment of migratory path taken by the oocyte nucleus. In addition, to limit the effects of excessive movements in the z-axis and in order to keep the oocyte within the range of the z-stack we perform z-sections over 40µm stack (41 sections 1µm apart), while stage-6 oocyte has a size of 20µm.

Point 5

I am unclear about whether I am supposed to evaluate the text on page 17.

We apologize for this, but we don't know what the reviewer is referring to as our manuscript was only 9 pages long.

Minor points

1: Under step on1, the preparation of 200 µl imaging medium is described. However, the sum of the three components is 234 µl. It should be made clear if some of the 200 µl Schneider medium

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is removed and replaced by fetal calf serum and insulin, or whether the total final volume is indeed higher than 200 µl

We have clarified this protocol section (lines 113-117):

Imaging medium preparation

1.1 Prepare fresh media on the day of use: pipet 200 µl of Schneider medium (containing L-Glutamine and 0,40 g/L $NaHCO_3$ complemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin).

1.2 Supplement with 30µL of insulin 10 mg/mL

1.3 Add 4µL of heat-inactivated fetal calf serum

2) *Step 2: I presume that there might be a challenge with applying too much silicon grease so that it spills over into the observation chamber when the coverslip is applied. Some guidance should be given on how much to apply and whether spillage into the imaging chamber is a problem or not.*

This is indeed one important step. We have modified accordingly the protocol section (lines 120-126) and illustrated this point in the new figure 4. We will also pay particular attention to this point when making the accompanying video.

Step 2: Observation-chamber preparation

2.1 With a pipette tip, apply a small amount of silicon grease all around the hole on the underside of the punctured slide (Figure 4D).

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3) *Movies 1 and 2: In what I downloaded, movie 1 is the cropped version of movie 2, not the other way round as described in the manuscript.*

We apologize for this and we have modified the movies accordingly.

4) *For the movies, it would be very helpful to explicitly state somewhere that the numbers in the upper left corners are the time (in hours) elapsed since the start of the imaging.*

We apologize for this and we have modified the movie legends.

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Reviewer 3

We agree with the different points raised. We modified the manuscript taking into account all the suggestions of the referee.

Point1

It would be great to have more detailed information on step 3 and step 4, such as the references of the different dissection tools (for instance, "forceps", "hook needle" and "thin needles").

We have modified accordingly the protocol section (lines 103-104) and illustrated this point in the new figure 4. In addition, all the precise references of the used tools are provided in the accompanying table of materials

For the dissection, it is recommended to use stainless steel forceps with a tip dimension of 0,05 x 0,02 mm, and 0,20 mm diameter needles for separation of the ovarioles (Figure 4B, C).

Point 2

The authors could add an image of the drosophila ovary development to visualize the different stages, identifying stages 6 and 7. This image could be fused/included together with Figure

As suggested, we have illustrated this point in the new figure 1.

Point 3

In the introduction, after line 76, the authors describe the design of the aluminium insert (which they refer to "piece of aluminium). This description should be part of the protocol since it is a key

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feature for the implementation of the method described.

We have modified accordingly the protocol section (lines 96-99):

For this live-imaging assay, a 1 mm thick piece of aluminum, which is nonreactive for the sample, has been cut into the dimensions of a microscopy slide. It has a 16 mm diameter hole in the center of the slide that has been counterbored to 0,85 mm. This counterbore has an additional 6 mm diameter hole with a depth of 150 µm (Figure 3A).

Point 4

In the introduction, line 81-85, the authors describe how to assemble the coverslip, add the sample and seal the chamber. This should not be part of the introduction since it is describe in step 4 to 6.

We have modified the text accordingly.

Point 5

In step 5, the authors should provide the reference for the permeable membrane.

We provide the reference in the table of materials

Point 6

In step 6, the authors should provide more information about the microscope they are using to allow a more efficient implementation by other labs.

sous la co-tutelle de

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We have modified accordingly the protocol section (lines 105-106): "The migrating nuclei are imaged on a spinning-disk confocal inverted microscope CSU-X1 equiped with a sCMOS PRIME 95 camera."

Furthermore, we have expanded the imaging section (lines 157-168). Especially we now give details on laser power and exposition time that we use.

Also, is there any temperature control?

We have modified accordingly the protocol section (lines 106-107):

Multi-position images were acquired by time-lapse every 15 min at 24 °C.

Point 7

Part of the originality of this protocol is to visualize nuclear migration in 3D. It would be interesting to add some 3D reconstructions to show this.

While we have performed few attempts of 3D reconstruction, we mostly analyze our movies manually as stated in the Image analysis (lines170-172). Also, we believe this would deserve a detailed description that would be beyond the scope of this publication.

Point 8

If the fly lines used are available in public repositories, please provide references number and/or other relevant information.

The flies are not available in public repositories but have been previously published and references are provided in this manuscript (Ref#9 and #10). All stocks are available upon request

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(Lines 89-94) : "To label the plasma membrane, it is recommended to use a *P[ubi-PH-RFP]* that encodes the Pleckstrin Homology (PH) domain of the Human Phospholipase C ∂ 1 (PLC ∂ 1) fused to RFP. This PH domain binds to the phosphoinositide PI(4,5)P2 distributed along the plasma membrane of the oocyte[9]. For the nuclear envelope, the *P[PPT-un1]Fs(2)Ket-GFP* protein-trap strain where GFP is inserted within the gene encoding the *Drosophila* ß-importin displays an homogeneous and intense signal[10]."

Point 9

How robust is the protocol? What is the % of ovaries at stage 6 that follow nuclear migration?

We have added some details in the results section (lines 194-198) : "Upon the observation of these degenerative egg chambers, the nuclei movements are no longer exploitable for analysis. Usually we do not observe any degenerated oocyte before 8 h of imaging and the rare cases of early degeneration are due to problems during the dissection or mounting steps. We consider that 50% of oocytes are still alive after 10h of imaging." And we modified accordingly the discussion section (lines 288): "On average, one third of the imaged nuclei are viable and can be analyzed."

Point 10

Movies 1 and 2 are switched.

We apologize for this and we have modified the movies accordingly.

Point 11.

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Authors should provide the Schneider medium composition information.

We have modified accordingly the protocol section (lines 113-115): “Schneider medium (containing L-Glutamine and 0,40 g/L $NaHCO_3$ complemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin).”

Point 2.

Figure 2 should have pictures of the device.

The device shown in the former Figure 2, now figure 3 is also presented with several pictures in the new figure 4

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