Submission ID #: 62533
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Project Page Link: https://www.jove.com/account/file-uploader?src=19072613

Title: An Adjustable High-definition Imaging System for Behavioral Studies of Drosophila Adults

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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? **No**

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?

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option.

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

Current Protocol Length

Number of Steps: 17
Number of Shots: 40

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Daxiang Yang:** Due to its awkward size, Drosophila is difficult to image. This method demonstrates imaging of the behavior or morphology of flies through an eyepiece of a stereo microscope.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.5.2*

OPTIONAL:

1.2. **Daxiang Yang:** This protocol can be used both for scientific research and teaching.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.3. **Daxiang Yang:** Demonstrating the procedure will be Ms. Li Tong and Ms. Weng Yujia, undergraduates doing research training under my guidance.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Construction of the Observing/Documenting System

2.1. To begin, make a fly behavior observation chamber, or FBOC using a translucent plastic bottle cap of about 17 by 22 millimeters or by cutting a section of 17 millimeters from the thick end of a 5-milliliter pipette tip **[1]**.

2.1.1. Talent shows translucent plastic bottle cap, and cut a section of 17 millimeters from the thick end of a 5 milliliter pipette tip.

2.2. Pour 1% agar into the FBOC to adjust its depth **[1]**. If food needs to be placed in the FBOC, pour the agar to obtain a depth of 12 millimeters. If food does not need to be placed in the FBOC, pour the agar to a depth of 5 millimeters to track the whereabouts of fruit flies more easily **[2]**.

2.2.1. Talent marking the height of the agar on the FBOC wall

2.2.2. Talent pouring agar into FBOC, Agar up to the depth of 12 millimeters and agar up to the depth of 5 millimeters

2.3. If using a pipette tip to make an FBOC, place the cut-off pipette tip section in a 35- or a 60-millimeter Petri dish **[1]**. Pour the 1% agar gel into the Petri dish to a thickness of about 5 millimeter **[2]** and wait for the agar to solidify and seal the bottom of the FBOC. Then, pour the agar gel into the FBOC to the desired thickness **[3]**.

2.3.1. Talent placing the tip section in Petri dish

2.3.2. Talent pouring the agar in the Petri dish

2.3.3. Talent pouring the agar gel into FBOC when agar in Petri dish solidified.

2.4. Make an FBOC base by boring a 10-millimeter-deep hole in the center of a piece of ethylamine foam sheet with the same diameter as the FBOC **[1-TXT]**. Insert the FBOC into the hole **[2]**.

2.4.1. Talent boring a hole in foam sheet **TEXT: Ethylamine foam sheet: 60 mm x 60 mm x 15 mm**

2.4.2. Talent inserting FBOC into the hole

2.5. Make fly food, if necessary, with yeast medium, artificial diet, or pure sucrose or glucose, depending on the purpose of the observation **[1]**. To visually determine whether flies are feeding **[1a]**, add food dyes to the food to a final concentration of 12.5 milligrams per 100 milliliters **[2]**. NOTE: Extra screen shot 2.5.1a is added

2.5.1. Talent preparing fly food

2.5.1a Added shot: LAB MEDIA: fly behavior.mp4.02:36-02:34

2.5.2. Talent adding food dye

2.6. Pour the prepared food into a Petri dish to a height of 8 millimeters **[1]**. After solidification, place food on a rectangle drawn paper and cut the food accordingly **[2-TXT]** and place it on a piece of plastic**,** and cut into a quadrangular pyramid or quadrangular frustum pyramid **[3]** to allow recording of fly behavior from different angles as the flies land on the food **[3a]**. NOTE: Extra screen shot 2.6.3a is added

2.6.1. Talent pouring prepared food into Petri dish

2.6.2. Talent cutting the solidified food **TEXT: Food size: 8 mm x 4 mm x 8 mm** NOTE: In the video 2-6-2, talent drawing an 8 mm×4 mm rectangle on the paper, place the paper under the food, and cut out the food according to this size. Hence, VO changed and exceeded the four-line limit

2.6.3. Talent placing it on a piece of plastic and cut it into a quadrangular pyramid

2.6.3a Added shot: LAB MEDIA: fly behavior.mp4.02:18-02:30

2.7. Cut a piece of plast, use tweezers to place the plastic and food in the center of the FBOC **[2]**.

2.7.1. Talent cuting a piece of plastic and using tweezers to place the plastic and food in the center of the FBOC **TEXT: plastic size: 9 mm x 5 mm**

2.8. Pour 1% agar gel into a clean empty bottle to a thickness of 1 to 2 centimeters and place it at room temperature for 1 to 2 hours **[1-TXT]**. Transfer flies to the bottle and place it at 25 degrees Celsius for 36 hours or more **[2]**.

2.8.1. Talent pouring agar gel into empty bottle **TEXT: 1 g agar/100 mL water, 600 µL of propionic acid**

2.8.2. Talent transferring flies to the bottle

2.9. Transfer one or more flies into the FBOC using an aspirator **[1]**. If using an aspirator is difficult, chill and inactivate the flies in crushed ice **[2]**, sort them on an ice pack **[3]**, and transfer them to the FBOC as previously described **[4]**.

2.9.1. Talent inactivating the flies on crushed ice

2.9.2. Talent sorting the flies on ice pack

2.9.3. Talent transferring the sorted flies to FBOC

2.10. After transferring the flies to the FBOC, cover it with 30 to 40 millimeters of UV or clear filter for the camera to form an FBOC complex **[1]**. Place the FBOC complex under the stereomicroscope for observation **[2]**.

2.10.1. Talent covering the FBOC with camera filter

2.10.2. Talent placing FBOC complex under stereomicroscope

2.11. Illuminate the FBOC. Mount mini-LED video lights to flash hot shoe mount stands **[1]**

and place the lights on the left and right sides of the FBOC **[2]**. Turn on the lights, and set the brightness to 100% and the color temperature to 5000 to 5600 Kelvin **[3]**.

2.11.1. Talent mounting mini-LED video lights to hot shoe mount stands;

2.11.2. Place mini-LED video lights on the left and right sides of the FBOC

2.11.3. Talent turn on the lights, setting the brightness and color temperature

3. Observation and Videography of Fly Behavior

3.1. Turn on the LED video lights, and adjust the stereo zoom microscope until the edge of the FBOC can be clearly seen with the naked eye **[1]**. Move the FBOC to the center of the field of view **[2]**.

3.1.1. Talent turning the LED lights on and adjusting the stereo zoom microscope

3.1.2. Talent moving the FBOC to the center of the field of view

3.2. Attach the clamp of the universal telescope digital camera adapter to an eyepiece of the stereo microscope **[1]**, then attach a compact digital camera to the adapter securely by alternately turning the camera mounting screw and camera fixing screw **[2]**.

3.2.1. Talent attaching the clamp of camera to the eyepiece of microscope

3.2.2. Talent attaching a compact digital camera to the adapter

3.3. Turn on the digital camera, and turn the horizontal/vertical fine-tuning knobs **[1]** until the FBOC edge clearly appears in the center of the bright circular field of view on the camera's LCD screen **[2]**.

3.3.1. Talent turning on the digital camera and turning the fine-tuning knobs

3.3.2. Clear FBOC edges on camera's LCD screen

3.4. Rotate the mode dial to **Aperture-priority auto Mode,** press **focus mode** on the multi selector, choose **Macro close-up,** and then press **OK** button **[1].** Move the zoom switch from the wide-angle end to the telephoto end **[2]** and zoom into the circular image until its central portion fills the full LCD screen **[3]**. Press the **Movie-record** button **[4]** and start recording the fly behavior **[4a]**. NOTE: Authors have modified the VO and hence the four-line limit exceeded and one extra screen shot 3.4.4a is added

3.4.1. Talent rotating the mode dial to **Aperture-priority** auto mode, pressing focus mode on multi selector and choose Macro close-up, and pressing the OK button

3.4.2. Talent moving the zoom switch from Wide angle to telephoto end

3.4.3. Talent zooming into the circular image and central image filling the LCD screen

3.4.4. Movie record button pressed

3.4.4a Added shot: LAB MEDIA: fly behavior.mp4.01:44-01:57

3.5. Turn the focus knob of the microscope until the flies in the FBOC are clearly visible **[1]**. Choose the fly behavior of interest for observation or video recording. Turn the zoom knob to zoom in and out **[2]** to achieve the desired magnification for observation or video recording **[2a]**. NOTE: Extra screen shot 3.5.2a is added

3.5.1. Focusing the flies in FBOC

3.5.2. Talent zooming in and zooming out and finally selecting the desired magnification for recording

3.5.2a Added shot: LAB MEDIA: fly behavior.mp4.00:05-00:15

Results

4. Results: Imaging System for Behavioral Studies of *Drosophila*

4.1. The representative photograph taken through the UV filter is clear and sharp **[1]**, very similar to the photograph taken when the culture vial is not covered **[2]**. The quality of the photo taken through the glass of the Petri dish is very poor and partly blurred **[3]**.

4.1.1. LAB MEDIA: Figure 5 *Video editor: Please highlight the second/middle image of the figure*

4.1.2. LAB MEDIA: Figure 5 *Video editor: Please highlight the first/leftmost image of the figure*

4.1.3. LAB MEDIA: Figure 5 *Video editor: Please highlight the third/rightmost image of the figure*

4.2. A photograph taken from the video recording showing the details of each part of the fly's body is shown here. Because the camera is connected to a zoom stereo microscope, it is very easy to shoot from panoramic to close-up shots using the zoom system **[1]**.

4.2.1. LAB MEDIA: Figure 6

4.3. Using this protocol, fly behavior can be observed and documented from multiple viewing angles **[1]**. For example, the female fly is constantly rubbing the ovipositor with her hind legs during the process of laying eggs. This detail of egg-laying behavior cannot be seen clearly from the side **[2]**.

4.3.1. LAB MEDIA: Figure 7

4.3.2. LAB MEDIA: Figure 7 *Video editor: Please highlight portion with white egg in between the legs of the fly in the figure*

Conclusion

5. Conclusion Interview Statements

5.1. **Daxiang Yang:** Using this simple and economical method, we can take a panoramic or close-up of the behavior or morphology of flies in all directions and obtain a good-quality video or photo.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: LAB MEDIA: Figure 6*