

AxioObserver Inverted Microscope - Color Camera Image Capture Instructions

1. Sign in the log book

Turning Hardware “On”

2. Power ON the appropriate camera (Orca is middle box; Color Orca is bottom box)
3. Power ON the Mac 5000 controller box
4. Power ON the fluorescence lamp AND select the appropriate light aperture setting.
5. Power ON the microscope (switch is flat, grey; located on back left of the microscope body)
6. “Wake-up” and then restart the computer so that it will recognize all the hardware you have turned ON.

Configure the Microscope to Begin Viewing with Transmitted Light

1. Lean back the tilt-back arm and place your sample on the stage.
 - if a slide, be sure that the mounting media is dry and that the coverslip is down
2. Manually rotate in the desired objective.
3. Select the desired light path – eyes or color Orca camera. Use the wheel on the left side of the microscope body to select the desired path.
4. Select the DIC filter cube for all transmitted light imaging.
 - Use the front two buttons on the focus knob on the right side of the scope to rotate the filter turret position. The cubes are labeled on the right side of the scope and will be displayed on the panel on the top of the tilt-back arm.
5. Insert additional components for DIC or phase imaging.
 - DIC: rotate the polarizer into place; select the appropriate condenser setting; insert the correct prism behind the objective
 - Phase: make sure you are using a phase objective; select the appropriate condenser setting
6. Turn on the TL light source to begin viewing the sample.
 - TL and RL buttons on the right panel, bottom front area, control the transmitted (TL) and the fluorescent (RL) light inputs to the sample
 - TL: lamp voltage control; press and hold > 1 sec initially and light will go to 3200K; when “ON”, a quick press will turn it off; a press > 1 sec will reduce the voltage to dim the light; when at the low voltage, press and hold for > 1 sec and it will return to 3200K. See additional documents for more information regarding the TL controls.
7. Focus on the sample using the digipot controller.
 - Do NOT use the regular focus knob on the left side of the microscope body.
 - The digipot can operate in COARSE, MIDDLE, or FINE. Use the toggle switch on the front of the digipot to choose which one. Be sure to always use FINE when using the 40x and 63x objectives.
 - Direction of rotation:
 - CLOCKWISE rotation: moves the objective closer to the stage; values on software get larger, more positive
 - COUNTERCLOCKWISE rotation: moves the objective away from the stage; values on software get smaller, more negative
8. Change the optivar to the desired magnification. Options are 1x, 1.25x, and 1.6x. This is encoded so the software will know that you have changed the optivar setting. However, the spatial calibrations in the software are designed for the 1x optivar setting ONLY.

Configure the Microscopy to Begin Viewing with Fluorescent Light

1. Lean back the tilt-back arm and place your sample on the stage.
 - if a slide, be sure that the mounting media is dry and that the coverslip is down
2. Manually rotate in the desired objective.
3. Select the desired light path – eyes or color Orca camera. Use the wheel on the left side of the microscope body to select the desired path.
4. Select the appropriate filter cube.
 - Use the front two buttons on the focus knob on the right side of the scope to rotate the filter turret position. The cubes are labeled on the right side of the scope and will be displayed on the panel on the top of the tilt-back arm.
5. Select the desired light source to begin viewing the sample.
 - TL and RL buttons on the right panel, bottom front area, control the transmitted (TL) and the fluorescent (RL) light inputs to the sample
 - RL: shutter control; press once to open the shutter, press again to close the shutter
 - Make sure that the aperture setting on the fluorescent lamp house is appropriate.
6. Focus on the sample using the digipot controller.
 - Do NOT use the regular focus knob on the left side of the microscope body.
 - The digipot can operate in COARSE, MIDDLE, or FINE. Use the toggle switch on the front of the digipot to choose which one. Be sure to always use FINE when using the 40x and 63x objectives.
 - Direction of rotation:
 - CLOCKWISE rotation: moves the objective closer to the stage; values on software get larger, more positive
 - COUNTERCLOCKWISE rotation: moves the objective away from the stage; values on software get smaller, more negative
7. Change the optivar to the desired magnification. Options are 1x, 1.25x, and 1.6x. This is encoded so the software will know that you have changed the optivar setting. However, the spatial calibrations in the software are designed for the 1x optivar setting ONLY.

Setting up the Software

1. Open the program, “Volocity”
 - Double-click on the icon on the desktop ONCE. Be patient, it sometimes takes a few moments to open.
2. Choose to create a new library or open an existing library
 - If creating a new library, then give an appropriate name and save the library in the “Data-Exchange” folder
3. Select “Window” → “Show video preview”. If you have both the color and black/white cameras turned ON, then you will need to choose the SOURCE (open the Preview; “Video → “Source” → then select the appropriate camera)..
4. Direct the light path to the camera using the black wheel on the left side of the microscope.
5. Click on the dummy objective bar to tell the software which objective you are using. The software will know the settings for the fluorescent cube and the optivar.
6. Select the appropriate light path.
 - There are 9 different light path settings. Position your mouse over the top of a path setting to find out what it is configured for.
 - SUGGESTION: there are light paths for each of the fluorescent cubes in the microscope, a fluorescent preview light path, and a DIC/phase light path. Use the preview path to find the sample, find area of interest and adjust exposure time. Then, switch to the cube specific path for actual acquisition.

- **What lights paths do:** provide “appropriate” pseudocolor; set software for fluorescent filter cube; set binning, light mode, gain, contrast, and exposure time; open the FL shutter. The DIC/Phase light path has an exposure time based upon a 3.5V light intensity.
- **What light paths DO NOT do:** change the filter cubes on the microscope, make settings non-adjustable. Be careful: you can change filter cube settings but not change the light path and end up confused about what channel/fluorophores you were imaging.
- DO NOT manually open or close the shutter.
- To close the shutter, click the circle on the bottom left of the control panel, beside the image of the mercury bulb. The circle will become gray. Click again to open the shutter.

Acquiring an Image

20. Set the acquisition parameters.

A. Adjust camera exposure time.

- Use the software slider or entering numbers manually. DO NOT oversaturate the image. If you see RED pixels on the preview image, then the image is oversaturated. Turn down the exposure time until there are no red pixels.

B. White balance the image.

- Automatic: “Video” → “Auto-white balance” (need focus and exposure good first). You can also access this by right-clicking anywhere in the right control pane.
- Manual: In the right control pane, there are 3 sliders for R, G, and B. You can try to adjust them manually. I recommend trying auto first.

C. Review and adjust the acquisition set-up.

- Right click in the control pane to get a menu to open.
- Select “Acquisition setup”
- Give the image an appropriate title
- Add useful, informative description
- Select the appropriate Z-step using the printed table for stacks.
- DO NOT change the “duration” or “change channels” boxes unless you are doing multi-color or time-lapse acquisition.
- Under “Manage shutters”, select the appropriate choice for your sample.

D. To capture a single-frame:

- Click the “camera” button located to the left of the red “record” button

E. To capture a stack of images:

- Move the focus and find the top of the sample. Top: numbers will get larger, or more positive.
- Left click on the box with up and down arrows beside the image of a focus knob. A small window will pop open. Click: “Set top”
- Move the focus to the bottom of the sample. Click: “Set bottom”.
- Left click the RED “Record” button at the top of the control panel to acquire the image stack.
- The image stack will automatically be saved into the library that you created or opened at the beginning.

Exporting an Image

21. From the Library view, select the files that you want to export.
22. Go to File → Export and select the folder where you want to put the images. Please put them in the “Data Exchange” folder. I recommend that you put them in the same folder that the Volocity files are located in as well.
23. Select the appropriate file format (TIFF or TIFF for publication are recommended).

Exporting an Image with Scale Bars

21. From the Library view, click on the image to “Open” it.
22. Make sure the image is at a zoom level of 100%. You can see what the zoom level is at the bottom right corner of the Volocity window. Use the magnifying glass and left click to zoom in; press the control button, left click and you will be able to zoom out.
23. Go to “Show scale” and adjust the size of the scale. You can also edit the color of the scale and whether there will be font associated with it.
24. Capture a snapshot of this image.
25. Now, you will see two scales of different sizes. Now go back to the menu and select “Hide Scale”. This will remove the floating scale and leave just the correct scale “stamped” onto the image.
26. Go to File → Export and select the folder where you want to put the images. Please put them in the “Data Exchange” folder. I recommend that you put them in the same folder that the Volocity files are located in as well.
27. Select the appropriate file format (TIFF or TIFF for publication are recommended).