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Introductory Hydra Activities V.3

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This is a series of introductory lab activities for BIOL309-03: Research Methods. The purpose is to master vocabulary related to Hydra anatomy, become familiar with compound microscopes and dissecting microscopes, and practice microdissections and micropipette use. The second section sets up a head and tentacle regeneration experiment that will be documented a week later to provide quantitative data for a statistics activity.

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Microscopes are delicate and expensive instruments. Please handle them with care at all times and follow these guidelines:

- Hold the microscope by its base and carry it with two hands.
- Avoid touching any of the optical components.
- Only clean lenses with a lens paper and lens clearer or DI water.
- When you are on high power (20X, 40X, or 100X) only use the fine focus adjustment. On 4X or 10X, you can use the course focus adjustment.
- Don't adjust any components if you don't know what they do or try to fix anything you don't know how to fix. Alert your instructor if there are any issues with the microscopes.
- When you are done using the microscope turn off the light, move the objective to 4X, and cover it with the dust cover.

Everyone's eyes are a different distance apart, so it's critical to adjust the distance between the oculars every time you use a microscope that someone else may have used before. If the oculars are too far apart or close together you will see double images or nothing at all.

Stereo dissecting microscope
lab equipment

Meiji Techno unknown [↗](#)

Any stereo dissecting microscope will do. This model might be discontinued.

Compound upright binocular microscope
Lab equipment

Olympus CX31 [↗](#)

Any compound microscope will do.

Multi-well plates, not treated
Lab consumable

Corning CLS3738 [↗](#)

60 x 15 mm plastic petri dish

Lab consumable

CellTreat T9FB2782491 [↗](#)

Can use any plastic petri dish

Disposable scalpel

Lab consumable

Feather 08-927-5A [↗](#)

Use blade shape #10. They can be cleaned and used multiple times.

100-1000 µl Micropipette

lab equipment

MiniOne M2011 [↗](#)

Optional. Only needed for dispensing rifampicin solution.

TipOne Pipette tips in racks, blue graduated 1000uL

Pipette tips in racks, 1000uL

TipOne 1111-2831 [↗](#)



Glass pasteur pipettes, 5 3/4 inch
glass consumables

Fisher Scientific 1367820B [↗](#)

Any brand of glass Pasteur pipette will do, just make sure you have bulbs that fit. Plastic Pasteur pipettes are not recommended because Hydra tend to stick to plastic.

Rubber bulbs for Pasteur pipette
Lab equipment

Heathrow Scientific HS20622B [↗](#)

Look for 2 mL latex rubber bulbs

Hydra with Bud
Preserved organism

Ward's Science 470176-910 [↗](#)

Hydra, Nematocysts
Preserved organism

Ward's Science 470181-506 [↗](#)

Hydra, General Structure

Preserved organism

Ward's Science 470181-502 [↗](#)

Can be cross-section or longitudinal section

Preserved cnidarian specimens

preserved organisms

Any NA [↗](#)

Set of preserved cnidarians (sea anemones, jellyfish, corals, Velella, etc.), can be from any vendor.

Scalpels, microscope slides, and glass Pasteur pipettes should be handled with care. Dispose of used Pasteur pipettes in the broken glass bin and dispose of all blades in the red sharps container.

Do not put any living organisms down the sink. Dispose of all media that comes in contact with Hydra in the "Hydra Waste" container.

Please read through the entire protocol before beginning.

Familiarize yourself with the components of the microscope and its basic operation and review the basic anatomy of Hydra.

Observation activities

30m

- 1 In this activity you will become familiar with the anatomy of Hydra including areas of the body, cell layers, and nematocysts, while practicing the new vocabulary we learned in lecture.

Learning objectives:

- Use a compound microscope to examine prepared slides
- Master vocabulary related to Hydra anatomy
- Correctly identify body parts and cell layers in fixed Hydra specimens
- Sketch biological structures under a microscope
- Identify similarities and differences in animal anatomy

Materials:

- Compound microscope

- Microscope slides with Hydra whole mount, cross section, and nematocysts
- Selection of preserved Cnidarian specimens
- Lab notebook, pen, and pencil (optional)

In this activity, you will be sketching biological structures that you observe under the microscope. Your sketches don't have to be "good", they just have to show the general shapes and relative positions of the structures you are asked to examine. You probably know not to write in pencil in your lab notebook, but you can make an exception here. It may be convenient to sketch the structures first with pencil then copy over the final lines in pen.

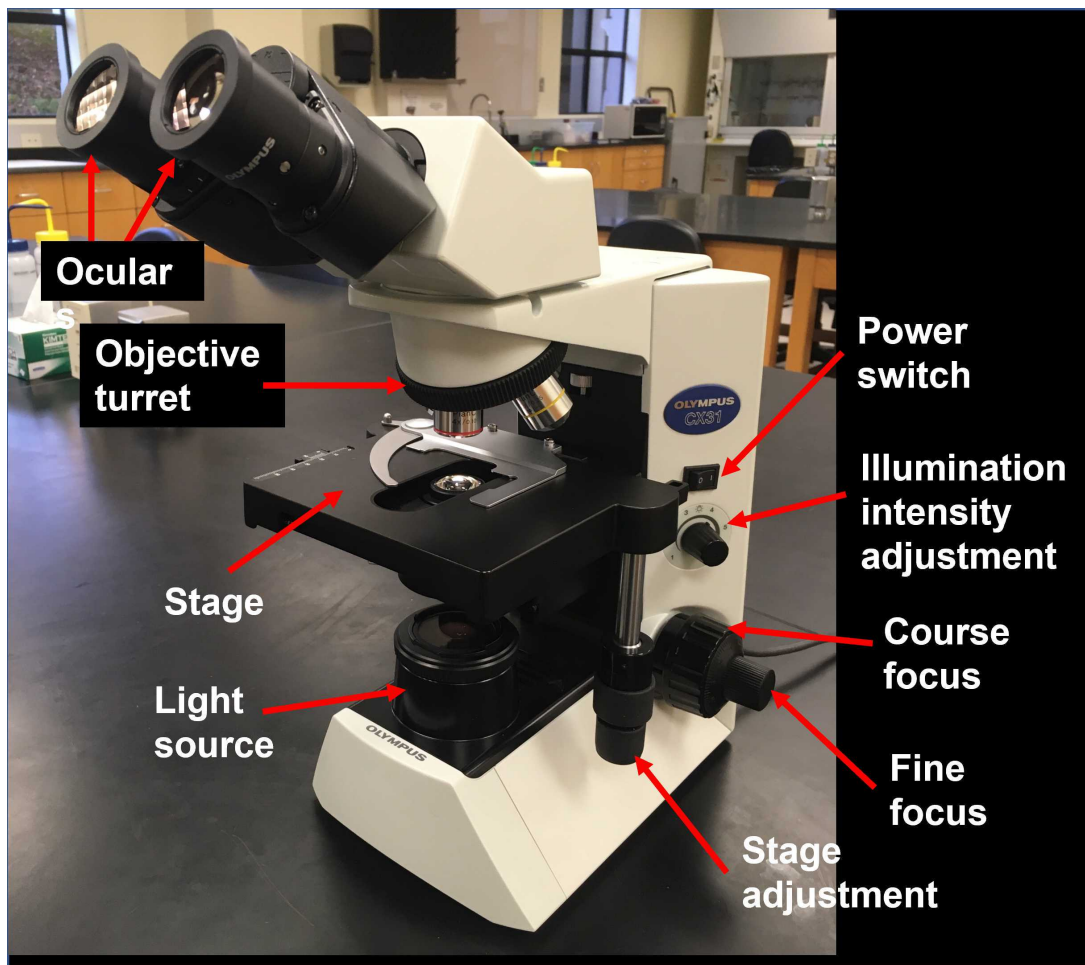


Figure 1. Parts of the compound microscope, just what you need to know for this activity.

2 Basic Hydra anatomy

Examine the whole mount Hydra slides with the 4X objective. Adjust the distance between the oculars and focus on the specimen if necessary. Sketch the whole Hydra in your lab notebook

and label the following body parts:

- Tentacles
- Hypostome
- Head
- Body column
- Peduncle
- Basal disk
- Bud
- Budding zone
- Label the hypostome and tentacles on the bud

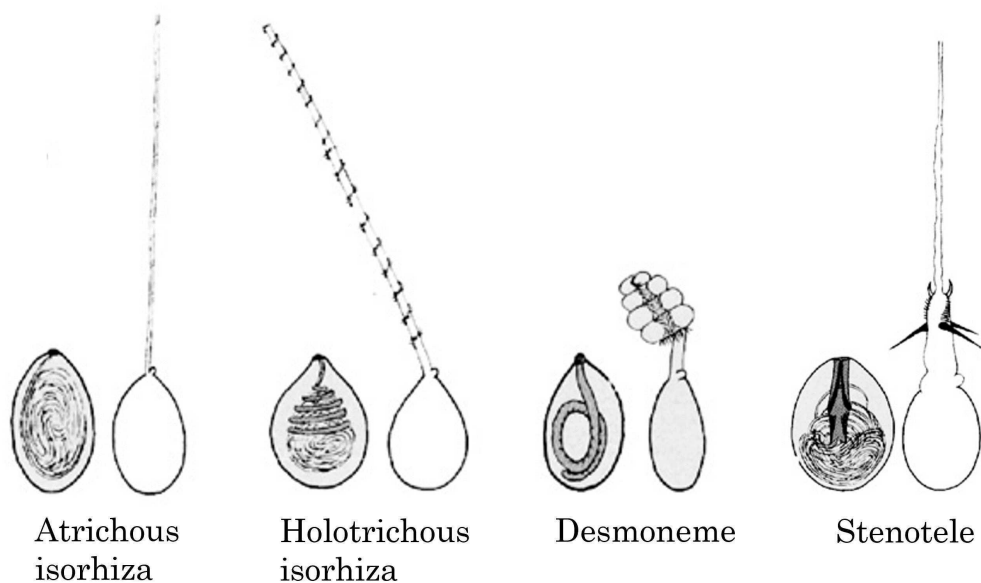
3 Body wall structure

Examine the Hydra cross section (transverse section) with the 40X objective. The cross section should already be centered in the field, so don't move the stage. Adjust the distance between the oculars and focus on the specimen if necessary (use only the fine focus adjustment). In your lab notebook, sketch just a section of the body wall and label:

- Endodermal cells
- Ectodermal cells
- Mesoglea

4 Hydra nematocysts

Examine the smear of Hydra nematocysts with the 40X objective. Adjust the distance between the oculars and focus on the specimen if necessary. Feel free to move the stage around to view different parts of the smear. Try to find at least two of the four different types of nematocysts (Figure 1) and sketch them in your lab notebook.



David, C. N., Adamczyk, P., Ozbek, S., Meier, S. (2008). Evolution of complex structures: Minicollagens shape the cnidarian nematocyst. *Trends in Genetics*, 24(9), 431-438.

Figure 2. Four types of nematocysts in Hydra.

Desmoneme: coiled filaments wrap around prey.

Stenotele: pierces prey cuticle and injects venom.

Holotrichous isorhiza: sticky filament with barbs used for defense.

Atrichous isorhiza: sticky filament without barbs used for locomotion..

5 Comparing Hydra to other Cnidarians

Examine the preserved specimens. In your lab notebook, make a list of the features that all of these animals have in common. What do they have in common with Hydra? What features make them appear different from each other and from Hydra?

Start your regeneration experiment

30m

- 6 Anyone who examines a large number of Hydra in a dish can clearly see that they do not all have the same number of tentacles. You may wonder, does each animal have a fixed number of tentacles? If we were to hypothesize that each animal has a fixed number of tentacles, it might follow that we cut a Hydra in half and allowed the foot-end to regenerate a head, this head would have the same number of tentacles as the head that was removed. We will keep the head-end of the Hydra to test whether the number of tentacles on an intact Hydra head spontaneously changes in the course of a week.

Learning objectives:

- Use a dissecting microscope to view live animals
- Become proficient with using an adjustable volume micropipette

- Practice moving Hydra with a Pasteur pipette
- Apply micro-dissection skills to create transverse sections of Hydra

Materials:

- Common dish with Hydra
- Dissecting microscope
- Scalpel
- 60 mm Petri dish
- Hydra medium
- 100-1000 μ L micropipette and tips
- 24-well plate
- Glass Pasteur pipette and bulb
- Waste container
- Permanent marker
- Labeling tape

For Hydra medium recipe and methods for maintaining Hydra in the lab, please see:



Low cost methods for Hydra care
by Callen Hyland

PREVIEW

RUN



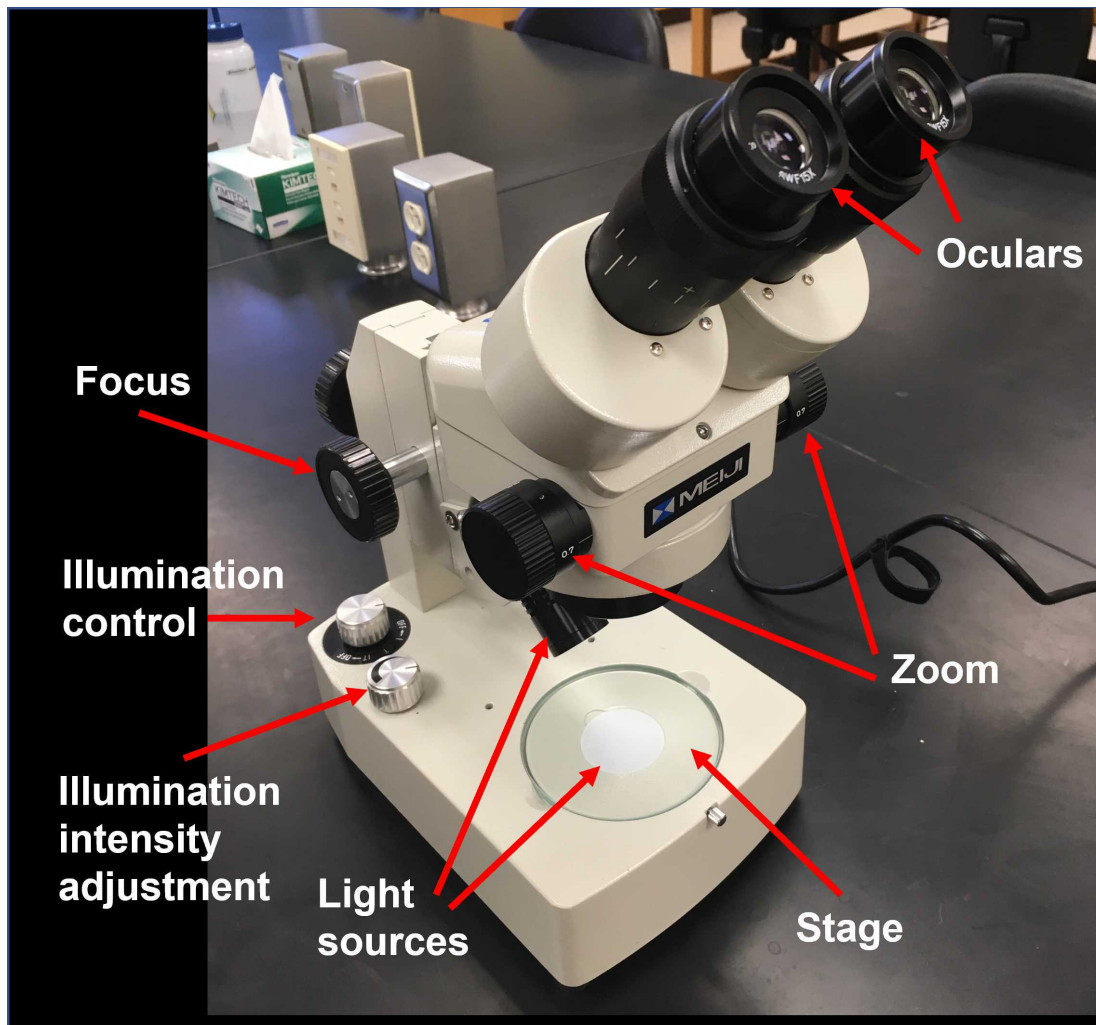


Figure 3. Parts of the dissecting microscope, just what you need to know for this activity.

- 7 Check your workstation to make sure you have all materials listed above.
Fill your Petri dish halfway with Hydra medium.
Use your glass Pasteur pipette to transfer six adult asexual Hydra without buds, one by one, from the common dish to your Petri dish.
- 8 Set your 100-1000 μL adjustable volume micropipette to 1000 μL . Get a new tip.
Fill 12 wells of your 24-well plate with 2 mL (2 X 1000 μL) of Hydra medium using your micropipette.
Dispose of used tips in the waste container.

12-well plates and 6-well plates can also be used, just adjust the volume of medium in each well.

Two students can share a 24-well plate.

- 9 Put a piece of labeling tape on the lid of your 24-well plate.
Use a permanent marker to label it with your name and date.
- 10 Place your Petri dish on the stage of your dissecting microscope and turn the light control knob to the "I" (incident light) setting.
Adjust the zoom to the lowest setting.
Adjust the distance between the oculars until you see a single field.
- 11 Adjust the focus knob to get a crisp image of one of your Hydra.
Try slowly zooming in on it, adjusting the focus as you go.
While doing the dissection, you'll have to find a magnification that works for you.
- 12 Count the number of tentacles that the Hydra has and record it in your lab notebook.
Since you are going to dissect six Hydra you may want to set up a table to record the following information for each Hydra:
 - Initial number of tentacles
 - Well containing head-end
 - Number of tentacles on head-end after a week
 - Well containing foot-end
 - Number of tentacles on foot-end after a week
- 13 Use your scalpel to cut the Hydra transversely (horizontally) in two. This is called a mid-gastric bisection.
Try to cut it right above the budding zone. In some adult Hydra, you can see a visible "bulge" near the budding zone. If this is not visible, just try to cut it into two equal parts.

Hydra are sometimes elongated and sometimes contracted. They are easiest to cut when they are elongated. If your Hydra is contracted, let it sit undisturbed until it elongates again before trying to make the cut.

Sometimes the Hydra will be floating on the surface of the water. Use your scalpel or the tip of your glass pipette to push them down to the bottom of the dish before cutting.

- 14 What happened to your Hydra right after you cut it?
Record any behaviors you observed in your lab notebook. Also record any "mistakes" that you make while cutting the Hydra (for example, cutting the same animal twice, or cutting in the

wrong place).

- 15 You should not have a head-end and a foot-end. Transfer both into separate wells of your 24-well plate.
Record which well the head and foot ends were placed in.
- 16 Repeat steps 11 through 15 for five more Hydra.
When you are done, place the lid on your 24-well plate and give it to your instructor.
Turn your microscope off when you're done and cover with the dust cover.

If the number of tentacles is not fixed for an individual Hydra, what factors do you think might effect the number of tentacles?
Are there any other control groups you think should have been included in this experiment?

Document results of your regeneration experiment

30m

- 17 We will allow the Hydra to regenerate for 7 days before counting the number of tentacles on the head and foot-ends. Get your 24-well plate that you used in the previous class session from your instructor.

Materials:

- Dissecting microscope
- Your 24-well plate
- Forceps (optional)

- 18 With the dissecting microscope, examine the Hydra in each well and record the number of tentacles in the table you started in step 12. If the Hydra are hard to see because they are stuck to the side of the well, you can use the forceps to push them into the center of the well.

Working with a partner, you can have one person observe and the other record, then switch roles.

- 19 When you're done, turn off the light on your microscope and cover the microscope with dust cover.
We will use the data you have recorded for statistical analysis to determine whether individual Hydra tend to regenerate the same number of tentacles that they had before bisection.