SOP: EVOS XL Core Imaging System

Purpose: Observe and capture images of cells in culture

Location: BHE B8A (cell culture room in BME Cellular/Molecular Teaching Lab)

PPE: fluid-resistant lab coat; nitrile gloves; safety glasses; long pants; closed toe shoes

Protocol for Use:

1. Remove the microscope cover. Turn on the mouse with the switch on the bottom.
2. Turn on the microscope with the switch on the bottom-right side of the unit. The dot at the bottom of the monitor will light up blue. After about ~15 seconds, the main interface will appear on the bottom of the monitor (**Figure 1**).
3. Plug a USB stick into one of the USB ports on the right side of the monitor.
4. Use the mouse to click on the “SETTINGS” icon. The “SETTINGS” window will appear (**Figure 2**).
    a. Image Resolution: select “3MP” to generate images with the highest resolution
    b. Contrast and Saturation: select “Defaults” (usually the default setting is sufficient)
    c. Color Balance: select “Cool”
    d. Image Save Format: select “JPEG”
    e. Quick Save: click “Edit” and enter your group name. Do not use spaces (dashes are OK). Then check “Enabled” and click “Save” at the bottom. Now each image will be saved automatically with this file name and a three-digit sequence number. The saved images will be stored in a folder called “EVOS” on the USB stick.
    f. Date and Time: this should already be set
5. Select the desired objective (4x: red, 10x: yellow, 20x: green, or 40x: blue) by manually rotating the objectives under the stage (**Figure 3)**. Turn the phase turret (**Figure 4**) to the position that corresponds to the objective (4/10 PH for 4x or 10x, 20/40 PH for 20x or 40x). It is generally easiest to start with low-power objectives (4x or 10x) to find the focal plane and then increase magnification. Remember

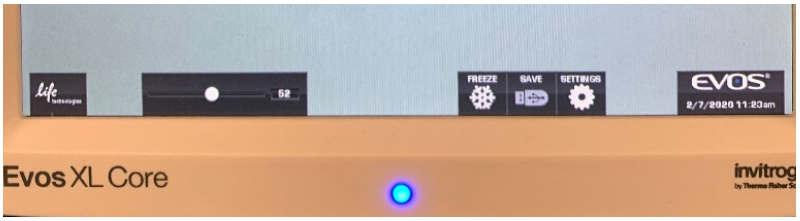

Figure 1: Main interface.

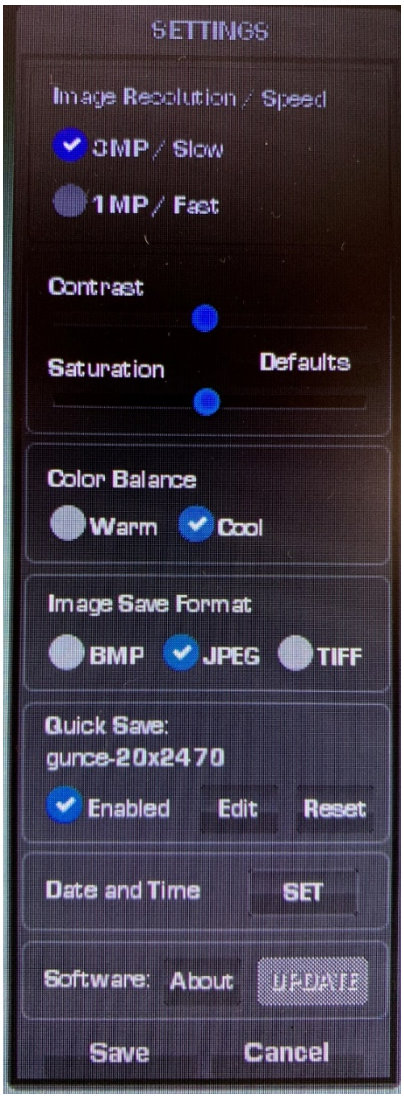

Figure 2: SETTINGS menu.

to adjust both the objective and the phase turret whenever changing magnification. You can also change the phase turret to brightfield (BF) for any of the objectives, but your image will have lower contrast.

6. Wipe the microscope stage with a Kimwipe sprayed with 70% ethanol. Do not spray ethanol directly on the stage as this could damage the objectives.
7. Place your sample on top of the stage and over the objective. If using a well plate or flask, secure the vessel with the stage clip (**Figure 4**). If using the stage clip, you can then move your well plate or flask with the knobs connected to the stage (top knob: Y-axis, bottom knob: X-axis). Otherwise, manually move your sample as needed.

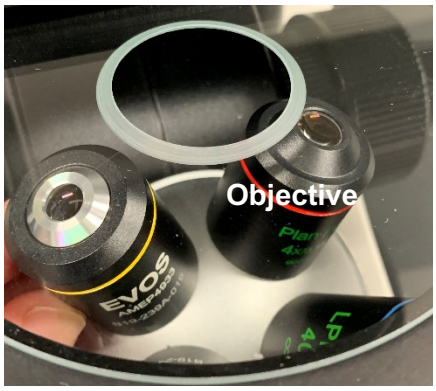

Figure 3: Objectives.

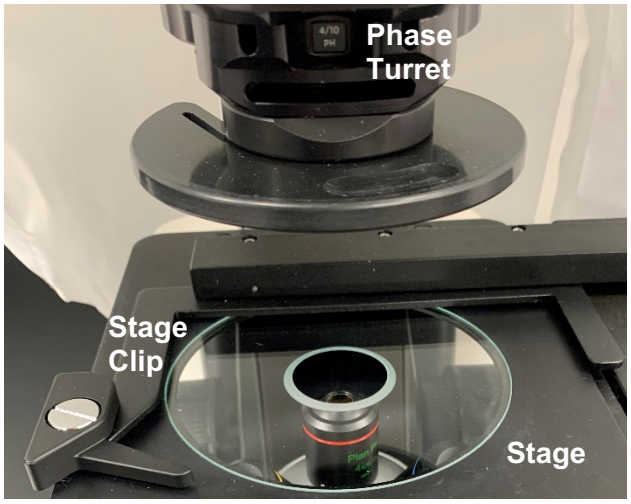

Figure 4: Stage and stage clip.

8. Use the monitor and the knobs on either side of the microscope to adjust the focus (**Figure 5**). The bigger knob is for coarse focus and the smaller knob is for fine focus.
9. As needed, adjust the light intensity by either scrolling the mouse on the sliding bar on the monitor or turning the dial located on the bottom of the microscope (**Figure 6**).

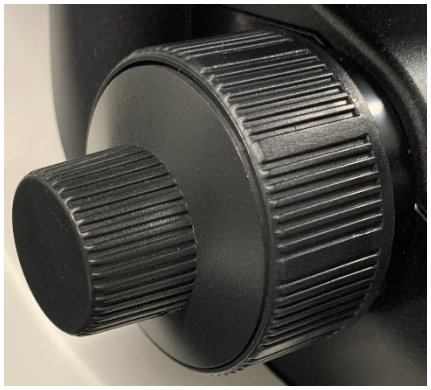

Figure 5: Focus knobs.

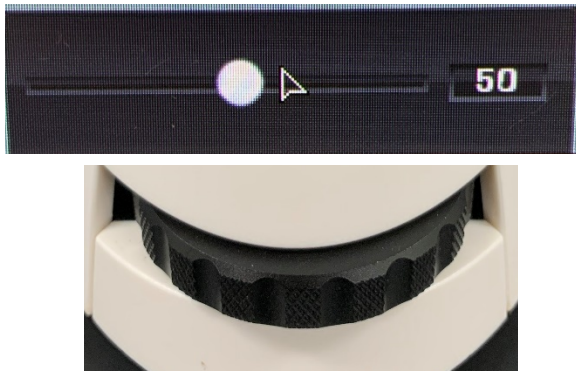

Figure 6: Slide bar on the monitor (top) or wheel on the bottom of the microscope (bottom) to adjust light intensity.

10. When you are ready to capture an image, either push the “FREEZE” button on the front of the microscope or push the “FREEZE” icon on the screen. To save the image, use the similar “SAVE” buttons on the microscope or the screen (**Figure 7**). With “Quick Save”

enabled, the image will be automatically saved as described in Step 4. If “Quick Save” is not enabled, a keyboard will appear to name the image.

Figure 7: Buttons on the microsope (left) and screen (right) for freezing and saving images.

11. To return to live viewing, either push the “FREEZE” button on the front of the microscope or push the “LIVE” icon on the screen (which toggles with “FREEZE”).
12. If image quality is low, click “SETTINGS” and adjust the cool/warm, contrast, and/or saturation bars (see Step 4) until you are satisfied with the image. Click “Save” at the bottom of the “SETTINGS” menu, then follow the steps above to capture an image.
13. When you are finished imaging your sample, remove it from the stage and wipe the stage with a Kimwipe sprayed with 70% ethanol. You can then image another sample or proceed to turn off the microscope.
14. When you are finished using the microscope, turn it off using the power switch on the bottom-right side of the unit and remove the USB stick. Turn off the mouse. Put the microscope cover back on.

Maintenance Schedule:

With each use: clean the microscope stage using a Kimwipe sprayed with 70% ethanol and always replace the cover.

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