BioRad Radiance2100 Confocal Instructions

MP (+/-Visible) Laser Instructions

Note: Before using the IR laser you must take and pass the HPO online laser safely training course.

Note: Make sure the proper light paths are selected. Also, take great care when placing samples on the stage and imaging them. The MaiTai laser is extremely powerful and PERMANENT EYE INJURY CAN BE INCURRED BEFORE PAIN IS FELT. Always start with the IR laser at a minimum setting.

Note: If using the external detectors the room light must be kept to an absolute minimum. The monitor should be in a low light mode and the curtain should be closed. Please see Tom for Individual instruction if you thing you need to use the external detectors.

1. Turn on Hg lamp - turn on blue switch on the front of the power supply
2. Turn on Nikon power supply
3. Turn on the Pockels cell power supply (ConOptics)
4. Turn on computer
5. Log on to Windows using your usual Hawk ID name, password and domain.
6. Turn on electronics box to the right of the air table. Flip switch down to activate.
7. Turn on desired visible lasers (if doing a multi-phase experiment) by depressing corresponding buttons on electronics box—top 633nn, middle 543, bottom Argon
8. Start the laser sharp software by clicking on the icon labeled **LaserSharp2000**. Log in using your confocal name and address. This may take several minutes. The icon labeled **No Hardware** is for offline editing and the laser, mercury lamp, and electronic box do not need to be turned on if using this option. Copies of the LaserSharp software for installation on other computers can be obtained from the CMRF.
9. Activate the MaiTai laser by clicking on **Both** of the start icons at the bottom of the control panel. This should be done as soon as possible to allow the laser to warm up and stabilize. For maximum stability this should be done as least 30 minutes before imaging is to commence.
10. Under **Methods** select the desired fluorophore(s)/detectors to be used and load the method. Methods can be created and edited as needed.
11. Set the objective lens, scan speed, box size and filter to the desired settings.
12. Locate sample using transmitted light or epifluorescence. **NOTE: It is very important that no transmitted or epifluorescence light be allowed into the scan box. Before either light is turned on the lever to the right of the binoculars must be pushed in. When switching to confocal mode, all lights must be turned off first, the filter should be set to the INT position, the shutter must be closed then pull out the lever to the right of the binoculars and proceed with the scan.** The epifluorescence filter head has 5 positions. Starting from the left the filters are for External detection (DDS), FITC, UV, Rhodamines/Cy3, and Internal detection (INT).
    If the 60x oil, 60x water or 20x multi-immersion lenses are to be used, back the stage down, place a drop of oil (or distilled water from the syringe, or glycerol) on the slide, rotate the lens in and raise the stage back up until the lens contacts the oil. Further raise the stage while looking through the oculars until the specimen comes into view. **Note; these lenses are very expensive. Utmost care must be exercised not to damage or contaminate the surface. The lenses should always be cleaned after every use by lightly dabbing with filter paper wetted with Kodak lens cleaner or sparkle. Please ask any staff member if you have questions.**

13. Move the filter slider to the INT position and close the shutter. Pull the eyepiece slider all the way out to the right.
14. Open the IR laser shutter by clicking on **Both** of the shutter icons at the bottom of the Control Panel menu.
15. Start scan by clicking on the laser symbol ("single exploration") for simultaneous acquisition or the pressing the space bar.
16. Select desired wavelength for IR laser and do nothing until the tuning message is gone. Adjust power as needed. For most imaging situations little power is needed, usually less than 5%. It is recommended to use the low power setting on the laser power control.
17. Open the **Irises**, completely and adjust **Gain**, and **Offset** level for each channel to optimize image. When possible avoid gain settings above 33 (image will be noisier.)
18. Preset **Kalman** to the desired number, usually at least 3, select **Kalma**n and wait for scan to stop.
19. Save the image by selecting (**FILE**) and (**SAVE**) to store the image. Choose drive C:\Experiments: (hard drive), E: (Zip drive) or your network shared drive. **Do not leave any data on C:!!! Burn it to a CD or DVD or copy to your shared drive and delete.** If for any reason **Save** does not work use **Save As.**

    Note: Each image or series of images is stored in a folder with the name of the image. If merged images are saved or if any action has been taken on the images (projection, rotations, measurements, etc.) the resulting files will be saved in sub-folders. In addition, files are stored that record all of the acquisition parameters. These files can be used to reset up the microscope to the exact conditions used to acquire the image (similar to the reuse command on the Zeiss 510) by opening up the stored image, right clicking on it and selecting (Restore Method.) The file format of the image itself is still .pic file that can be read by ImageJ or Confocal Assistant in the usual manner.

    Note: Images can also be directly converted to Tiff (or other format) by right clicking on the pane to be saved and selecting export. This will result in smaller files but all image acquisition parameters and scale will be lost.

20. To further manipulate the stored images right click on the image and choose the desired command. The same functions can be accessed via the icons at the top of the screen.
21. To scan a new image the saved image should be closed and a new experiment window opened. New scans can not be started until this is done.
22. When finished, exit the laser sharp software, logout of windows and select shutdown from the start menu. When directed, turn off the computer. Finally, turn off the laser and mercury lamp power supplies.

    Note: If another user is signed up within the next 2 hours, log out of LaserSharp and Windows, leaving the system on.

    If another user is signed up more than 2 hours later, turn off entire system.

    Log on to the CMRF website scheduler and check the schedule if you are not sure when the next user is scheduled.

23. If the 60x oil, 60x water or 20x multi-immersion lenses were used, gently dab off excess oil with lens tissue or a cotton swab and clean with. **DO NOT USE KIMWIPES.** If lenses are very dirty please contact a staff member.