

Langerhans Lab Protocols

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Gonopodium Lateral Distal Tip Imaging Protocol

Starting steps:

1. Log in to computer H as Admin.
2. Turn on the microscope by switch at back.
3. Open the Leica software.
4. Proceed as below.

Photographing with Leica S8 microscope w/ TL RCI base and DFC 425 camera:

Software – preparation:

- Be sure the Leica microscope stand is turned on using the switch on the back, before logging in to the Leica program.
- Open the Leica software package (LAS V3.7) on the desktop.
- There are four tabs at the top of the window: Setup, Acquire, Browse, and Process.
 - Set the capture location (where you want your images to be saved) on the Browse page in the Folders box on the left side of the screen by selecting the appropriate folder (usually C:/gonopodia/[collection#]/raw) and clicking the “Set capture location” button.
 - If “Set capture location” is not available, go into Options -> Preferences -> capture photos to fixed destination.
 - Set the name of your images in Options -> Preferences -> Images. Generally, the name of the photo will be the specimen ID number.
 - Save as file type Tiff- 8 bit.

Software – camera settings:

- In the Leica software program, go to Acquire -> Camera and there will be a box in the top left of the screen with a 2x4 rectangle of buttons. Set the camera to Brightfield by clicking on the bottom right icon in that rectangle.
- In Acquire -> Mic1, adjust the CT knob (color temperature) approximately to “1:00”. You can also set this by using the dial on the right side of the base of the microscope.
- Light intensities and temperatures can be saved as presets here.
- The viewing field can be white balanced by placing white paper on the stage, and clicking the “white balance” button on the right side of the computer screen in the Leica software program, and dragging a square on the white.
- The camera base can be turned on and off from here. It is recommended to turn the base off using the software before exiting the program after each use (this way the switch will always be turned to the on position on the base, but powered on and off with the software).

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Microscope settings:

- *Gambusia hubbsi* are always photographed at 128x magnification (1.6x lens attached and magnification set to 8.0; $10 * 8 * 1.6 = 128$), but this will vary by species and specimen, depending on the size of the gonopodium tip.
 - If the 1.6x lens is not attached to the bottom of the microscope, the lens should be in a drawer just to the left of the microscope and computer (this is where it is kept, so you can also return it to this drawer if you need to remove it).
 - If a gonopodium image is taken at a magnification other than 128x, this needs to be noted in the filename so that scale can be properly set for morphometrics.
- Center the gonopodium in the picture plane as much as possible, while ensuring that all relevant structures are shown.
- Adjust the focus to bring the initial region of interest into sharp focus (the software allows you to alter the size and rate of the live-view window if desired). To modify your focusing abilities, you can change the precision of the manual focus handles by holding one and gradually turning the other (this will rarely be necessary; this alters the torque so that turning the handles can result in either large or small changes in focus).
- On the left hand side of the microscope base, the OB dial should be set to 45, and pushed as far away from you as possible.
- Keep the red 'scope cover over the top of the 'scope, to reduce overhead light on the specimen.

Positioning the Specimen:

- Set the fish on its right side in a Petri dish so that its head is facing left.
- Squirt a little water on the bottom of the Petri dish and on the fish so that the gonopodium (and body) doesn't dry out during the process, and also because the best images are often acquired while the gonopodium is completely immersed in fluid.
- The lateral side of the gonopodium must be parallel with the microscope/camera lens. This can be achieved by strategically laying pins or toothpicks on the fish and the gonopodium. Place one pin across the gonopodium near its insertion with the body and place one pin across the gonopodium near the tip (but out of camera view). This forces the gonopodium to rest against the flat surface of the Petri dish, and provides a nice lateral view for the camera.
- Position the specimen so that the key points (see figure) are centered in the screen. This usually means that the tip is near the right edge of the screen.
- To obtain an accurate lateral view of the gonopodium distal tip, be sure that the gonopodium is not twisted or tilted in any way. At the beginning, this will take some practice.
- Make sure the gonopodium isn't damaged and that all the key points, ridges, etc. (see figure) are clearly visible and not damaged. If it is damaged, don't photograph that specimen.
- If the fish/image is jumpy on the screen, press on the fish body to release air.

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Software – taking photographs:

- Though you can click “Acquire Image” in the lower left hand side of the “Acquire” screen, it is generally easier to simply hit F3 on the keyboard.
- Depending on the particular specimen, you will usually want to take 3-5 photographs of the gonopodium, focusing on different areas in each picture.
- It is generally recommended to start with the gonopodium out of focus and slowly turn the black fine focus knob (only turn one at a time, not both) until the first section of the gonopodium comes into focus. Acquire this picture, and then continue to slowly turn the fine focus until a different section is in focus, capturing a photo each time. Do this until each section of the gonopodium is in focus for one picture. Try to do this in as few photos as possible (facilitated by having the gonopodium as parallel as possible to the camera lens) because this usually results in a higher-quality stacked photo.
- In the Leica software, click the Browse tab to see the images you have taken. Click through one specimen’s images to see the areas in focus in each picture, to make sure you have all of the gonopodium in focus.

Image Stacking (create one composite image from several gonopodium photos):

- Open the program Helicon Focus (HF).
- In HF there is a right side bar where you can locate the folder in which your photos from Leica are saved.
- Select the images you wish to stack for a single composite by clicking in the little box next to each photo.
- After all desired photos are selected, click Run on the upper right side of the screen.
- Visually check the image for clarity of key points. If any are not clear, re-take an image with that point in focus, then re-run the stacking.
- Save the photo as a .tif file to the appropriate folder (usually the gonopodium_lateral folder for that collection).

Closing steps:

1. Turn off lights through Leica software -> Acquire -> Mic1 -> Power
2. Turn microscope off by switch at back.
3. Close software.

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The final image should look like this (resolution reduced and top & bottom cropped):



Key morphometric points that should be visible and undamaged:

