Two-photon Microscope Start Protocol

1. Check the labels of the current filter cubes. Change the filter cubes if needed, and change the labels respectively.
2. If you use channel 3 and 4, remember to pull the second Dichroic Mirror bar out.
3. Turn on the power supplies of the system sequentially from #1 to #7 (with red tape) one by one. **Wait several seconds in between each power supply. Wait #4 touch pad to show "Start Operation" before proceed to #5.** If you don't need epifluorescence, do not turn on #2.
4. Open the FV31S-SW software on the desktop of the computer.
5. HW configuration window will show up during software initialization:
   - Check Initialize Resonant Scanner
   - Check Enable XY Stage Control
   - Uncheck Start Mechanical Origin Search
6. Go to Layout on the upper-left corner. Select Default, and click Apply.
7. Turn on Lasers. Go to Tools → Configuration → IR Laser Emission → Turn on the laser(s) you want to use.
8. Select the Filter Cubes. Go to Tools → Configuration → Filters (on top) → RNDD Filters (on left) → Select the filter cubes you put in.
9. Change image saving folder directory to your lab's folder.
10. **Wait the lasers to be fully warmed up before this step!**
    If you have an ObservationMethod saved, go to ObservationMethod next to Ocular. Load your specific imaging setting.
11. If you do not have an ObservationMethod saved, do the rest.
12. Tune Lasers. Adjust laser wavelength to your targeting excitation wavelength.
13. Auto-alignment (no need for Insight laser >1100nm). Wait lasers finish tuning, go to Fine Adjustment, click Start Active Alignment.
14. Select Coupling Mirror. Go to Lightpath. Select the right Coupling mirror at the bottom of the IR Laser Setting (on left) based on the laser wavelength. Double check if the light pathway is correct.
15. Focus under eyepiece. Go to Ocular. Select Ocular. Select right Filter. If you use Wide Field external light source, select Empty. Observe through eyepiece and find your focus.
16. Change Ocular to LSM to start laser scanning imaging.

Reminders:

- If you change the laser wavelength during imaging:
  1. If it's more than 50nm change, remember to auto-align the laser.
  2. Please check the Lightpath, especially the coupling mirror, to make sure the laser can be directed to main scanner at that wavelength.
- The default value for the Offset of every PMT is 3%.

Two-photon Microscope End Protocol

Cleaning:

1. Lower the stage.
2. Remove your animal or sample preparations at the end of imaging. No animals can be kept at the core.
3. Remove and turn off objective heater if you used.
4. Remove isoflurane if you used. Turn off the air tank. Turn off the water bath.
5. Clean up your surgery station and put contaminated trash to the biohazard bag.

System shut down:

At the end of the imaging session, check PPMS calendar to see if there are users afterwards.

If there is another user within 2 hours:

1. Turn off the Z-Brightness. Do NOT close the software. Do NOT turn off lasers.
2. Transfer your local data to the server.
3. Log out PPMS.
4. Gently clean the objective with lens paper using distilled water only. Leave the objective on the system.
5. Clean the stage with alcohol.
6. Close the filter cube box.
7. Close the imaging rig curtain.

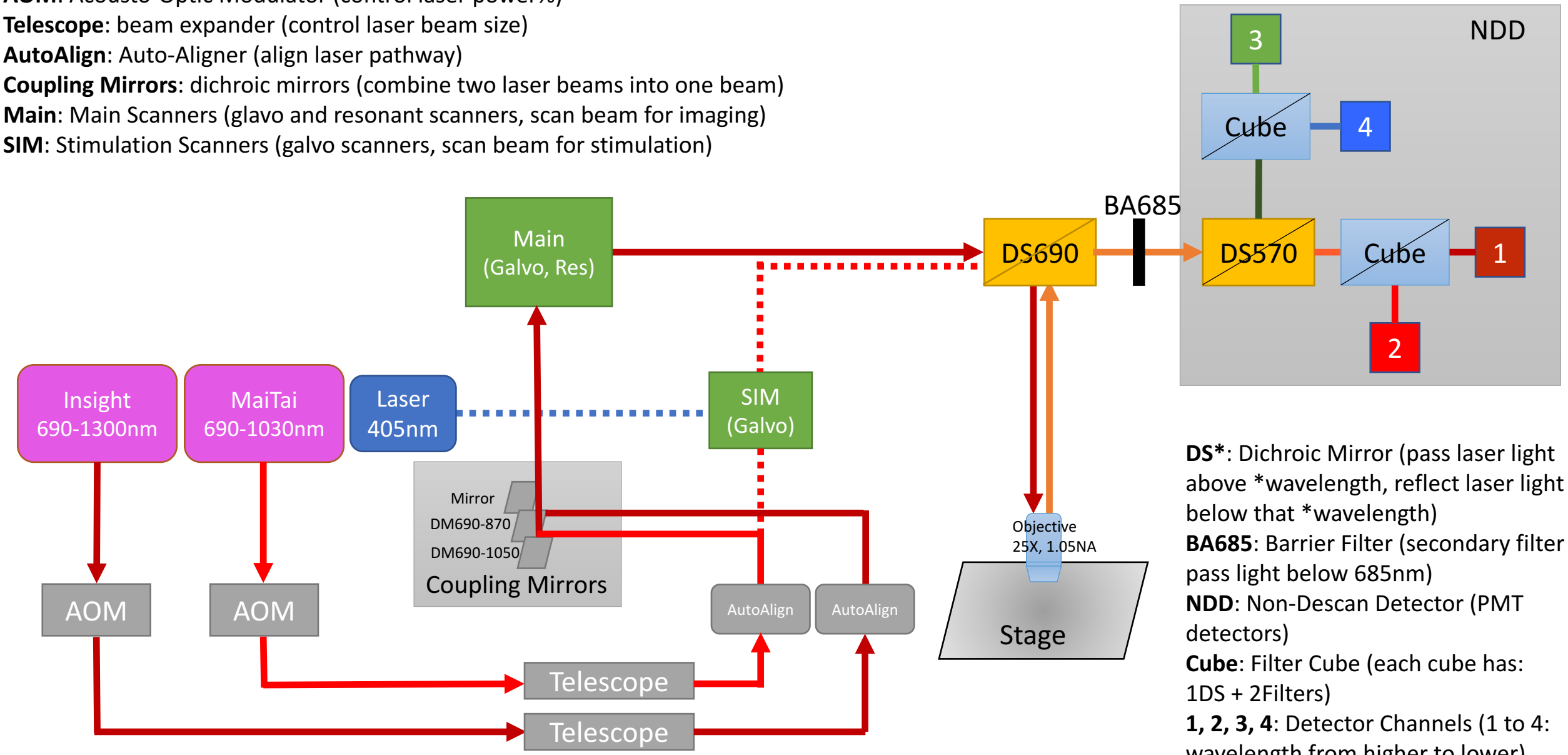
If there is no user within next 2 hours:

1. Turn off the Z-Brightness.
2. Turn off the lasers. Go to Tools → Configuration → IR Laser Emission → Turn off Lasers.
3. Close the FV31S-SW software.
4. Transfer your local data to the server.
5. Log out PPMS.
6. Turn off the touch pad on the screen and switch off on the back of the pad.
7. Turn off the rest of the power supplies from 1 to 7.
8. Gently clean the objective with lens paper using distilled water only. Leave the objective on the system.
9. Clean the stage with alcohol.
10. Close the filter cube box.
11. Close the curtain.
12. Turn off the room light if you are the last one of the day.

Please remember to turn off Air Tank!

Please remember to turn off Water Bath!

AOM: Acousto-Optic Modulator (control laser power%)
Telescope: beam expander (control laser beam size)
AutoAlign: Auto-Aligner (align laser pathway)
Coupling Mirrors: dichroic mirrors (combine two laser beams into one beam)
Main: Main Scanners (glavo and resonant scanners, scan beam for imaging)
SIM: Stimulation Scanners (galvo scanners, scan beam for stimulation)



DS*: Dichroic Mirror (pass laser light above *wavelength, reflect laser light below that *wavelength)
BA685: Barrier Filter (secondary filter pass light below 685nm)
NDD: Non-Descan Detector (PMT detectors)
Cube: Filter Cube (each cube has: 1DS + 2Filters)
1, 2, 3, 4: Detector Channels (1 to 4: wavelength from higher to lower)

URMC Multiphoton Core OlympusFVMPE-RS Two-Photon Microscope LightPath (03/07/2018)

Cube Information (01/12/2018)				
New cube name	**Color**	**Bandpath**	**DM**	**Old cube**
B/G	B	420-460	DM485	Cube 1-1
	G	495-540		Cube 1-2
CFP/YFP	C	460-500	DM505	Cube 2-1
	Y	520-560		Cube 2-2
SHG/G	SHG	370-410	DM485	Cube 3-1
	G	495-540		Cube 3-2
R/fR	R	575-630	DM650	Cube 3-3
	fR	645-685		Cube 3-4
CFP(420-500) /YFP(519-549)	C	420-500	DM509	Cube 4-1
	Y	519-549		Cube 4-2
SHG/B	SHG	370-410	DM405	Cube 5-1
	B	425-465		Cube 5-2
nR(573-613) /fR(634-686)	nR	573-613	DM640	Cube 5-3
	fR	634-686		Cube 5-4
G/R	G	495-540	DM570	Cube 1-4
	R	575-630		New