Fluorescence Microscope (EVOS FLc)

Brief user manual

INSTRUMENT SAFETY GUIDELINES - The fluorescence microscope is designed and only to be executed in accordance with this manual and company SOP. Failure to observe the following precautions could result in instrument failure and personal injuries:

- The fluorescent microscope is electrically powered. To avoid electric shock, please observe all standard precautions and general safety guidelines for electronic instruments.
- The objective lens should be handled carefully to avoid damage. The lens should be cleaned using optical tissues and recommended solvents.
- Do not look directly into the LED cubes, especially while using the UV LED cube (DAPI).
- If any liquid such as water or organic solvent is spilled on the instrument, wipe it off immediately.
- Do NOT use flammable sprays (hair sprays, insecticide sprays, etc.) near the product.

In an emergency: If a problem is encountered, turn OFF the power switch according to the following procedure.

- Press the power switch, located on the lower section on the right side of the instrument, to the "O" position.
- Remove the power cord from the lower section on the right side of the instrument.

Operation:-

1. Turn on the main supply after which, the microscope with the **power switch** on the right side of the base.

2. Plug a USB flash drive into one of **the USB ports** on the right side of the microscope (near the screen).
3. Place the **sample** on the stage using a vessel holder or glass slide if needed.
4. Set the magnification (10X, 20X, 40X, 100X) with the **objective selection wheel** on the front of the microscope.
5. Put the **light cube selection lever** (left side of the base) all the way toward the front of the microscope (the channel bar will highlight the "transmitted" position in the screen).
6. Turn on illumination with the **LIGHT ON** button located on the left side of the control bar.
7. Focus the sample with **focusing knobs** on both sides of the stage.
8. Optional: To take a picture of the transmitted light image, click the **Capture button** on the control bar shown on the screen
9. Place the **light shield box** on the stage, over the samples.
10. Bright field option (white light) provides gray scale image.
11. Move the **light cube selection lever** to desired fluorescence channel (the channel bar will highlight the selected light cube).
12. With the **Find and Focus tab** active, turn on the fluorescence illumination using the LIGHT ON button.
13. Adjust the focus as required.
14. Adjust the **Illumination intensity slider** on the control bar as needed.
15. Click the **Capture button** for capturing live images.
16. Repeat steps 10-14 to acquire each fluorescent channel (depending on the requirement).
17. Click the **Overlay tab** to show all channels in color overlay mode.
18. Adjust the **Brightness and Contrast** for each channel to bring them to desired balance.
19. Click the **Save button** to save the colour image or bright field image.

Tips/ Notes:

1. Use **Color Adjustment** button to fine-tune live image brightness, contrast, saturation, and hue prior to capture.
2. In **Find & Focus** mode, the exposure time is set to 100 ms to assist real-time focusing, moving the stage, etc. with an illumination level of approximately 60% of the light used for capture in order to minimize photobleaching and

phototoxicity. Using direct **Capture**, will result in brighter illumination and longer exposure time providing the required quality.

3. With **longer exposure times** (more than 200 ms), there will be a lag between moving the focus knob and seeing the focus change onscreen.
4. Following **light cubes are installed** with respective excitation and emission wavelengths:
    - DAPI: 360 nm excitation, 447 nm emission
    - GFP: 470 nm excitation, 525 nm emission
    - RFP: 530 nm excitation, 593 nm emission
5. For best imaging, **avoid direct light sources near** the microscope to reduce the effect of ambient lighting.
6. **Time Lapse feature** allows the instrument to record time lapse images. Set the time interval and duration in secs/mins setting the number of images as well. The instrument takes multiple images and converts the file into an avi video file.
7. The **size bar** can be changed as well as **grid image** can be selected by going to the image review tab.