Olympus Fluoview Fv10i LIV (Drug Discovery Building, DD 521C)

Before using these instructions make sure you have had at least one training session on using the system. If you wish to image live cells under normoxic or hypoxic condition contact the Facility manager for a free training.

Please report any problem with the microscope or any other component immediately to the facility manager: Monika Gooz (beckm@musc.edu) (843) 494-3700. Thank you.

Operating instruction:

(For fixed specimen start at step #4.)

1. If you image live cells first open the **CO_2 tank** main metal valve fully. The outgoing pressure should be ~25-30 psi. Then **turn on the gas regulator** (white box to the right of the microscope) by pushing the orange button to maintain 5% CO_2 inside the chamber for live cell imaging experiments that need CO_2 incubation.
2. If you wish to image your cells under **hypoxic condition** open both the CO_2 and the N_2 tank. Then turn on the gas regulator and set up your condition (1-2.5% O_2).
3. To humidify the chamber **pipette distilled water into the small reservoir** in the back of the imaging chamber. Water and transfer pipette is located next to the microscope. Cover the reservoir with the metal lid to prevent water spray onto your specimen.
4. Select the right specimen **stage adapter** for your specimen and insert the adapter with your specimen into the system.
5. Cover the specimen with the black cover with the glass mesh top for all live cell imaging experiments.
6. The white computer **login** screen will prompt you to enter your e-mail address and iLab password. This account is used for tracking users and their usage time for billing.
7. Once logged on the computer, launch the FV10i software (Fv10i-SW.exe).
8. On the next log on screen simply hit OK (be sure the user ID show “Guest”)
9. Select the fluorophores from the dye data base that you want to observe in your specimen.
10. Select **acquire map image** tab and select the fluorophores or bright field to locate your specimen. Select the position on stage that you would like to observe and hit START. Wait

for a minute as the system locates and focuses on the specimen and creates an image map. Then move to the NEXT tab.

11. In the **observe** tab select the image acquisition mode on the top left then double click on the region that you want to observe.

12. The Navigation tool at the left upper corner can help you to navigate through this page. If necessary, change the objective lens to **60X** water-immersion and **drop water** on the lens. You may need to readjust the focus using the **depth tool** at this point.

13. Select in the **other parameter** tab 1024x1024 images with confocal aperture of one. Select the scan speed that you want to use to acquire your images.

14. Press **control h** to bring up the range indicator. Use detector sensitivity, laser power and histogram to adjust for fluorescence intensity. You have to click on the each channel and then adjust that channel. Do this while scanning.

15. Select the upper and lower limit for your z-stack and the time for your time-lapse images.

16. Select the folder where you want to save your images.

17. Click **one shot** for acquiring single images or **series** for acquiring time lapse or z-stacks.

18. Once you are done acquiring images exit the software, log off and take your specimen out of the chamber.

19. **Save all your data to USB memory or external drive**. The facility is not responsible for storing images.

20. Clean the objective lens using the provided lens cleaning paper and the Zeiss cleaning solution.

21. Close the CO_2 tank then turn off the regulator. Close microscope lid securely and leave the machine on.

22. **Clean up** the microscope area, trash used paper, Kim-wipe etc. into the trash. The facility provides red biosafety bins if you wish to discard your cells. Pipet tips are collected in paper boxes inside the cell culture hood.