Zeiss LSM 780 FLIM, startup and measurement

Startup

- Make a Rhodamine 110 slide (droplet) - lifetime is known, diffusion time is known
- Use high precision 1.5 cover slip
- Go to FCS (zeiss software), default GFP setting
- Count Rate is used (CPM, counts per minute)
- Adjust the correction collar to highest CPM (optimum is between two worse extremes)
- Start Experiment

- Go to Picoquant SymphoTime software
- FCS tab, test, start
- Select channel - data channel, but see CPM of each one, first!
- Pico-FCS-measurement (not test), point, 30 seconds
- TCSPC resolution 128 ps (faster fit...)
- Start/stop; select right data channel! (in the right screen)

- We have CW laser (to test); therefore the Histogram (blue line) is just a high plateau
- Save measurement (eg. called 2%, for laser intensity)
- Zeiss ZEN software:
- use pre-set: "485 external laser, external port"
- info: pulse-rate is 32 MHz

- Measure dark background, set no laser power for 485
- Register Counts

- Now use Erythrosin Dye slide (coverslip 1.5 high precision, droplet) which has a short lifetime, quenched with KI solution (potassium iodide)
- get IRF
- 60% laser = OK
- but can use optical attenuation (big rotary button: stepwise eg 0->1, small button: continuous adjustment)
- Attenuation 1 = approx. 0.9 µW

- Laser power meter: Simply switch on, can set 485
- Measure and mark laser power.

- Change to scan
- pre-set Zeiss settings eg. "488 26x265"
- there, can also pick pre-sets for FLIM
- Detectors: are still PMT, with gain 600-700

Measurement

- "start" in symphotime (1)
- "start" in zeiss software (2)
- "stop" in symphotime
- save image, name it, copy data to word file: Picture of cells, Picture of histogram

- Do that for each acquisition separately
- info: for a 2-component fit, >1000 photons per pixel are needed
- ROI data is displayed in histogram, but warning: has to be told to use that ROI for the fit

- Select experiment
- Analyze
- Calculate fast FLIM
- Fitting model
- Import the measured IRF (from the set-up step)
- Initial fit (change # of parameters used, if necessary)
- Evaluate amps + their error range
- Evaluate mean/average lifetime
- "shift" indicates delay between IRF/population of the early lifetimes that were fit