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Title: Applications of Immobilization of Drosophila Tissues with Fibrin Clots for Live Imaging

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NOTE: This is an APF

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?

   Yes

   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.

   N/A

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**.

   ☒ Author interview statement opt out. Statements removed completely.

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

Current Protocol Length

Number of Steps: 24
Number of Shots: 31

Introduction

1. Introduction

1.1. This protocol describes sample immobilization using Fibrin clots. Samples are transferred to a drop of Fibrinogen containing culture medium on the surface of a coverslip, after which polymerization is induced by adding thrombin.

1.1.1. *3.3.1.*

1.2. Nicolas Loyer, a Senior Research Assistant in Jens Januschke's lab, will demonstrate the procedure. *Video Editor: Please Show this as a title card or text overlay.*

Protocol

2. Dissection of *Drosophila* Larval Brains

2.1. Under a dissection microscope **[1]**, use a brush to transfer L3 larvae into a 9-well borosilicate glass dish containing PBS **[2]**. Stir to detach most of the fly food from the larvae **[3]** and transfer it to another well containing culture medium **[4]**.

2.1.1. WIDE: MOV_0077.MP4. Establishing shot of talent at the microscope.

2.1.2. SCOPE: MOV_0088.MP4 00:00:03-00:00:14. Talent transferring larvae to the glass dish.

2.1.3. SCOPE: MOV_0088.MP4 00:00:14-00:00:18. Talent stirring the larvae.

2.1.4. SCOPE: MOV_0088.MP4 00:00:19-00:00:23. Talent transferring the larvae to another well.

2.2. Use forceps to grasp a larva across its entire diameter, in the middle of its body length, and transfer it to another well of the borosilicate plate containing 200 microliters of fresh culture medium. Do not release the larva **[1]**.

2.2.1. SCOPE: MOV_0037.MP4 00:00:08-00:00:21. Talent transferring the larvae to another well with medium.

2.3. To cut the larva in half across its entire diameter, either grind the grasped area with lateral movement of the forceps tips or slide one tip of another pair of forceps between the two forceps tips holding the larva **[1]**.

2.3.1. SCOPE: MOV_0037.MP4 00:00:24-00:00:34. Talent cutting the larvae in half.

2.4. Use the forceps to hold the larva by its cuticle and another pair of forceps to peel apart the cuticle without pulling on the brain. Repeat this until the nerves originating from the brain are visible and the organs connecting the brain to the mouth parts can be accessed **[1]**.

2.4.1. SCOPE: MOV_0036.MP4 00:00:44-00:00:58. Talent peeling apart the cuticle.

2.5. While holding the nerves originating from the brain with forceps, cut the connection between the brain to the mouth parts with the other pair of forceps, separating the brain from the rest of the cuticle **[1]**.

2.5.1. SCOPE: MOV_0036.MP4 00:01:29-00:01:50. Talent separating the brain from the cuticle.

2.6. Hold the brain by the axons coming out of the ventral nerve cord and pluck the surrounding organs by gently pulling them away from the brain **[1]**. Then, grasp the connection between the eye imaginal disks and the brain with the other pair of forceps and cut this connection without pulling on the brain **[2]**.

2.6.1. SCOPE: MOV_0036.MP4 00:02:03-00:02:28. Talent pulling organs away from the brain.

2.6.2. SCOPE: MOV_0036.MP4 00:02:29-00:02:46. Talent cutting the connection between the eye imaginal disks and the brain.

2.7. Make sure that the brain is appropriately cleared of other tissues and not damaged. Discard the brain if it shows signs of damage **[1]**.

2.7.1. SCOPE: MOV_0036.MP4 00:02:58. Appropriately cleaned brain.

2.8. Pipette the coating solution with a P20 in and out to coat the pipette tip **[1]**, then aspirate the brain with the coated tip along with 3 microliters of medium and pipette it out in another well containing 200 microliters of clean culture medium **[2]**.

2.8.1. MOV_0094.MP4 00:00:00-00:00:08. Talent coating the pipette tip.

2.8.2. SCOPE: MOV_0036.MP4 00:03:06-00:03:22. Talent pipetting the brain into a well with culture medium.

2.9. Repeat this process until enough brains are dissected, occasionally replacing the culture medium of the well in which the dissections are performed **[1]**.

2.9.1. MOV_0096.MP4 00:00:30-00:00:45. Talent at the microscope, performing the dissection.

3. Immobilization on Coverslip with Fibrin Clots

3.1. Use a P20 pipette with a coated tip to transfer the dissected brains into another well of the borosilicate plate containing 200 microliters of culture medium and Fibrinogen **[1]**.

3.1.1. SCOPE: MOV_0053.MP4 00:00:00-00:00:13. Talent transferring the brain to a well with Fibrinogen.

3.2. Aspirate one brain along with 9 microliters of culture medium and Fibrinogen **[1]**. With the end of the tip almost touching the cover glass bottom of the cell culture dish, pipette out the contents so that the culture medium touches the coverslip immediately after exiting the pipette tip **[2]**.

3.2.1. SCOPE: MOV_0053.MP4 00:00:15-00:00:23. Talent aspirating the brain.

3.2.2. SCOPE: MOV_0053.MP4 00:00:30-00:00:40. Talent pipetting the brain and medium into the bottom of the dish.

3.3. Use one tip of a pair of forceps or a closed pair of forceps to gently push and position the brain within the culture medium and Fibrinogen drop. If the ventral part of the brain has to be imaged, induce Fibrinogen clotting to properly orient the brain within the clot **[1]**.

3.3.1. SCOPE: MOV_0053.MP4 00:01:03-00:01:10. Talent positioning the brain in the Fibrinogen drop.

3.4. Push the brain to one side of the drop. Touch the edge of the drop on the opposite side of the brain with the pipette tip and add 1 microliter of Thrombin **[1]**.

3.4.1. SCOPE: MOV_0053.MP4 00:01:45-00:01:54. Talent adding Thrombin.

3.5. Wait for 1 to 2 minutes for the Fibrinogen to start polymerizing, resulting in the formation of a cloudy precipitate at one side of the drop. Then, gently push and tuck the brain into the Fibrin clot, making sure that the ventral side is as close as possible to the coverslip without deforming the brain **[1]**.

3.5.1. SCOPE: MOV_0053.MP4 00:02:58-00:03:05. Talent pushing and tucking the brain into the clot.

3.6. Pipette out 1 microliter of Thrombin solution close to the side of the brain not tucked into the Fibrin clot **[1]**.

3.6.1. SCOPE: MOV_0053.MP4 00:03:28-00:03:40. Talent pipetting Thrombin to the side of the brain.

3.7. Wait for 2 to 3 minutes for the second Fibrin clot to set, then press on the edges of the clot to make it adhere more strongly to the coverslip, taking care not to deform the brain. If the brain appears to be too far from the coverslip, bring it closer by pressing the Fibrin clot to the brain **[1]**.

3.7.1. SCOPE: MOV_0053.MP4 00:04:55-00:05:41. Talent pressing on the edges of the clot.

3.8. To induce the Fibrin clot for imaging the dorsal part of the brain, position the brain in the center of the drop, dorsal part facing the coverslip. Using a p10 pipette, touch the edge of the drop on the opposite side of the brain with the pipette tip and pipette out 1 microliter of Thrombin **[1]**.

3.8.1. SCOPE: MOV_0101.MP4 00:00:02-00:00:15 THEN 00:00:38-00:00:46. Talent positioning a brain for dorsal imaging and adding Thrombin.

3.9. Wait for 2 to 3 minutes for the Fibrinogen to start polymerizing, resulting in the formation of a cloudy, fibrous precipitate. Then, press on the edges of the clot to make it adhere more strongly to the coverslip, taking care not to deform the brain **[1]**.

3.9.1. SCOPE: MOV_0101.MP4 00:03:38-00:03:57. Talent pressing on the edges of the clot.

3.10. With the end of the tip positioned about 0.5 centimeters above the clot, gently pipette 390 microliters of culture medium without Fibrinogen dropwise on top of the clot. Do not add the culture medium on the sides of the clot as it may detach it from the coverslip **[1]**.

3.10.1. SCOPE: MOV_0101.MP4 00:04:12-00:04:28. Talent pipetting medium with Fibrinogen on top of the clot.

3.11. To wash out the excess Thrombin, remove 300 microliters from the dish then add 300 microliters of clean culture medium, making sure that the clots remain fully immersed **[1-TXT]**.

3.11.1. MOV_0101.MP4 00:04:42-00:05:42. Talent removing and adding medium to the clots. **TEXT: Repeat 2 X**

4. Addition of Reagents during Live Imaging

4.1. To avoid temperature changes causing changes of focus, keep the solution with the reagents at the same temperature as the culture dish **[1]**.

4.1.1. Solutions and culture dish at the same temperature. NOTE: Authors did not provide footage for this, please use the beginning of MOV_0083 for this VO.

4.2. Remove the lid of the culture dish without displacing the dish itself. For easier removal, place the lid of the 35-millimeter dish upside-down on top of the dish before imaging **[1]**.

4.2.1. MOV_0083.MP4 00:00:04-00:00:09. Talent removing the lid of the culture dish.

4.3. Position a P1000 pipette tip close to the surface of the medium inside the culture dish and gently release the adequate volume of reagent solution onto it without generating strong fluxes that could detach the clots **[1]**.

4.3.1. MOV_0083.MP4 00:00:09-00:00:24. Talent adding reagents to the culture dish.

4.4. To homogenize the solution within the culture dish, use a P200 pipette to slowly pipette a small amount of the solution in and out five times **[1]**. Replace the lid without displacing the culture dish and resume imaging **[2]**.

4.4.1. MOV_0083.MP4 00:00:25-00:00:40. Talent pipetting the solution.

4.4.2. MOV_0083.MP4 00:00:40-00:00:50. Talent replacing the lid.

Results

5. Results: Application of an ATP analog on immobilized egg chambers during live imaging

5.1. When 1-NAPP1 *(spell out '1-N-A-P-P-1')* was added to Fibrin-immobilized larval brains expressing GFP-tagged Bazooka, brains carrying an analog-sensitive mutation of aPKC *(pronounce 'A-P-K-C')* displayed a contraction of the neuroepithelium **[1]** as well as bright clusters of Baz in neuroblasts and their progeny **[2]**.

5.1.1. LAB MEDIA: Figure 6 A. *Video Editor: Emphasize the apkcas4 images.*

5.1.2. LAB MEDIA: Figure 6 B. *Video Editor: Emphasize the bright clusters in the bottom right image.*

5.2. Neuroblasts kept dividing throughout the time-lapse, indicating that the tissue remained healthy, and brains showed little drift despite the culture medium change **[1]**.

5.2.1. LAB MEDIA: Video 1.

5.3. Similarly, application of 1-NAPP1 to ovarioles expressing GFP-tagged Bazooka induced the contraction of analog-sensitive aPKC mutant follicular cells but not of controls **[1]**.

5.3.1. LAB MEDIA: Video 2.

5.4. A hyperstack displaying both lateral and focus drift **[1]** was first corrected for lateral drift **[2]**, then focus drift, resulting in a substantial reduction of the movements observed in the original hyperstack **[3]**.

5.4.1. LAB MEDIA: Video 3. *Video Editor: Emphasize the left panel.*

5.4.2. LAB MEDIA: Video 3. *Video Editor: Emphasize the middle panel.*

5.4.3. LAB MEDIA: Video 3. *Video Editor: Emphasize the right panel.*

Conclusion

6. Conclusion Interview Statements

6.1. When attempting this protocol, it is important to tuck the brain into the partially polymerized Fibrin clot while the clot is still malleable but solid enough to stably hold the brain. Experiment with manipulation of empty clots and gently probe the clot while it is polymerizing to check how sturdy it is.

6.1.1. *3.5.1.*