

Instructions for the Yokogawa CSU-X1 spinning disk confocal microscope

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Check-in and Start up

There are 3 remote switches and a power strip that supply power to all the components.

1. make sure the power strip for the computer is off, see **Caution** in the Check out procedures for the known issues
 - In case the power strip is left on
 - i. if computer is still on, shutdown Windows, then turn off the power strip
 - ii. if computer is off, turn off the power strip
2. remote switch labelled MICROSCOPE: power up to turn on the DAQ, microscope, piezo stage, laser interlock box, and the epifluorescence LED
3. If you need the CSU-X1, power up the switch labelled SPINNING DISK to turn on the X1, laser launch, Prime95B camera, and LiveSR. Please wait 1 min for the Prime95B camera to initiate before proceeding to the next step.
4. If you need the Baker Lab additions, power up the switch labelled WIDE-FIELD to turn on the Celesta, Fusion camera, and filter wheel. The Celesta takes about 30 s to fully initialize.
5. turn on the power strip for the computer and monitor
6. log into Windows and then launch Nikon Elements

Check out

1. lower the nosepiece (objective turret) e.g., set the focus (Z2) to 500 μm
2. remove your prep
3. if applicable, remove oil/immersion fluid on immersion objectives and clean (see [Appendix](#))
4. switch to the 10 \times or lower magnification objective
5. save your experiments and quit Nikon Elements
6. copy your data to external storage media or network file servers, as needed
7. submit your usage using the google form
8. shutdown Windows
9. after the computer has shutdown, switch off the power stripe

Caution: Leaving the computer or the power strip on can cause problem.

- o If the computer is on, the DAQ will not load when the next user turns on the MICROSCOPE remote switch. The user will need to restart the computer to establish the connection to the DAQ.
 - o If the computer is off but the power strip is left on, activating the MICROSCOPE remote switch will cause the DAQ to boot up the computer. Now when the user turns on the Prime95B camera via the SPINNING DISK remote switch, windows will not see it because the camera needs to be fully initialized first for windows to load the driver during boot up. The user must then restart the computer to load the camera driver in order to use the spinning disk. It is a known annoyance that we have no means to overcome.
10. power down the remote switches: [SPINNING DISK, WIDE-FIELD], and MICROSCOPE
 11. put the cover back on the microscope
 12. clean up the work area

Appendix

Content: **Köhler illumination** • **immersion objective**

Köhler illumination

1. set microscope to diascopic mode and the optical path to 100% Eye e.g., select an EYE-BF OC in the software
2. set condenser turret to position 1 (Open)
3. open fully both field and aperture diaphragms
4. set the microscope focus (Z2) to 500 μm
5. select the 10x objective
6. load a sample and bring an object of interest to focus
7. close the field diaphragm, note the out of focus image of the edge of the diaphragm
8. use the condenser focus knobs, adjust the condenser height to bring the edge of the diaphragm into focus
9. use the 2 mm Allen wrench(es) to center the image of the diaphragm, open the field diaphragm to about 90% might help
10. open the field diaphragm to just beyond the field of view
11. close down the aperture diaphragm until the intensity of the illumination just starts to dim-this is about 70% of the objective pupil plane
12. when switching to a different objective, you might need to readjust to optimize the image quality

immersion objective

setup

1. locate the object of interest with the 10× or 20× and center it in the field of view
2. record the XYZ position
3. set the microscope focus (Z2) to 500 μm
4. switch in the immersion objective
5. use the stage XY control to move the sample out of the way to expose the objective
6. apply an appropriate amount of immersion medium
7. recall the recorded XYZ position
8. check the focus of the object of interest

clean up

1. set the microscope focus (Z2) to 500 μm and remove the sample
2. blot to remove the immersion medium by gently pressing the designated lens tissue against the objective lens
3. apply the appropriate cleaning agent to the special cotton swab (Puritan 869-WC, cotton without glue or binder), the cotton should be damp but not dripping
 - water: 95% ethanol
 - GenTeal: water followed by 95% ethanol
 - Immersol W: 95% ethanol
 - Nikon F oil: no need to clean
4. gently roll the cotton swab on the front lens from one side to the other, you might need to do it twice to cover the whole surface of the lens
5. repeat the above step with a fresh cotton swab and cleaning agent