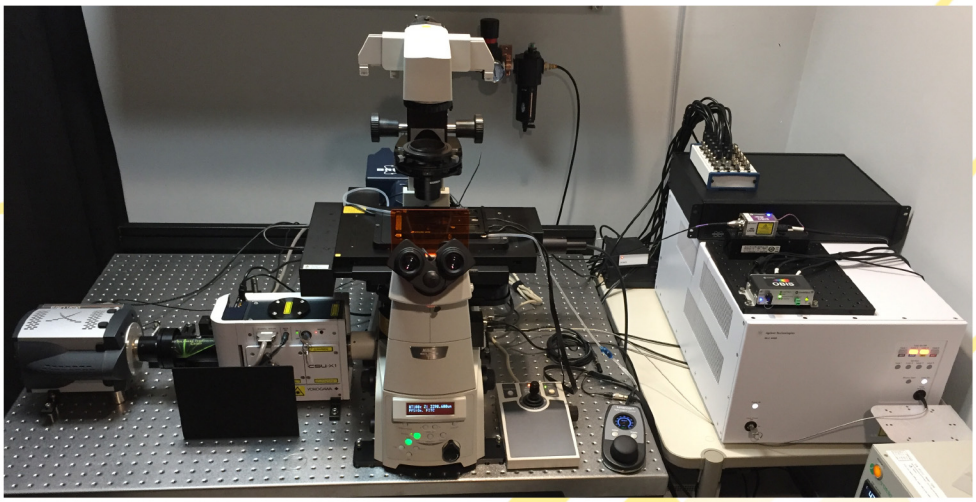
Spinning Disk Confocal Quick Start Guide



1. **Remove dust cover** and place in lower (3rd) drawer of cabinet.
2. **Flip the red switch** on the front of the line conditioner to the on position (**Figure 1**, below)
   - Location: On shelf underneath microscope.
   - The microscope will initialize (<30sec).

Figure 1

3. **Turn the key on the Agilent laser launch** from the off (vertical) to on (horizontal) position AND turn on the laser(s) you wish to use by pressing the button(s) of the corresponding lasers (**Figure 2**, below).
   - If the button of the laser begins to flash, this is NORMAL and will appear solid after firing (<20sec)

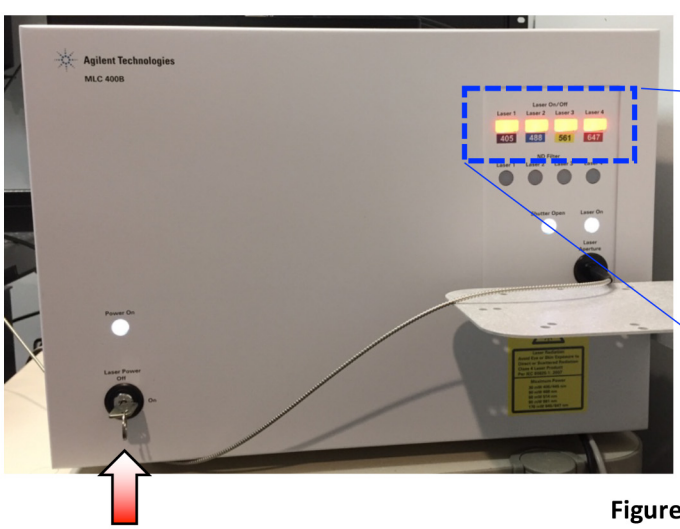


Figure 2

(**OPTIONAL**) If you will be needing the photo-stimulation laser (OBIS 405, which is separate from the imaging 405) for FRAP, photo-conversion, photo-activation, and/or ablation, simply turn the OBIS Control Unit rocker switch to the on (up) position and WAIT until the status light switches from **AMBER** to **BLUE** **THEN** turn the key from the STANDBY (horizontal) to ON (vertical) position (**Figure 3**, see below).

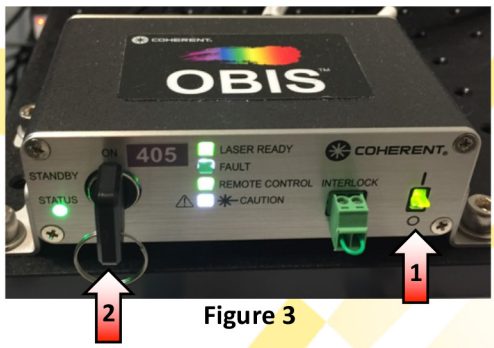


Figure 3

4. **Turn computer on** (if not on already). This will take the longest of any of the startup.
5. Make sure that the **objective is in the "Escaped" position** (all the way down) by pushing the Escape button on the right side of the inverted microscope (**Figure 4,** below).
    - If you started up the system, the objective will be escaped by default.

Figure 4

6. **Decide which stage insert you will need** for your sample (**Figure 5**)
    - Inserts are located in the 2nd drawer of the file cabinet.
    - Use the two thumbscrews at the bottom left and top right of the insert to install on stage

The incubator/insert holder is mounted inside a sensitive piezo [z] stage to allow for very fast and accurate acquisition of image stacks. **DO NOT apply more downward force than necessary** when installing your stage insert or it can be damaged (**$15,000!**).

35mm Dish

Standard Slide (2 parts)

Chamber Slide* (read footnote!)**

Figure 5

7. **If you plan to do live cell experiments** (and thus need the incubator), switch on the incubator by pressing the large orange "POWER" button at the top left of the incubator located behind the left monitor screen (**Figure 6**, right).
    - It is ok that the values displayed don't read 37C as these are all calibrated so that the sample will be at 37C.
    - The default values should read as pictured in **Figure 6**.
    - The lens heater can be left off.

Figure 6

Figure 7

8. If using an oil immersion objective, **apply a SINGLE drop of Type NF2 oil** (**Figure 7**, left) to the front lens of the objective.
    - It is not recommended to image a sample that has already been imaged using another type of oil, however, if this cannot be avoided, be sure to **thoroughly remove all previous oil from the coverslip** with Windex® (or other cleaner) to avoid mixing different types of oils (which causes emulsions and significantly degrades image quality).

9. **Position a [clean] sample in the appropriate holder** and bring the objective up to begin imaging.

If the system has been on before you, pressing the "Refocus" button to bring the objective up will cause the objective to return to the position it was in previous to your session, which may cause damage to your sample and the objective if this position is too high. To ensure this doesn't occur, hold down the "Escape" button and tap the "Refocus" button to reset (refer to **Figure 4**). This will now allow you to manually bring the objective up using the focus knob from the escaped position.

***USE EXTREME CAUTION: When using the chamber-slide insert, you will not have access to a significant portion of the perimeter of the wells with the higher NA lenses as the metal insert surounding the chamber-slide bumps into the objective and causes damage due to the small working distance. You may need to switch to a another insert to allow for full access to the perimeter.**