SOP: EVOS M7000 Fluorescence Microscope

Purpose: Observe and capture images of cells stained with fluorescent labels

NOTE: These are abbreviated instructions for fluorescence imaging of slides. Refer to the full manual for additional details and functionalities: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018326_EVOS_M7000_Imaging_System_UG.pdf

Location: BHE B7A (microscopy room in BME Cellular/Molecular Teaching Lab)

PPE: Nitrile gloves when handling slides; long pants; closed toe shoes

Protocol for Use:

1. Turn on the computer with the power button on the front bottom right of the computer.
2. Turn on the microscope by flipping the switch behind the microscope. The 3x3 dotted icon on the front of the microscope will light up blue.
3. Double click on the M7000 software icon. For 20-30 seconds, the software will initialize.
4. Remove the dark box from the microscope stage and mount your specimen. If you are imaging slides, place the double slide holder on the stage and insert the slides **face down** with the label on the right side (**Figure 1**). Put the dark box over your specimens and remove the opaque cover piece on the top.

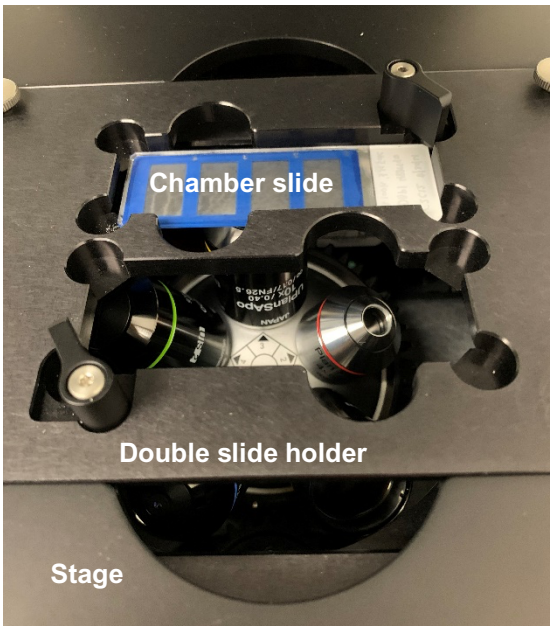

Figure 1: Imaging a chamber slide.

5. In the software, click the Capture tab (**Figure 2**) and then click Vessel. Select the vessel that matches your specimen. If you are imaging slides with the double slide holder, select Slides, Holder: Double Slide | Generic Slides.
    a. If you are imaging slides with four chambers, select “4 Chamber Lab-Tek II NUNC 155382” for the Top and Bottom Slides
    b. If you are imaging slides with coverslips, select Slide – Facing Down Generic for the Top and Bottom Slides
6. Click the button for your desired Objective. For fluorescence imaging, it is generally easiest to start with the 4x objective to find your field of view and focal plane. Then, you can increase to a higher power objective as needed.
7. Next, select your desired filter cube by clicking that button in the Light source menu. If you stained for DAPI, it is generally easiest to start with that channel.

8. Use the Light button to toggle the light on and off (the light is on when the button is blue). Turn the light on to see a live image of your specimen in the software.
9. With the light on, adjust the field of view, brightness, and focus until you are satisfied with your image:
    a. To adjust field of view, click and drag the image with the mouse; drag the image on the monitor with your fingers; or click your mouse onto a specific region of the slide using the map in the upper right corner.
    b. To adjust focus, scroll your mouse over the Coarse and Fine bars. Coarse will make large changes and fine will make small changes. You can also click AutoFocus and let the microscope focus for you, although this isn't always effective, especially if you are very far from the focal plane.
    c. To adjust brightness, scroll your mouse over the Bright bar. Try to use the entire dynamic range, i.e., the signal should be bright but not overly saturated. In Settings → General → Saturated Pixels, you can click Show saturated pixels to make any saturated pixels visible in your image with a chosen color. You should adjust your image until you see only a few specks of saturated pixels.

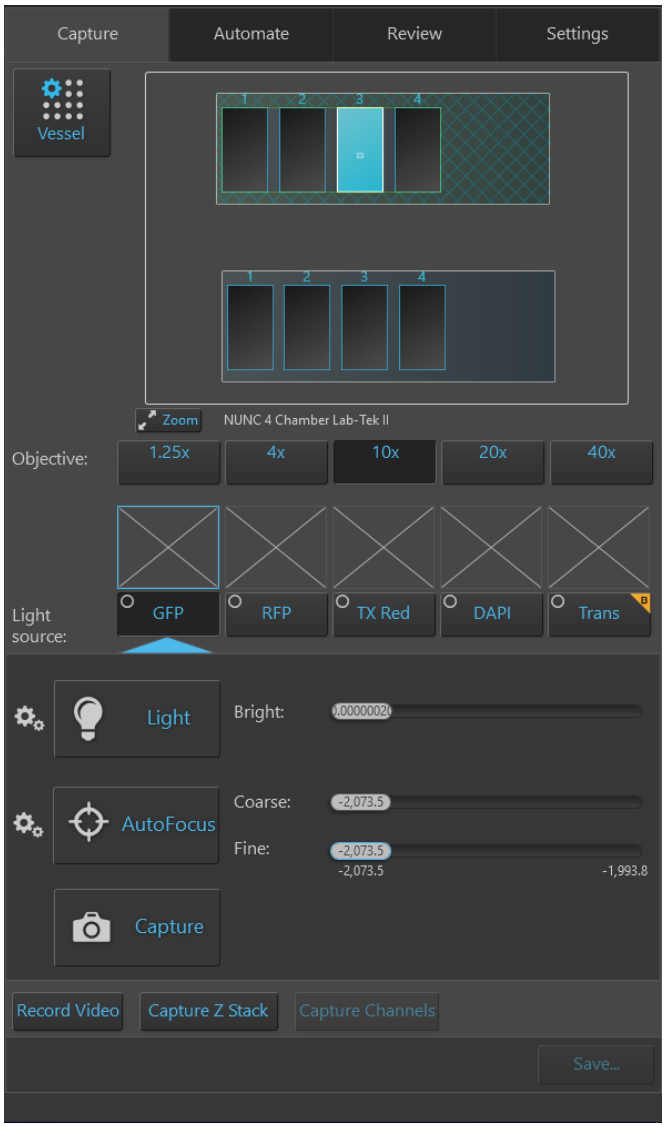

Figure 2: Capture tab for acquiring images.

10. When you are satisfied with your settings, click the small circle in the Light source box for that channel, which will add a check mark.
11. If you have another color channel to image, click that Light source box. Adjust the brightness of the light and, when you are satisfied, click the small circle in that Light source box to add a check mark. Repeat for any other channels.
12. Click Capture Channels to capture an image of all the checked color channels with your preferred settings.
13. *Optional:* Add a scale bar by clicking the scale bar icon on the bottom right corner of the image. However, once you save an image with a scale bar, the scale bar is permanent, which can be problematic for image analysis. Thus, if you do save an image with a scale bar, also save the image without a scale bar.

14. Click Save… In Location, click Browse and find your USB stick. In Prefix, enter any critical details you want saved with your image, such as objective, chamber number, image number, etc. Ensure the file format is TIF and check only the box Displayed image → Merged image. Click Save.
15. When you are finished collecting all images, remove the specimen from the stage, put the dark box and its cover back on the stage, and cover the microscope. Close the software, turn off the microscope, and shut down the computer.

Maintenance Schedule:

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

As needed: if objectives are dirty, spray lens paper with 70% ethanol, gently wipe the objectives, then wipe with a dry piece of lens paper

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