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A low cost time-lapse imaging system for Hydra

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We use this protocol and it's working

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Abstract

Hydra is a genus of freshwater cnidarian polyp known for their ability to regenerate their entire body from tissue pieces. Despite their simple body plan and tissue organization, Hydra is capable of complex behaviors, including capturing and consuming live prey, somersaulting locomotion, and phototaxis. Hydra make ideal model organisms for college and high school biology labs because they are easy to grow in large numbers and regeneration experiments can be carried out with minimal resources. The inexpensive setup described here allows time-lapse imaging of the behavior of Hydra, or other small invertebrates, with a cell phone camera. An imaging chamber is constructed out of cardboard. The sample is illuminated by a USB-powered LED panel and magnified by an inexpensive plastic lens. We also provide example movies and instructions for importing cell phone videos into ImageJ.

Image Attribution

Callen Hyland

Guidelines

For general guidelines for maintaining, working with and disposing of Hydra, please see <u>Low Cost Methods for</u> **Hydra Care**.



Materials

Materials for making the imaging chamber:

- Imaging chamber pattern files (download from Thingiverse)
- Corrugated cardboard, 2 or 3 mm thick
- Scissors, boxcutter, laser cutter, or Cricut cutter
- Glue or double-sided tape
- A6 LED light panel with micro USB cable (example)
- USB wall adaptor
- 25 mm plastic lenses (example)
- Translucent vinyl sheet (example)
- Optional: rubber bumpers (example)

Materials for behavioral imaging:

- Hydra and Hydra medium
- Glass Pasteur pipette and bulb
- Plastic or glass petri dish
- Cell phone camera
- Optional: ruler

Software:

- iOS or Android camera app
- Optional: <u>Lapselt</u>
- FIJI

Troubleshooting

Safety warnings



Use caution when working with glass Pasteur pipettes and sharp items like razor blades and scalpels and dispose of these materials in the proper container.

Before start

Assemble and test the imaging chamber before starting an experiment.



Assemble the imaging chamber

Cut all cardboard panels for the outside of the imaging chamber. You can use the *.dxf files with a laser cutter or Cricut cutter. If you are using a boxcutter or scissors, print out the *.pdf files, cut out the shapes, and use these to draw outlines on the cardboard to be cut.

30m

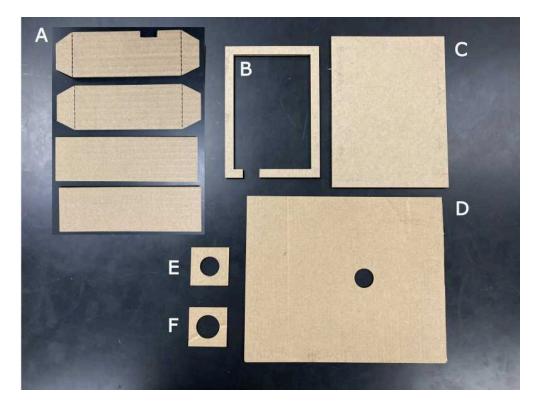


Figure 1. Cut out cardboard parts of imaging chamber. A. chamber walls, B. light panel support, C. base, D. phone platform, E. lens support, F. lens spacer.

2 Glue the light panel support to the base, making sure it's centered. This creates the base assembly.



Figure 2. Light panel support attached to base.

- 3 Optional: add four rubber bumpers to the corners of the base. This will keep the imaging chamber from moving on the bench.
- 1m

4 Glue the lens spacer to the phone platform centering the holes.



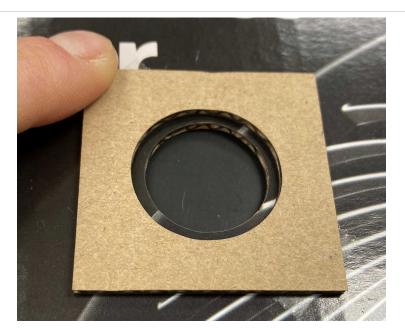


Figure 4. Lens support attached to phone platform.

5 Position the lens inside of the lens spacer with the concave side facing away from the phone platform.





Figure 5. lens inserted in the spacer.



Note

The recommended lenses are inexpensive and easy to purchase because they are mass produced for VR goggles. The focal length is 45 mm and the diameter is 25 mm. If you are using lenses with a different diameter, the size of the hole in the phone platform, lens spacer and lens support will have to be adjusted. If you are using a lens with a different focal length, the height of the walls will have to be adjusted.

6 Glue the lens support to the lens spacer, securing the lens between the lens support and the phone platform. Be careful not to get glue on the lens. This creates the phone support assembly.



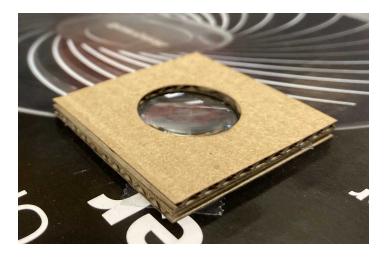


Figure 6. Lens attached to phone platform assembly.

7 Assemble the walls of the chamber. Make cuts halfway through one wall of the cardboard to fold the flaps to contact the long sides of the walls. This creates the chamber walls assembly.



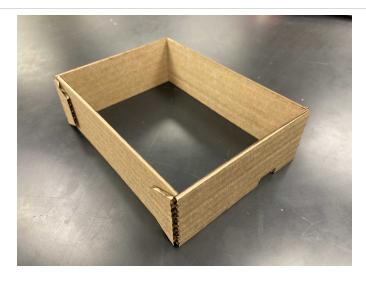


Figure 7. Chamber walls assembly.

8 Cut the vinyl sheet to 84 mm X 100 mm and adhere to the LED light panel. You can use the lightpanel_film.svg file with a Cricut cutter.



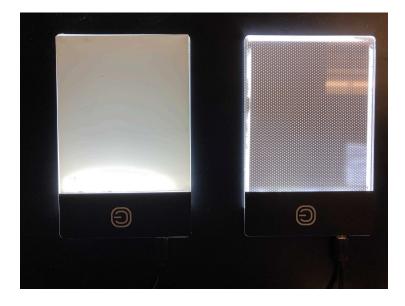


Figure 8. Left: light panel with vinyl sheet attached. Right: light panel without vinyl sheet.



Note

The A6 LED light panels recommended here were chosen because they are inexpensive and do not heat up the sample during imaging. Because the LEDs are located to the side of the panel there is a slight gradient of light intensity in the background (see example data below). If you need uniform background illumination, consider a different light source.

Image a sample

9 Place the light panel on the base assembly, connect the micro USB cable, and plug the cable into the wall adaptor. Turn on the light panel.

1m



Figure 9. Light panel on base assembly.

10 Place the Hydra in a petri dish with Hydra medium and place the dish on light panel, then place the chamber wall assembly on the base assembly.



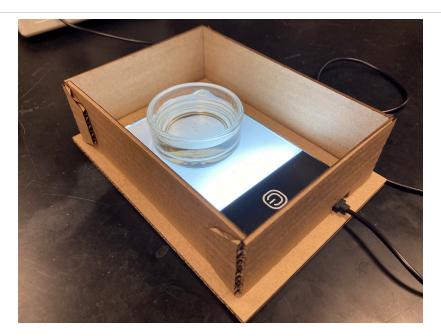


Figure 10. Sample in imaging chamber.

11 Place the phone platform assembly on top of the chamber walls.

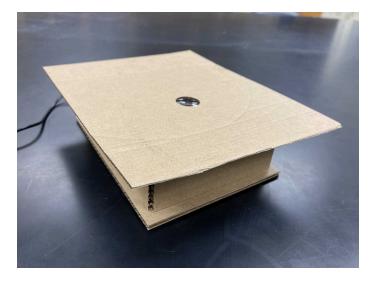


Figure 11. Assembled imaging chamber.

Note

For some experiments, you may want to let the Hydra acclimate to the new environment before starting the recording.

On your phone, open the camera app or Lapselt time-lapse app. With the phone's camera centered on the lens, move the phone platform until the sample is centered on your phone's screen.

2m



Figure 12. phone camera aligned with lens and centered on sample.

Set the desired time-lapse parameters on your phone and start the acquisition. The example movie below was acquired with the Lapselt app with 5 second interval for 30 minutes.





Note

You can zoom in on your sample for acquisition, but keep in mind that images of the ruler for setting the scale will need to be acquired with the same zoom settings.

Import video into FIJI

14 The iPhone camera app exports time-lapse movies in *.mov format and Lapselt exports movies in *.mov or *.mp4 format. Follow these steps to import movies in these formats



into FIJI.

15 Open FIJI. Go to the Help menu and select Update.



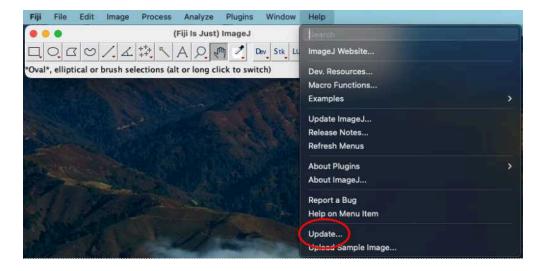


Figure 12. Help -> Update.

Add FFMPEG to the list of sites FIJI will download updates from. After the updater finishes running, click Manage Update Sites. From the list, check the box next to FFMPEG, then Apply and Close.



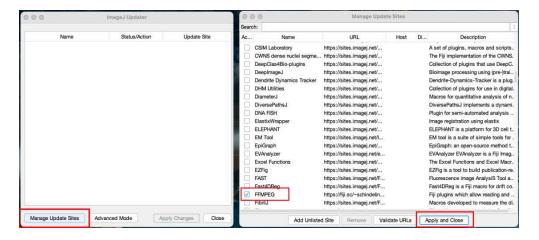


Figure 13. Add FFMPEG to update sites list.

17 Close and restart FIJI. Go to the File menu. Select Import → Movie (FFMPEG). This option will only be available after FFMPEG is added to the list of update sites.



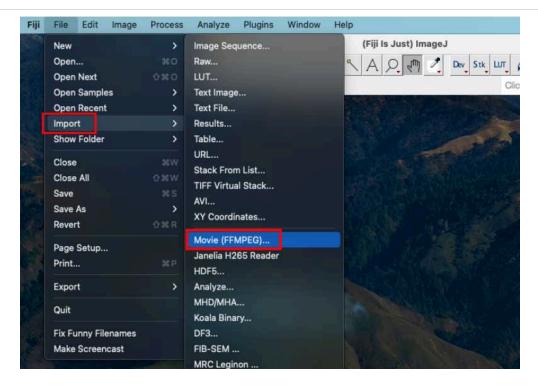


Figure 14. Import your movie as FFMPEG.

18 Navigate to your movie file and click Open.

1m

Optional: calibrate distance

- 5m
- Take a picture of a ruler with millimeter scale using the same camera settings as your time-lapse acquisition. Import the image into FIJI.
- 2m
- Zoom in on a centimeter in the middle of the field of view. Use the line tool to measure the number of pixels in one centimeter. Draw the line from the center of one centimeter line to the next.
- 1m
- Go to Analyze → Set Scale. The length in pixels of the line you drew should be filled in. Change the Known Distance to 10 and the Unit of Length to mm. Check the Global box.

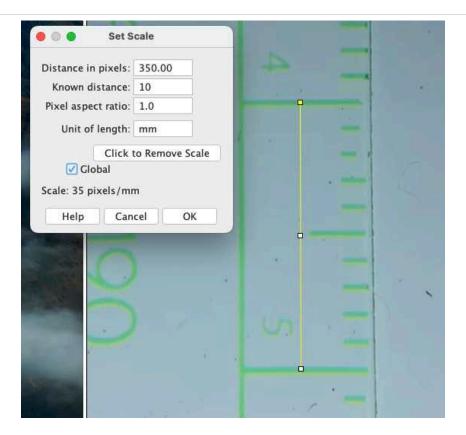


Figure 15. Setting a scale according to measured distance.

- 22 Open your time-lapse movie or image. The scale you set should be applied to the new image.
- 1m
- 23 Optional: add a scale bar. Use the rectangle tool to draw a rectangle approximately where you want the scale bar. Go to Edit → Selection → Specify. Check the Scaled Units (mm) box, then adjust Width, Height and X and Y coordinates to have the scale bar with the size and position that you want, then click OK.



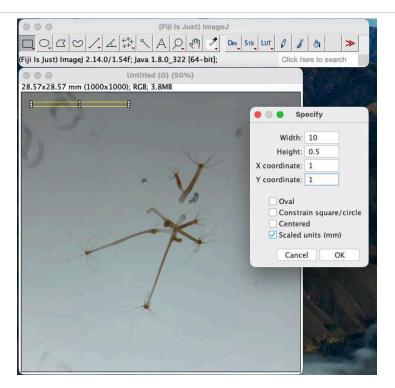


Figure 16. Specify dimensions of the scale bar.

24 Use Ctrl + F to fill in the rectangle to create the scale bar. Colors for filled shapes can be adjusted by going to Edit \rightarrow Options \rightarrow Colors.





Figure 17. Scale bar is 1 cm.