Spinning Disc CARV II (Drug Discovery Building, DD 526)

Before using these instructions make sure you have had at least one training sessions on using the system.

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.

BD Biosciences CARV II for video rate confocal imaging operating instructions:

1. Turn on the Halide Mercury fluorescence lamp first.
2. Turn on the CCD camera and CARV spinning disc unit (black switches located at the back of these units).
3. Switch on the Axiovert 200M microscope using the green button on the right hand side of the microscope.
4. The computer will prompt you to enter your username (e-mail address) and password that was created for ilabs. This account is also used for tracking users and their usage time for billing.
5. Select the objective lens that you intend to use for the experiment. Use the black buttons labeled as Objectives on the right hand side of the microscope for this. Make sure you use the appropriate immersion medium. Do not use the wrong medium as it will damage the lens. The following objectives are available on the microscope:
    A. 4X dry lens
    B. 10X dry lens
    C. 20X dry lens
    D. 40X oil immersion lens (use Zeiss 518F immersion oil)
    E. 63X oil immersion lens (use Zeiss 518F immersion oil)
6. After selecting objective mount the specimen on the microscope stage and bring it in focus either using halogen light or the mercury fluorescence lamp.
7. For the halogen light push the black halogen button located on the right hand side of the microscope to turn it ON.
8. Use the lowest black button located on the left hand side of the microscope to select binocular.
9. Use the center black button on the left hand side of the microscope to select front port binocular.

10. Looking through the binoculars bring the specimen into focus and go to step 15.

11. If you use mercury lamp to bring specimen in focus, then use the center black button located on left hand side of the microscope to select 100% side port.

12. On the CARV control box select the appropriate excitation, dichroic and emission filters:

    Position 1: DAPI – UV excitation/Blue emission
    Position 2: GFP – Blue excitation/Green emission
    Position 3: Texas Red – Green excitation/Blue emission
    Position 4: Mito Deep Red – Red excitation/Deep Red emission

13. Open the shutter for the lamp from the CARV control unit.

14. Select binoculars to view the specimen on the CARV control unit. Looking through the binoculars on the CARV bring the specimen into focus.

15. After bringing the specimen into focus, launch IPLab software on the computer. Click on **Continue** on the first window that pops up. This will allow IPLab to persist with the objective lens that you have selected.

16. Make sure the lights in the room are turned OFF or are very dim.

17. Using the Zeiss-Axiovert200 LP button on the top right hand corner make sure you have selected 100% side port left option.

18. Click on F1/Live Capture button on the bottom right hand corner of the computer screen.

19. Select Confocal Disk in for confocal or leave it out for regular fluorescence.

20. Select from the following filter sets:
    A. DAPI – UV excitation/Blue emission
    B. GFP – Blue excitation/Green emission
    C. Texas Red – Green excitation/Red emission
    D. Empty – Red excitation/Deep Red emission

    This will set up the excitation, dichroic and emission filters.

21. On the Acquire Preview window select Exposure time.

22. Click on **Continue** to begin scanning.

23. Using the fine focus knob on the microscope or the Zeiss-Z stage button on the upper right hand corner try to fine focus the specimen.

24. Click **Cancel** on the Acquire window to stop acquiring image.

25. You are now ready to start collecting images. Adjust exposure times and binning number based on your experimental specimen.

26. Save all your images under your file in D:\UserData\. Use the export function to save them in other formats like TIFF, JPEG. Ask the Core personnel to show you how to use this function.

27. Once finished with the experiment close all images on screen and exit the IPLab software. Save all your data to CD, DVD or USB memory. The facility is not responsible for storing images.

28. Turn OFF the computer.

29. Turn OFF the microscope, CARV unit and the CCD camera.

30. Turn OFF the fluorescence lamp light.

31. Remove your specimen and clean the lens using only Zeiss lens cleaning solution.

32. Clean up the workspace and cover the microscope before you leave.