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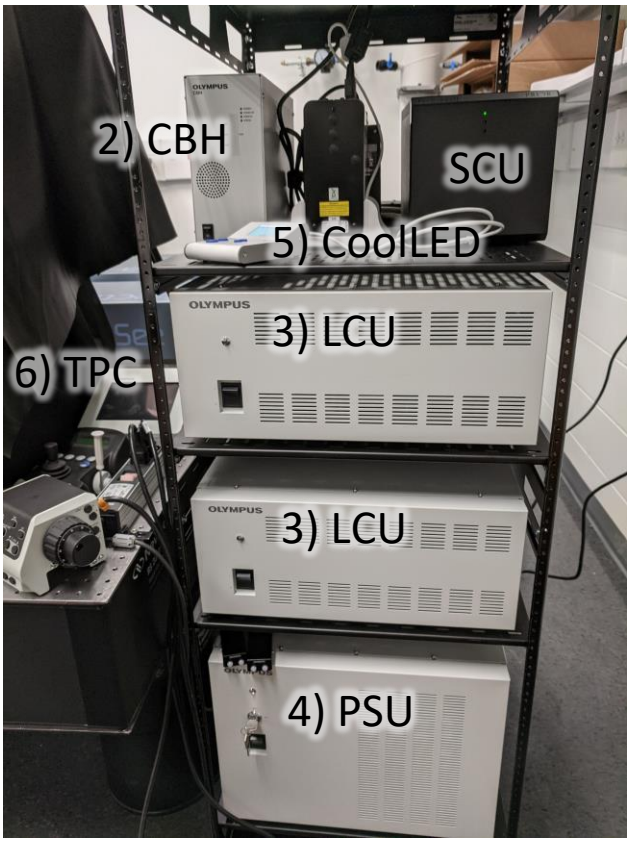
Olympus Multiphoton Microscope: Turn-on Sequence



1) Turn on computer if it is off. Log on to BRC Login.
2) Turn on Control Box House (CBH). Wait for *beep* and then 5 more seconds.
3) Turn on both Laser Control Units (LCU's), one for each laser. Wait for ~30 sec until sounds have stopped.
4) Turn on the Power Supply Unit (PSU). The key should always be set to "ON".
5) Turn on the Epi Light Source (CoolLED) for standard fluorescence through the eyepieces.
6) Turn on the Touch-Pad Control unit (TPC) via a single button push on the back right side. Do not double-press. The screen will appear blank for a while. Wait until "Start Operation" appears, but do not press it!
7) Open the Fluoview (FV) control software using the "Acquisition" icon on the desktop. (Press OK for the HW configuration window with default settings.)
8) Turn on lasers via Tools -> Configuration -> IR laser emission
9) Align lasers for both lasers via Tool Window -> IR Laser Setting -> Fine Adjustment -> Start Active Alignment.
10) Set data folder you will be using via Tools -> Configuration -> Preference -> File/Folder

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8) Turn off lasers via Tools -> Configuration -> IR laser emission
7) Make sure you have all your data saved on the fileshare. Close the FV acquisition software.
6) Turn off the Touch-Pad Control unit (TPC) via a single button push on the back right side.
5) Turn off the Epi Light Source (CoolLED).
4) Turn off the Power Supply Unit (PSU), leaving the key set to "ON".
3) Turn off both Laser Control Units (LCU's), one for each laser.
2) Turn off the Control Box House (CBH).
1) Log out of the computer.
0) Bring stage down via hand crank and clean up!

Rough imaging sequence. Most of these windows should already be open.

1) **Ensure stage is fully down using hand crank.** Set objective to ~middle of travel using Olympus touchpad. Reset focus in Acquire -> Z-Section box -> Register origin.
2) **Find focus** using Tool Window -> Ocular. Adjust the stage to the rough location using the hand crank.
3) **Switch to acquisition mode** using Tool Window -> LSM.
4) **Ensure correct objective** at Tool Window -> Microscope -> Objective Lens.
5) **Set up lasers** using Tool Window -> IR Laser Settings. Select wavelengths and coupling mirror. Do a fine adjustment on both lines.
6) **Make sure the correct filters are entered into the system** using Tools -> Configuration -> Filters (tab) ->RNDD filters.
7) **Set up channels and tracks** using Tools -> Dye & Channel Settings. (This will not be active if you are in the Ocular mode. See #3.) If you have the correct filters entered, you can also load an Observation Method under Tool Window.
8) Ensure Tool Window -> LSM Imaging and Tool Window -> PMT Setting are available for **scan adjustments during imaging**.
9) Window -> Live button is used for **live scanning**. If the Live button is not active, make sure you are in LSM (see #3), and make sure you have at least one channel active.
10) For **data acquisition**, use LSM Start under Tool Window -> Acquire -> Normal(tab). For acquiring Time or Z series, set the appropriate radio button under Tool Window -> Series and fill out the relevant boxes. To acquire a Z series, you must register three points: origin, start and end.

Multiple positions or tiling

1) Set up positions using map tab under live mode. You will have to zoom in with the scroll bar in order to see positions/tiles. Use the following buttons to register single positions or tiles.

Note that **Load Acquisition Parameters** means take those stored in the position list and populate current scan parameters. **Update Acquisition Parameters** means take current scan parameters and update the currently selected position. Live scan uses current scan parameters, not saved ones! (Very confusing . . .)

For example, height differences can be entered by changing the registration origin and updating the acquisition parameters while a position is selected.

2) Use MATL tab in acquire menu to execute.

Easy targeted stimulation or photobleaching with time series

1) Set up time series
2) Use Tool Window -> LSM Stimulation to define ROI and stimulation laser properties.
3) Use Tool Window -> Synchronization and choose LSM imaging Base Method. Choose number of initial frames before stimulation under Stimulation: Wait:
4) Use Sync tab under Acquire window to execute.