Updated BioRad LaserSharp2000 Confocal Instructions

1. Turn on Hg lamp - turn on black switch on the power supply
2. Turn on laser - turn on switch on the front right of the power supply.
3. Turn on electronics (scan) box under the monitor. Flip switch down to activate.
4. Turn on monitor and computer.
5. Log on to Windows using "**confocal**" as the both the name and password, and select "**M83133"** as the domain
6. 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.
7. Under **Methods** select the desired fluorophore(s) to be imaged load the method. Methods can be created and edited as needed.
8. 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 open position, the shutter closed then pull out the lever to the right of the binoculars and proceed with the scan.** The epi-fluorescence filter head has 4 positions. Starting from the left the filters are for FITC, Rhodamines/Cy3, Cy5 and open.

9. Set the objective lens, scan speed, box size and Kalman to the desired settings.

   If using transmitted light the silver bar to the left of the transmitted detector must be moved to a horizontal position to allow light to pass through.

   If the 60x oil or 100x oil lenses are to be used back the stage down, place a drop of oil on the slide, rotate the 60x 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.

10. Move the filter slider to the open position, close the shutter and turn off the transmitted light (if used)
11. Pull the eyepiece slider all the way out to the right
12. Start scan by clicking on the laser symbol ("single exploration") for simultaneous acquisition or the pressing the space bar. If imaging sequentially start the scan by clicking on the "multiple exploration" button for a sequential scan.
13. Adjust **Iris**, **Gain**, and **Offset** (previously know as black level) level for each channel (or mixer) to optimize image. Avoid gain settings above 1400.
14. Select (**Kalman,**) average several frames, usually at least 3, and stop the scan.
15. Save the image by selecting (**FILE**) and (**SAVE**) to store the image. Choose drive D: (hard drive), F: (Zip drive) or your network shared drive. When your login

profile was created the default was set to either D:\Experiments\User name or the zip drive. **Under no circumstances should data be stored on the C:!!! It will be deleted immediately!!** This system also has a 52x CD burner so it is anticipated that users will save the data on D:, burn it to a CD and delete the files on D:. 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 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.

16. 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.
17. 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.
18. 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.

19. If the 60x or 100x lens was used gently dab off excess oil with lens tissue or a cotton swab. **DO NOT USE KIMWIPES.** If lenses are dirty please contact a staff member.