

How to start MIRAVA abberior:

1. Switch on the THOR ABS (on the left – nr. 1 = LED light source for fluorescence).
2. Switch on OLYMPUS touchpad (on the right – nr. 2, for objectives, focus, BF/FL)
3. Switch on the PC (if not in sleep mode)
 - login as Abberior admin with password Abberior (please do not use your personal login)
 - Start the Inspector LIGHTBOX software, select AutoAlignment (first user on given day) or New Sample

AutoAlignment:

AutoAlignment needs to be done by the first user of the day, autoalignment slide is in the fridge, let it warm up to RT. Put 2 drops of OLYMPUS (!!) oil onto the coverslip, choose 60xOIL objective. Press Escape button (touchpad nr. 2) to lower it, insert the autoalignment slide, then press START (touchpad nr. 2) to find focus automatically. The system finds the focus (no human help needed). Go to Lightbox SW, select AutoAlign (top menu), press run and wait, until completed. If you need to use another objective, CLEAN with LENS tissues the 60x OIL objective and change the objective on the touchpad.

New sample:

Select objective on the touchpad (2), put immersion the slide. Press Escape button to lower the objective, insert slide (coverslip with immersion down), then press START to find focus. The system finds the focus (no human help needed). In Lightbox software: top menu: New sample, select ALL dyes you plan to use. Choose confocal mode, STED or FLIM as needed. For FLIM (Timebow), use hybrid (HYD) detector. For STED, go for APD.

Lasers are switched on/off automatically.

How to turn off

Press Escape button (touchpad nr. 2) to lower objective, remove your sample

- switch off Lightbox software, wait until is fully terminated
- turn off OLYMPUS touchpad (2)
- turn off THOR ABS (on the left – nr. 1)
- PC to sleep mode
- DO NOT turn off any keys or buttons in the black boxes below the system

General:

Objectives: use 40x water for thicker samples

Lasers available: 405 nm, 440 nm, 488 nm, 561 nm, 640 nm

FLIM = Timebow. Use hybrid detector (or MATRIX), but always the same for Ctrl+test set).

Detectors:

They are lined behind pinhole as follows: Pinhole-APD1-APD2-MATRIX-PMA1-PMA2, so that the colors are automatically assigned RED to APD1, YELLOW/ORANGE to APD2, GREEN to MATRIX, UV to PMA1. By clicking on (for example) MATRIX in imaged dye of your choice, you shift the whole sequence towards the end (for example: I want RED for MATRIX, so Yellow will be shifted to PMA1).

- APDs: most sensitive for RED and ORANGE
- MATRIX detector – improves signal-to-background ratio (measures background and sample separately). Less sensitive for RED and ORANGE. Practical: Use higher laser power and accumulation (Matrix is less sensitive). May be used for confocal, STED and FLIM (for FLIM, it gives slightly different lifetimes than hybrid detector, so always use the same detector for 1 comparison).
- Hybrid detectors: very fast, for FLIM, less efficient for RED

Other tips:

The higher STED, the higher laser power and accumulation needed

What helps to subtract background: lower pinhole + increase STED (to 30%)