

Langerhans Lab Protocols

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X-Ray Imaging Protocol

Preparation and Calibration:

1. The X-ray tube must warm-up before use after significant gaps of non-use (about once every day should suffice). Initial warm-up time might take as long as 30 minutes; subsequent warm-ups might be as short as 10 min. To warm-up and operate the X-ray device:
 - a. Close the cabinet.
 - b. Switch the POWER key from Off (O) to On (I).
 - c. Turn OPERATE dial from Off to On.
 - i. If the INTERLOCK light does not come on, check that the doors are securely shut.
 - d. Switch on the Detector (sitting on top of the control unit) using the power switch in the back.
 - e. Push the X-RAY ON switch and it will light up, automatically starting a 10-30 minute warm-up sequence. During warm-up, the orange Aging light will be on, and it will cycle through increasing calibration points of voltage and current. When it is done warming up, the green Standby light will be on, the Aging light will be off, and the X-ray will turn off. **DON'T GO TO STEP 3** until the Aging light is off and Standby is on.
2. Boot up the laptop, log in as X-Ray User (no password needed), and open the Viva software on the desktop. It should automatically connect to LN-RAD-G1; if not, select link to Virtual CP in the Acquisition Mode tab.
3. Once the unit is warmed up, do Imaging Calibration:
 - a. Go to Acquisition, then to Mode Settings (F2), and change the number of calibration frames to 4 (from 4-8 frames works well).
 - i. Click Apply → Click Ok
 - b. Click on Acquisition, then Gain Calibration (F4), and make sure X-Ray is off.
 - c. Click Continue and it will do the offset calibration automatically.
 - d. Turn the X-Ray on, wait until 45kV and 40mA are reached, and click Start to resume the gain calibration.
 - e. After it finishes, turn the X-Ray off and click Finish then Close.
 - i. If you get an error (no calibration), go back to step b.
 - f. You should perform the calibration at the kV and μ A setting you will use for imaging (or similar). If you change the setting dramatically, you may want to perform a new calibration.

Operation:

To acquire an image:

1. Choose shelf height: see pg 3 for Field of View dimensions. Higher shelf level (i.e., closer to X-ray source) gives higher magnification, so choose the highest shelf level that will accommodate your region of interest.
 - i. Level 0 = shelf is at level 1, specimen is on top of inverted petri dish (there is a specific, labeled dish to use in the X-ray table drawer). See Notes section about shelf level labels.
 - ii. For *Gambusia*, generally use L1 or L0 for males, L2 or L3 for females.
- b. Place a specimen in a petri dish (unless the specimen is too large). There is a specific, labeled dish to use in the X-ray table drawer. If the specimen doesn't fit in the petri dish, set it on the X-ray shelf without a dish.
- c. Place the specimen inside the cabinet:
 - i. **DO NOT TOUCH** the little window at the top
 - ii. dorsal side away from door, head/anterior to the right
 - iii. directly underneath the x-ray source. The target area is marked on the shelf (see Notes).
- d. Click Acquire Image (F5), turn the X-ray on, click Start, wait until the image appears, then turn the x-ray off.

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2. To take the best images, adjustments may be necessary in:
 - a. voltage – use 45kV for *Gambusia*; higher # will go through denser material, lower will allow imaging of soft tissue. Use the up & down arrows while the X-ray is on to adjust.
 - b. current – use 40 μ A for *Gambusia*. Use the up & down arrows while the X-ray is on to adjust.
 - c. distance of specimen from the source -- closer to X-ray source gives higher magnification, but also reduces field of view
 - d. frames per second -- located in Mode Settings; 0.2-0.5 is recommended
 - e. number of frames to capture -- located in Mode Settings; 1 is usual; setting this number higher will mean that multiple images are taken which are then averaged, possibly resulting in a clearer image
 - f. placement of the detector at the bottom of the cabinet -- recommended position is indicated with black corner frames marked onto floor of cabinet
 - i. one axis is longer than the other, so orient the specimen accordingly
 - g. if image has strong dark \rightarrow light gradient across image background, do Offset Calibration (F3), then Gain Calibration (F4)

To save images: (see X-ray Template Protocol.docx for spreadsheet info)

1. *Before* you modify the image, save it as a 16-bit .raw file. Don't save it in Viva's proprietary format to facilitate use in other photo-editing software programs.
 - a. Label pictures with specimen number and magnification (shelf) level in the filename (e.g., 13289_L2PD.raw). Level is denoted as L0, L1, L1PD, etc. The "PD" denotes that the specimen was in a petri dish (otherwise assumed the specimen was directly on shelf).
 - b. Save the image in X-ray folder -> your name folder -> collection ID # folder.
2. Also save a .jpg of each image, *after* adjusting the image:
 - a. Use the box tool to frame the specimen as closely as possible, then click "A W/L" (= auto W and L adjustment).
 - i. W corresponds to contrast, L corresponds to brightness
 - ii. you can adjust W and L manually using the sliders below the A W/L button
 - iii. "invert W/L" makes the image white on black (like a traditional X-ray)
 - b. after adjusting, click Save As -> .jpg -> Save
 - i. After clicking Save, set quality to 10.

When finished:

1. Turn the X-ray off, turn the OPERATE dial to off, turn the key from on(I) to off(O), and turn off the detector on top of the x-ray control console.
2. Fill out the Langerhans Lab X-rays.xls spreadsheet on the laptop desktop indicating which X-Rays you did and what collection they are from. You should also use this spreadsheet to indicate your near-future plans for x-imaging to avoid duplicating x-rays among lab members / students.
3. Fill out X-Ray Log of Use in the sleeve on the Detector every time you use the machine. EHS requires us to keep this information and they can request it from us at any time.

To polish images:

Do not change the .raw image. You can create a new .jpg from the .raw using Photoshop if your adjustments go badly. Open .jpg image in FastStone (or other) application, then:

1. Rotate image so that the head faces left and the dorsal side is up.

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2. Adjust brightness/contrast if necessary. You should be able to see all the bones in the specimen if possible. One indicative region is the pharyngeal jaws and teeth below the otoliths (large black ellipses in the back of the head)—this region should be clear and not appear as a large dark region.
3. Using the Crop tool, trim the image so that the specimen fills the frame, and replace the original .jpg image with the cropped one.

Notes:

- The **shelf levels** are denoted as L1 (highest placement), L2, L3, etc., and are labeled with tape inside the cabinet. These labels are located on the shelf holders (so that the appropriate label is covered up by the shelf) as well as beside the holders to clearly indicate which level the shelf is on. L0 is the setting with the specimen placed on an inverted, large plastic petri dish on L1 (labeled dishes are in the drawer of the X-ray table).
- **Target placement zones** are marked on the shelf in colors that correspond to the shelf level label tape (blue = L1, green = L2, black = L3, red = L5). + sign in the center = image center. Note that image center shifts slightly with level changes, so that the blue + of L1 is not the same as the red + of L5.
- Close cabinet doors gently as air pressure can disturb the specimen.
- The .raw image displayed on the monitor is the view of the specimen *from underneath*. That is, the image on the screen has the short axis reversed compared to what you see when you look down at your specimen. Look for the guide written in the corner of the shelf (Top, Bottom, Left, & Right).
- IP Address: 192.168.2.11 for TCP/IP – Local Area Connection 2.
- 768 X 960 – Position of Exact Middle of the Image.
- To open RAW files in Photoshop, ImageJ, or other program, use the settings: Width: 1536 pixels, Height: 1920 pixels, 1 channel or image, 16-bit (unsigned), IBM-PC byte order (or Little-Endian), no header. The image may appear black or with poor brightness/contrast (it is the raw data after all), but using auto-adjust levels in Photoshop or auto brightness/contrast in ImageJ usually gets it in the right ballpark very quickly.

Questions?

- Check the manual in the envelope on the side of the cabinet.
- Check the software manual located on the desktop of the Laptop.
- Contact Gary Korkala, whose card is affixed to the cabinet. Garrett, who works with him, can also help with software issues.

Scale Information on Next Page...

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Scale Information:

Magnification	Scale (mm/px)	Field of View (1920x1536)	
		Height (mm)	Width (mm)
L0	0.013063	25.08	20.06
L1	0.016488	31.66	25.33
L1PD	0.016221	31.14	24.91
L2	0.023229	44.60	35.68
L2PD	0.023015	44.19	35.35
L3	0.030165	57.92	46.33
L3PD	0.029896	57.40	45.92
L4	0.036697	70.46	56.37
L4PD	0.036495	70.07	56.06
L5	0.043571	83.66	66.93
L5PD	0.043478	83.48	66.78
L6	0.050377	96.72	77.38
L6PD	0.049999	96.00	76.80
L7	0.077185	148.19	118.56
L7PD	0.076866	147.58	118.07