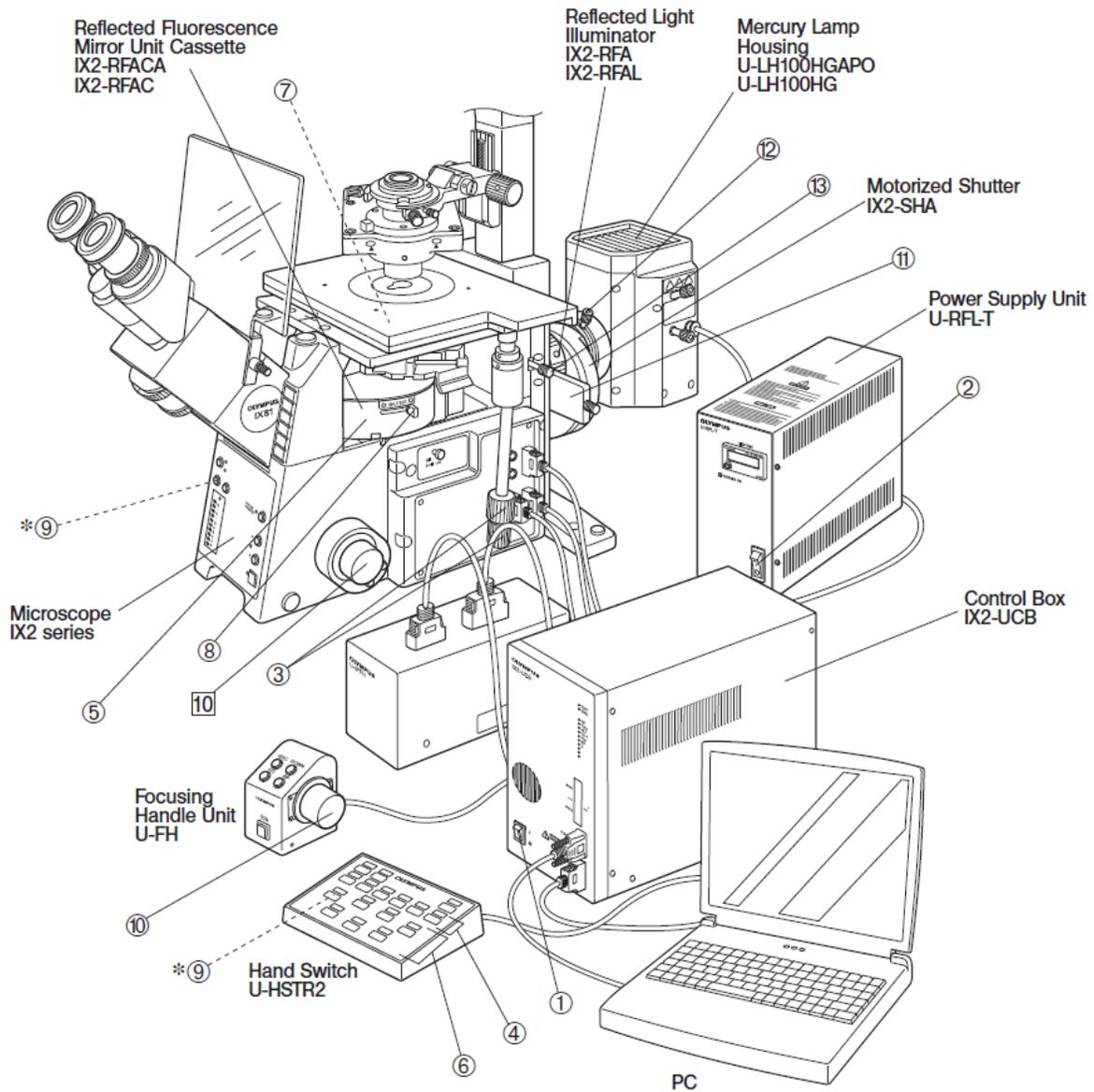


Leach Fluorescence Microscope (IX81 Olympus):

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UMBC 7/27/17



1. Turning the Unit On

- 1.1. Sign in on the instrument log sheet.
- 1.2. From left to right, turn on the power supply unit, control box, camera, and stage control box. Then turn on the computer.
- 1.3. Login with your UMBC username and password

****Only people that have been trained and granted access to the computer will be able to log on.****

1.4. Open Slidebook software.

2. Sample Loading

- 2.1. Select the stage attachment appropriate for your sample.
- 2.2. Lift the microscope column up on its hinge out of the way, careful not to touch the lenses.
- 2.3. Pull current stage attachment up to pop it out of the setting.

*****Notice the left corner of the stage inset, closes to the eye piece, has a small metal bouncer spring. This locks the stage attachment still on the moving stage. If the stage attachment is not properly set, your sample will not be level with the objective and your images will not be able to focus.*****

- 2.4. Place your desired stage attachment piece on the stage platform so that the left edge is at the center of the stage platform opening, teetering on the right edge of the stage platform opening.
- 2.5. Place your left hand on the left side of the stage attachment and apply moderate pressure as you use your right hand to push the stage attachment left into place in the stage inset.
- 2.6. The stage attachment should click into place. Press on each corner of the attachment to make sure there is no movement. The attachment should be flush with the stage platform.
- 2.7. If the stage attachment teeters, then it is sitting above the spring and is not held in place. Start over with the attachment to the right of the inset and slide it into position.
- 2.8. Load your sample onto the attachment.

****Note live fungus, yeast, and bacteria are not allowed on this microscope. Fungus, yeast, and bacteria must be fixed before being imaged.****

*****If any solution spills on the microscope objectives below the sample, inform the current microscope manager or Dr. Jennie Leach immediately. The water and salts will quickly fuse the metal objective threads to the microscope carousel causing major damage.*****

3. Viewing Specimen

3.1. Brightfield

- 3.1.1. Lower the microscope column on its hinge into its lowest position.
- 3.1.2. Adjust filter wheel for phase contrast or DIC microscopy with intended objective in mind. See "Understanding Microscopes and Objectives" document for Leach Microscope objectives and filter turret settings.
- 3.1.3. In Slidebook software, click the Focus button (icon is an "F" with a blue circle around it).
- 3.1.4. Click the objective you would like to use, it will be set automatically.
- 3.1.5. To initially focus your specimen click 100% Eyes to look through the eye pieces not the camera.
- 3.1.6. Slide the Lamp slider to 20%
- 3.1.7. Halfway down the window are 6 boxes designating the available filters. For brightfield, click Open.

- 3.1.8. To the right of the filters click the Open Bright button. When the light is turned on, the little lightbulb next to the button will be illuminated.
- 3.1.9. Look through the eyepiece and use the focus dial to bring your specimen into focus. You can toggle between course and fine focus using the button below the focus dial that says "Focus". You may want to adjust the lamp power.
- 3.1.10. Use the joystick to move the stage. You can toggle between course and fine movement by pressing the button on top of the joystick.
- 3.1.11. Once your specimen is in focus, click the 100% Camera button to see your specimen image in the software. You will need to adjust the focus using fine focus to adjust from the eyepiece to the camera.
- 3.1.12. On the live camera feed red may appear. You must lower the Lamp slider to allow less light in. Otherwise when you capture the image, anywhere the red is will be whited out.

3.2. Fluorescence

- 3.2.1. The microscope column is entirely for brightfield imaging, the fluorescence unit is under and behind the stage. You do not need to lower the column on its hinge for fluorescence work.
- 3.2.2. In the Slidebook software, click the Focus button (icon is an "F" with a blue circle around it).
- 3.2.3. Click the objective you would like to use, it will be set automatically.
- 3.2.4. To initially focus your specimen click 100% Eyes to look through the eye pieces not the camera.
- 3.2.5. Slide the Lamp slider to 0% to prevent any random light from entering the imaging field.
- 3.2.6. Halfway down the window are 6 boxes designating the available filters. The fluorescent filters are DAPI, FITC, Txs Red, and CY5. Select the filter you would like to use.
- 3.2.7. To the right of the filters click the Open Fluor button. When the light is turned on, the little lightbulb next to the button will be illuminated. Be sure not to click the Open Bright button, this will turn on the brightfield, but if the lamp is set to 0% no light should enter.
- 3.2.8. Look through the eyepiece and use the focus dial to bring your specimen into focus. You can toggle between course and fine focus using the button below the focus dial that says "Focus". You may want to adjust the lamp power.
- 3.2.9. Use the joystick to move the stage. You can toggle between course and fine movement by pressing the button on top of the joystick.
- 3.2.10. Once your specimen is in focus, click the 100% Camera button to see your specimen image in the software. You will need to adjust the focus using fine focus to adjust from the eyepiece to the camera.
- 3.2.11. On the live camera feed red may appear. You must lower the Exposure slider to allow less light in the camera. Otherwise when you capture the image, anywhere the red is will be color saturated.
- 3.2.12. With the Exposure bar you want to increase the exposure as high as possible without any red in the image. This will give you the best contrasting image. Remember the exposure number, it maxes out at 500.
- 3.2.13. When you are happy with the image in the live camera view, turn off the fluorescence by clicking "Close Fluor". This will minimize photobleaching.

4. Image Capture

- 4.1. Click the camera icon to open the Image Capture window.
- 4.2. You will be prompted to save this Slidebook. Click yes. This saves all your slidebook image files so that you may open the file at a later date to adjust the contrast, color, scalebar etc. Save this as a more general name such as the date.
- 4.3. For basic image capture, ignore the top half of the window and move to the white box with the filter options. Check the box for the filters you want to use. For each filter you check, you need to input the Exposure number in the box to the right.
- 4.4. At the bottom of the window in the Image Information box, enter the Name to identify your sample image. Be specific (ex: 2D F12 24hr well1 img1).
- 4.5. For fluorescence make sure you lower the dark box window to reduce outside light.
- 4.6. Click Start.
- 4.7. The captured image will appear. On the menu bar click Annotations → Scale Bar. You can adjust the scalebar size, color and position by clicking the View Setting icon on the image window (icon on the far right).
- 4.8. Click Annotations → Set Default Setting. This will save the scalebar setting to every image you take. Otherwise you will have to add the scalebar to each image before you export it.
- 4.9. Click the Adjust Renormalization icon (looks like a black mountain with a red and green bar). This is how you adjust the background and brightness of your fluorescent image. If you have multiple filters you will have to make adjustments to each filter by selecting them one at a time from the dropdown menu.
- 4.10. Move the left red bar to the right so that it is just left of the highest peak. This will reduce background signal.
- 4.11. Move the right green bar to the left to brighten color. Use your own digression to have minimal background and optical detail.
- 4.12. Click OK on the Adjust Renormalization window to close it.
- 4.13. Click the Set Default View button to save the adjustments to the image (icon looks like a finger nail).
- 4.14. Exit the image window and click the Focus button to start your next specimen image search.
- 4.15. Take all your images. When done, on the menu bar click View → Export → All images as Tiffs. It will prompt you where to export your images to. In the D drive, enter your Lab's folder, then enter your user folder and click OK. Your images will be exported with the names you gave them.
- 4.16. Exit from Slidebook and you will be prompted to save your Slidebook, click Yes to save all your images for editing.
- 4.17. Use a flash drive to take your exported Tiff images off of the computer.

5. Shutting Down

- 5.1. Close the Slidebook software and shutdown the computer.
- 5.2. Turn off the four boxes in opposite order, from right to left starting with the stage controller box, then the camera, the control box, and finally the power supply unit.
- 5.3. Sign out on the instrument log sheet.
- 5.4. Remove your sample from the microscope.

- 5.5. Turn off the lights and deadbolt the door.
- 5.6. Return the shared key to its storage location.