Nikon 2 Photon manual

1. Turn on the button on the power extension cord.
2. Switch on PCs (windows 7 on both), password **Vilkommen2014**.
3. Check beam splitter in front of the detector, choose the one you need.
4. Switch on an Andor camera (left from the Nikon microscope)
5. Turn on Mai Tai laser: wait for warm up and then turn the emission on (COM port 1, power >2.7 is good), choose your wavelength (λ).
6. Start Micromanager, choose EYE and focus on your sample (eye). Filters to use UV, FITC(495/519), TRITC (550/573), and Cy3 (549/562).
7. For two photon measurements, switch on **PMT2** software as admin on the left PC. Adjust wavelength according to the laser set wavelength (λ), switch on PMTs and HV, and adjust laser intensity (0-10).
8. Start **VistaVision** software; go **to Imaging if you need or to FastFlim**.
9. **For measurements** Choose filters for Channel 1 (direct) and for Channel 2. **Dichroic mirror OUT on the stage for λ in 800 range**. Open Mai Tai laser shutter, choose two-photon option in Micromanager, open a shutter in micromanager and make Laser ON in PMT2.
10. For FLIM measurements: choose external clock for 80MHz, then upload and do calibration with you substance (Florocine 4.05ns lifetime, Rhodamine B 1.68ns lifetime etc). After calibration do your FLIM measurements.
11. **To turn off**: Close shutter, switch off the laser, close Mai Tai software, close micromanager, VistaVision, PMT2, switch off Andor camera and switch off power extension cord.
12. **Do the data transfer through VDS (use icon on the desktop-right click), fill the paper log.**

In the case of problems contact: Vita Solovyeva (vita@bmb.sdu.dk) or Jonathan Brewer (brewer@bmb.sdu.dk)