FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67546
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20601578

Title: Surgical Removal of a Complex Sensory Organ in Highly Regenerative Ctenophores

Authors and Affiliations:

Orianna A. Duh, Magy Hanna, Allison Edgar

Department of Biological Sciences, New Jersey Institute of Technology

Corresponding Authors:

Allison Edgar allison.edgar@njit.edu

Email Addresses for All Authors:

Orianna A. Duh oad8@njit.edu
Magy Hanna mh688@njit.edu
Allison Edgar allison.edgar@njit.edu

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**

   Can you record movies/images using your own microscope camera?

   Yes but it would be great if you could bring a your kit as a backup or in case the resolution on ours is insufficient. Our scope is set up for 2 observers and has an additional C-mount, but actually the C-mount directs the light from the primary observer's left eye so your scope kit could be an improvement.

   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.

   If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

   Enter make and model of microscope.

   Leica M125

   If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

   3.2-3.6

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

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

Current Protocol Length
Number of Steps: 11
Number of Shots: 25

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: For the interview question 1.1, 1.2, 1.3 please only use the below clips. Previous clips had issues.

1.1. **Allison Edgar:** Our lab is interested in understanding the origins and evolution of animal life history strategies, meaning reproduction, growth, and development, including regeneration. Ctenophores are excellent, phylogenetically informative models for these phenomena.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 1.* NOTE: Use C076_C842_0721RZ_001 (1.1 take 4)

What are the most recent developments in your field of research?

1.2. **Allison Edgar:** We are still learning a lot about ctenophore regeneration and life history, even though people have been studying both for over a hundred years. A paper from last year showed that when two ctenophores are fused during regeneration, some of their behaviors synchronize.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. NOTE: Use C076_C842_0721RZ_001 (1.1 take 4)

What significant findings have you established in your field?

1.3. **Allison Edgar:** We showed that the dramatic changes in body shape that take place as our study species grows happen after the onset of reproduction, which is quite different from our usual ideas about metamorphosis.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. NOTE: C076_C844_0721FF_001. (1.3)

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Preparation of Dissection Needles and Measuring Pipettes

Demonstrator: Orianna Duh

2.1. To begin, adjust the Bunsen burner to produce a sharp blue cone of flame **[1]**. Hold the center of a glass capillary micropipette in the flame until it softens under gentle pressure **[2-TXT]**. Remove the pipette from the flame and immediately pull both ends straight apart **[3]** to create a short, strong needle **[4]**. Alternatively, 26-Gauge ½ *(half)* inch needles can also be used effectively, particularly for the larger end of the focal size range **[5]**.

2.1.1. WIDE: Talent adjusting the Bunsen burner flame to achieve a sharp blue cone.

2.1.2. Talent holding the center of a glass pipette in the flame. **TXT: For animals over 1 mm, use calibrated soda lime glass micropipettes**

2.1.3. Talent pulling both ends of the softened pipette to form a needle.

2.1.4. CU: A shot of the formed needle.

2.1.5. CU: A shot of the 26-Gauge ½ inch needle.

2.2. To prepare measuring pipettes, cut plastic transfer pipettes using sharp scissors or a razor blade to form openings with the preferred internal diameter **[1]**. Prepare at least one pipette for the top and one for the bottom of the desired size range **[2]**.

2.2.1. Talent using scissors or a blade to cut the end of a plastic transfer pipette.

2.2.2. A shot of two finished pipettes, each indicating a different size range, placed on a table (or any other surface).

3. Animal Preparation, Dissection, and Mounting Procedure for Imaging

Demonstrators: Magy Hanna and Orianna Duh

3.1. Select animals that appear morphologically normal and are of the desired size and stage. Verify that the animals do not pass through the bottom-of-the-range sizing pipette but fit comfortably into the top-of-the-range sizing pipette **[1-TXT]**.

3.1.1. SCOPE: Talent looking through the microscope, selecting the appropriate animal, and measuring its size. File name 000002_20250721_141929.MP4: 00:00-00:10 **TXT: Animals of ~0.5 – 3 mm in diameter have been found to be optimal for mounting on slides**

3.2. Use a transfer pipette wider than the animal's body to move it into a 35-millimeter

polystyrene dish filled with sterile seawater **[1]**.

3.2.1. SCOPE: Talent gently transferring an animal into the dish filled with seawater using a wide-bore transfer pipette. File name 000002_20250721_141929.MP4: 00:10-00:20

3.3. Under a dissecting microscope **[1]**, roll one animal onto its adesophageal side to access the aboral organ easily **[2]**. Focus the microscope on the aboral organ **[3]**.

3.3.1. Talent positioning the dish under a dissecting microscope.

3.3.2. SCOPE: Talent rolling the animal onto its adesophageal side. File name 000002_20250721_141929.MP4: 00:20-00:55

3.3.3. SCOPE: Microscopic view focused on the aboral organ of the animal. File name 000002_20250721_141929.MP4: 00:55-2:00

3.4. Using the non-dominant hand, gently hold the animal in place with one glass needle and with the dominant hand, insert the tip of a needle just below the aboral organ **[1].** Make an oblique cut from one edge to below the base **[2]**. Withdraw the needle **[3]** and make a second cut to remove a wedge-shaped piece containing the aboral organ structures **[4]**. Examine both the animal and excised tissue to verify successful removal **[5]**.

3.4.1. SCOPE: Talent using the non-dominant hand to stabilize the animal with a glass needle and with the dominant hand inserting the tip of a needle just below the aboral organ. File name 000002_20250721_141929.MP4: 02:00-2:20

3.4.2. SCOPE: Talent making the first oblique cut. File name 000002_20250721_141929.MP4: 03:00-03:15

3.4.3. SCOPE: Talent withdrawing the needle. File name 000002_20250721_141929.MP4: 03:15-04:30 (3.4.3 and 3.4.4)

3.4.4. SCOPE: Talent making the second cut and removing the tissue wedge.

3.4.5. SCOPE: Both the animal and removed tissue being examined under magnification. File name 000002_20250721_141929.MP4: 04:30-05:00

3.5. Use the same transfer pipette to move the dissected animal into a new dish filled with sterile seawater **[1]**.

3.5.1. SCOPE: Talent transferring the animal using the pipette into a clean dish filled with seawater. File name 00001_20250721_135813.MP4 - 3:45 - 4:15

3.6. Next, treat a standard microscope slide with a silanizing agent, such as a water-repellent glass treatment, and allow it to dry completely **[1]**.

3.6.1. Talent applying silanizing agent to a slide. NOTE: 3.6.1. is filmed with wrong slate number- Please use clip C076_C825_07212B_001

~~3.6.2. SCREEN: To be provided by authors: Time lapse of dried slide (<1 min at room~~

~~temperature).~~ NOTE: Not filmed, its the same as 3.6.1

3.7. Use a transfer pipette to place one postsurgical animal onto the prepared slide **[1]** and confirm that the water beads up with the animal inside **[2]**.

3.7.1. ~~SCOPE:~~ Talent transferring the animal to the treated slide. NOTE: This is not a scope shot

3.7.2. SCOPE: A shot of the animal placed on the prepared slide and the water droplet forming a bead or dome-shaped droplet. File name 00001_20250721_135813.MP4 – 06:36-06:45

3.8. Place approximately 1.5 milligrams of modeling clay on each corner of the coverglass to form feet that will serve as a spacer to lift the coverglass away from the animal **[1]**. Gently place the coverglass, clay-side down, over the animal and roll it into position **[2]**. Press gently to slightly compress and hold the animal in place, adjusting as needed for optimal imaging **[3]**.

3.8.1. Talent placing modeling clay on the corners of the coverglass.

3.8.2. SCOPE: Talent positioning the coverglass onto the slide and rolling the animal into view. File name 00001_20250721_135813.MP4 – 07:25-07:45 and 08:05-08:25

3.8.3. SCOPE: Talent gently pressing and repositioning the animal for best imaging. . File name 00001_20250721_135813.MP4 – 08:50-09:05

3.9. For imaging longer than 30 minutes, apply a thin layer of petroleum jelly around the edges of the coverglass to prevent the specimen from drying **[1]**.

3.9.1. Talent applying a thin layer of petroleum jelly around the edges of the coverglass.

Results

4. Results

4.1. Wound closure was visibly completed within 20 minutes after aboral organ removal in cydippid-stage *Mnemiopsis leidyi*, with tissue spanning the wound site **[1]** that was initially open and exposing the gut **[2]**.

4.1.1. LAB MEDIA: Figure 5. *Video Editor: Highlight the bottom image (t = 20 min).*

4.1.2. LAB MEDIA: Figure 5. *Video Editor: Highlight the middle image (t = 0 min) and the red circle.*

4.2. Regeneration progressed gradually, with early tissue contraction evident by 60 minutes post-surgery **[1]** and full regeneration of the aboral organ observed by 72 hours **[2]**.

4.2.1. LAB MEDIA: Figure 6. *Video Editor: Highlight the two images labelled 0 min and 60 min.*

4.2.2. LAB MEDIA: Figure 6. *Video Editor: Highlight the three images labelled 0 min, 60 min, and 72 h.*

4.3. In lobate-stage *Mnemiopsis leidyi*, wound closure progressed steadily and was complete by 240 minutes post-surgery **[1]**, with full regeneration of the aboral organ clearly visible at 120 hours **[2]**.

4.3.1. LAB MEDIA: Figure 7. *Video Editor: Highlight the two images labelled 0 min and 240 min.*

4.3.2. LAB MEDIA: Figure 7. *Video Editor: Highlight the three images labelled 0 min, 240 min, and 120 h*.

1. Bunsen burner
Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/bunsen-burner Collins Dictionary+13Cambridge Dictionary+13Cambridge Dictionary+13
IPA (American): /ˈbʌn.sən ˌbɝː.nɚ/
Phonetic spelling: *BUN-sən BUR-nər*

---

2. Micropipette
No direct link was found from Merriam-Webster or Oxford, but the pronunciation is standard in scientific contexts.

- **Pronunciation link:** No confirmed link found
  IPA (American): /ˌmaɪkroʊˈpaɪˌpɛt/
  Phonetic spelling: *my-kroh-PY-pet*

---

3. Aboral (as in "aboral organ")
Pronunciation link:
https://www.merriam-webster.com/dictionary/aboral
TheFreeDictionary.comYouTubeTheFreeDictionary.com+10Merriam-Webster+10Wiktionary+10
IPA (American): /æbˈɔr.əl/
Phonetic spelling: *ab-OR-əl*

4. Mnemiopsis leidyi
Pronunciation link:
https://animals.jrank.org/pages/1447/Pronunciation-Guide-Scientific-Names.html (entry lists *nee-mee-OP-sis LEE-dee-eye*) howjsay.com+13animals.jrank.org+13marinespecies.org+13
IPA (American): /nəˌmiːˈɒp sɪs ˈliːˌdiː ˌaɪ/
Phonetic spelling: *nuh-MEE-op-sis LEE-dee-eye*