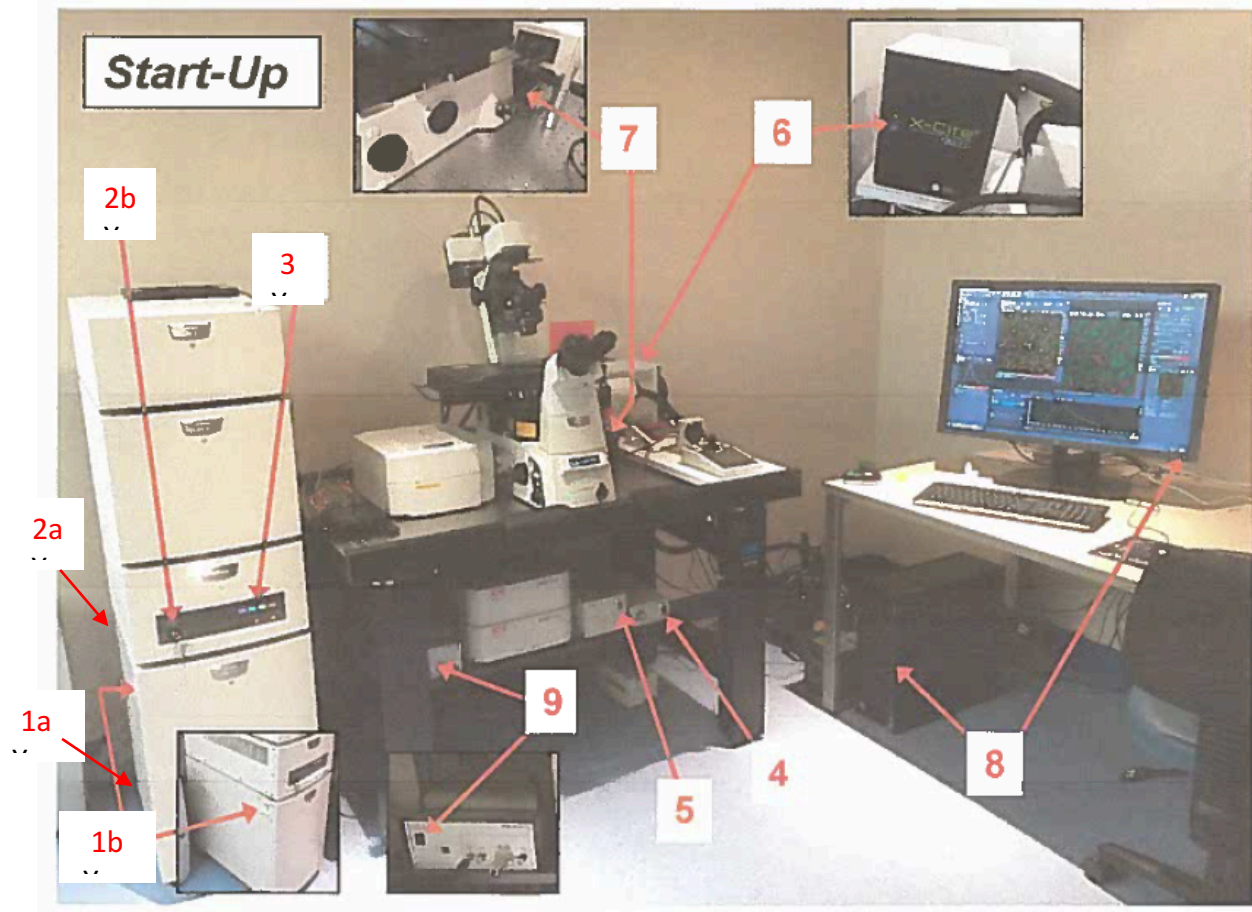


# A1R/A1

## Start-Up & Shut-Down Procedures



- A. Main power, laser and detector units: turn on switches from back to front starting with bottom unit by following these steps.
  - 1a. Turn on the rear power switch for the A1R main power supply (back bottom).
  - 1b. Turn on the A1R main power supply (recessed push button on left side)
  - 2a. Turn on the LUN4 laser launch main power switch at the rear of the unit.
  - 2b. Turn on LUN4 laser launch by turning key  $\frac{1}{4}$  turn clockwise.
  3. Individual lasers may be turned on/off by pushing the corresponding indicator buttons.
- B. Halogen light and motorized stages located on lower shelf.
  4. Turn on the the X-Cite 120LED widefield fluorescence light source.
  5. Turn on the motorized stages- two power supplies.
  6. This is the position of the X-Cite 120LED widefield fluorescence light source turned on in step 4.
- C. Microscope, PC and optional accessories.
  7. Turn on the Ti-E microscope (rocker switch at rear/right). Listen for Ti-E microscope to initialize.
  8. Turn on PC and monitor.
  9. Turn on the Piezo-Z power supply (if needed).

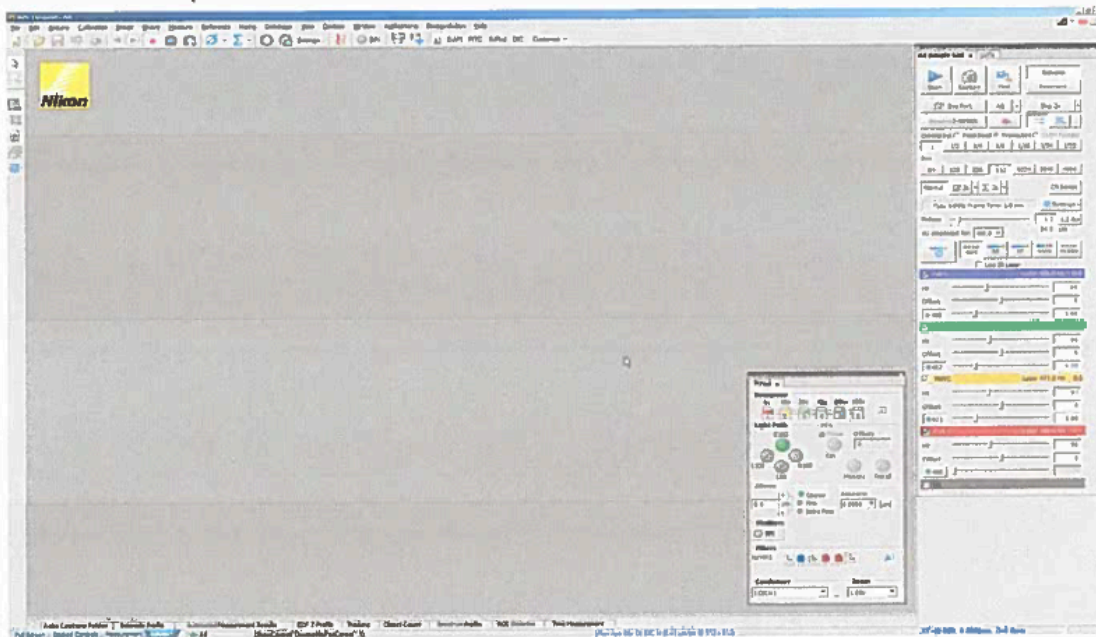
10. Turn on Live Cell Incubation System (if needed; not shown. Ours is located on top of AIR detector unit).

D. Software

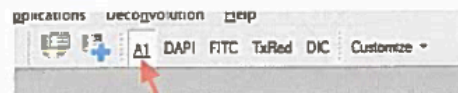
11. When the PC has booted up, start NIS-Elements AR software by double-clicking the NIS-Elements AR" short-cut icon on the desktop. Select Confocal from the drop down menu (should be the default). You will get an error if you try to run the NIS-Elements software when the confocal hardware is not started up with steps 1-8. We also have a Nikon Elements Analysis shortcut (NIS-Elements AR Analysis) that can be used when you are analyzing images but do not have the full confocal system running.

START UP COMPLETE

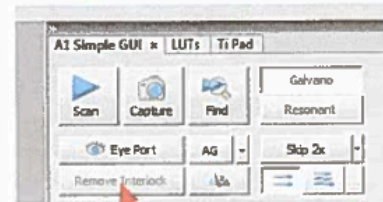
12. You will need the "A1 Simple GUI" window to control the A1. If it is not displayed upon start-up, right-click on the grey desktop, and select "A1 Simple GUI" from the Acquisition Controls. Also convenient are "Ti Pad" to control the microscope functions, "A1 Scan Area" to adjust scan area size, position, and rotation, and "ND Acquisition" for multi-dimensional acquisition.



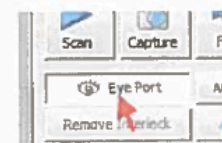
13. Click on the "A1" Optical Configuration (OC) button in the top tool bar. This will automatically direct the microscope light path to the left camera port and moves the fluorescence turret to an open position.



14. Click on the "Remove Interlock" button in the A1 Simple GUI. It is likely colored red initially, and may take a second or two before turning grey. Once the interlock is removed, the system is ready for confocal scanning. You can press the "Scan" button or make adjustments to the A1 Settings.



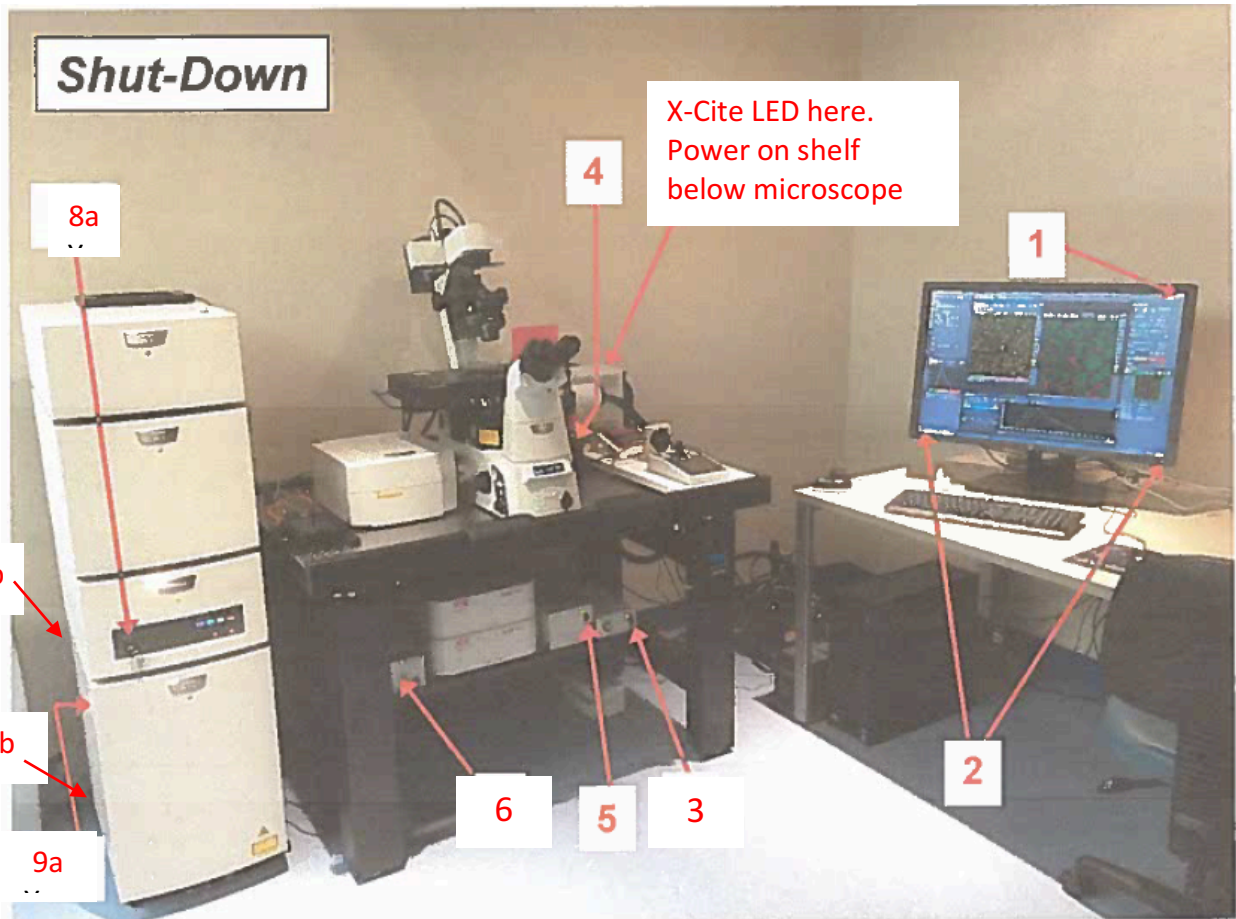
15. The easiest way to switch between confocal scanning and ocular viewing is by using the "Eye Port" button, located just above the "Remove Interlock" button. In order to observe the sample through the eyepieces, click on "Eye Port".



16. Click on the desired eyepiece viewing OC (DAPI, FITC, TxRed or DIC). You can switch between different eyepiece viewing modes by clicking different OC buttons. When you select one of the widefield fluorescence modes, you also need to manually open/close the 120LED shutter (by pushing the remote control knob) and adjust the illumination intensity (by rotating the knob).



17. At this point, the "Eye Port" button has been programmed to act as a simple, one-click toggle function between confocal (A1) scanning mode and eyepiece viewing mode. When you want to return to confocal imaging, simply click on "Eye Port" again. This will switch the system back to "A1 mode" and automatically removes the interlock. If you click the "Eye Port" button again, the system will return to the last eyepiece viewing mode that was used.



For Shut-Down of the software and hardware work backward from the start up steps. Before starting these steps, make sure to a) lower the objective to the lowest position, b) remove slide and clean oil from objective, c) turn objective to open position.

1. Exit out of NIS-Elements AR software.
2. Shut down PC and monitor.
3. Turn off the X-Cite 120LED widefield fluorescence light source.
4. Turn off the Ti-E microscope (rocker switch at rear/right).
5. Turn off motorized stage power supplies (2 switches).
6. Turn off Piezo-Z power supply (if needed)
7. Turn off Live Cell Incubation System (if needed; not shown)
- 8a. Turn off the LUN4 laser launch by turning key  $\frac{1}{4}$  turn counter-clockwise. Note that when the LUN4 is turned back on, only those lasers which were turn on during the last power-down sequence will automatically turn on (colored buttons).
- 8b. Turn off power switch at the rear of the LUN4 laser launch (rocker switch).
- 9a. Turn off the A1R main power supply (recessed push button on left side).
- 9b. Turn off A1R main power supply switch at rear of unit (rocker switch at bottom).
10. Cover Ti-E microscope. Clean up any slides or prep materials.