

Olympus 2 Photon manual

1. Turn on button on a power extension cord if it was off. (On top of microscope box)
2. Choose **B** in the laser switch.
3. Switch on PC, password **!simfcs1**.
4. Check beam splitter in front of the detector, choose the one you need. 460 (Laurdan) or 550 (Green blue)
5. Switch on an Andor camera.
6. Turn on Mai Tai laser (warm up and then ON), COM port 9, ($\lambda=780\text{nm}$). (Press YES when it says there is an error and ask if it should retry)
7. Choose filter on the microscope (ring beneath objectives): (A UV I), (B 2 photon II), (C Bodipy III), (D DiI IIII)
8. Start MicroManager, start the **LSMdec2015** software, press [**Visible**], choose eye on the microscope, and focus on your sample (eye or Andor camera).
9. Verify the position of the quarter waveplate (for producing circular polarized light, to the right of the scan mirror box). It should **not** be in the light path for linear polarization experiments.
10. Start **VistaVision** software.
11. **For measurements** open Mai Tai laser shutter, turn the [LSM/EYE] knob on the microscope to LSM.
12. In the **LSMdec2015** software switch to LSM-mode, turn on PMTs (start with gain 600), choose desired filters, adjust laser power. Finally open shutter in LSMdec2015 and start acquisition in VistaVision.
13. **For polarization rotation experiments** use **Thorlabs Kinesis** software. Go to [Settings], [Advanced], and enable triggered rotation (relative fall). Choose step in degrees (10 degree correspond to 20 degree rotation in the image). Start experiments from 46 degree (which

corresponds to horizontal polarization in image). Disable rotation after acquisition in same menu.

14. **To turn off:** Close shutter in PMT and in MaiTai software, switch off the laser, close Mai Tai software, close micromanager, close VistaVision, go back to visual and close **LSMdec2015**, switch off Andor camera, go to eye on the microscope, and switch off power extension cord.

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