Using the FluoroLog 3

Keep the lab tidy!

Do not leave your samples here.

Do not leave used glass slides, gloves, optical components, posts or whatever else here.

This is not a wetlab – prepare your samples elsewhere

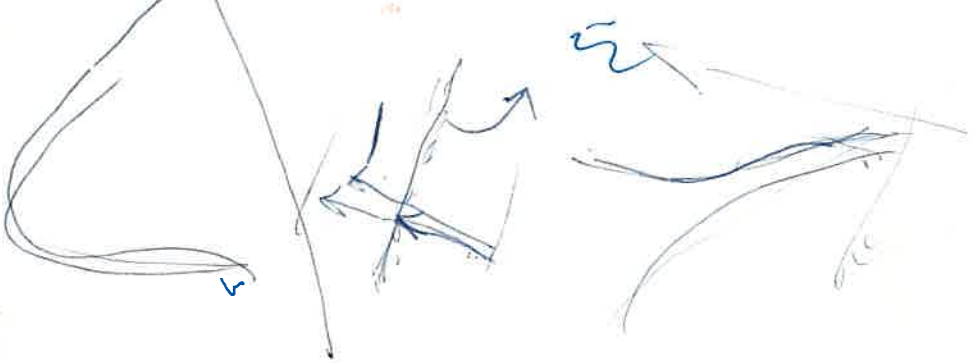
Do not wear gloves while using the instrument – no one wants parts of your sample transferred to the keyboard or other components

Start-Up

- Start all cooling first – detector cooling (if necessary) and ventilation fan in lamp
  - InGaAs detector and CCD camera must be cooled by liquid nitrogen
  - PMT is (automatically) electrically cooled, and no action is needed
  - Lamp fan is turned on with the main power-switch on the lamp housing
- Start Xenon lamp. It will take about 20 minutes to stabilize
  - Important: make sure the air-inlet on the back is not blocked!
- Enable your detector of choice:
  - PMT is automatically initialized, no action needed
  - CCD camera is initialized with the TriAx, no action needed
  - InGaAs needs external power – switch on PS-1 power supply on the table
- Start instrument controller (black computer under the table)
  - It is finished booting when you hear one beep, followed by a double beep
- Start TriAx controller (white "computer" on the table)
- Start measurement computer (black computer on the table)
- Measurement software can be found on the desktop ("FluorEssence")

Shut-Down

- Shut off lamp but NOT main power to the lamp housing
  - Let the ventilation fan for the lamp run for ***at least*** 20 minutes after shutting off the lamp
  - After the lamp has cooled down, main power to the lamp can be turned off
- Detectors, controllers, and power supply can be turned off safely simply by turning off their respective power switches
- Close the FluorEssence program
- Turn off the measurement computer



Detectors & Accessories

The instrument is equipped with 3 detectors:

- PMT – Covering the range from 250-850 nm
- InGaAs – Covering the range from 850-1400 nm
- CCD camera – Covering the range from ~300-1000 nm

Choose your detector appropriately!

- PMT and InGaAs use the lateral exit port of the TriAx, while the CCD is on the axial exit port
- Choosing between CCD and PMT/InGaAs is done by checking the appropriate box in the software
- Choosing between PMT and InGaAs also requires setting the 45° mirror on the lateral exit appropriately. Mirror down: PMT – Mirror up: InGaAs
- You can *not* use more than one detector per measurement!

Notes:

- PMT saturates at approximately 2 million counts – keep the signal below this!
- In the range from about 830-880 both PMT and InGaAs have extremely poor sensitivity, and CCD camera should be used instead.
- The detected signal can (and should) be corrected for both lamp intensity and detector sensitivity
- Corrections for detector sensitivity works very poorly around 850 nm! Corrections will often be grossly over-estimated in unpredictable/unreliable ways. If you need to measure emission spectra with PMT or InGaAs in this range, consider dropping the correction and just report raw data
- Only the PMT has a pre-installed correction file!
- Correction for the lamp spectrum (strictly necessary for excitation measurements!) is set in the software by simply dividing the signal ("S1", "S2", or "A", depending on detector) with the intensity measured on a diode in the excitation monochromator ("R")
- CCD camera and InGaAs must be cooled by liquid nitrogen to decrease thermal noise to useable levels. No cooling is necessary for the PMT.
- InGaAs has a separate power supply which must be turned on before use ("PS-1")

Polarizers

- The instrument is equipped with polarizers before and after sample
- The excitation polarizer is substantially smaller than the physical size of the beam resulting in the beam being clipped. This cannot be altered, and you must figure in an intensity loss of around 100-200 times after insertion of polarizers. Adjust integration times and slit sizes accordingly to get a reasonable signal.
- Polarizers are fully automatic, and controlled from the software under the "accessories" tab. Set polarizers as "in", and set the desired angle – 0 degrees corresponds to vertical polarization.
- Polarizers are meaningless when using the fiber-optic insert – e.g. for integrating sphere measurements.

Software and Calibrations

- The software is an Origin shell, and all data from an experiment series are saved in a single .opj project file. If you need spectra as individual asci files, you need to manually export the data using the export function
- Do not do data analysis in the measurement software. Strange things may happen.
- Save regularly! The software doesn't crash very often, but it happens.

- After opening the software, click on the "M" on the utility bar on the top of the window. This will start initialization of the instrument components.
- Almost all initialization problems can be solved by making sure all instrument components are switched on, and/or by restarting the computer and all components. Instrument-software miscommunication happens, and this is by far the easiest fix...
- Select the appropriate experiment (typically "Spectra", and either emission or excitation spectra)
- Set emission grating in the Monochromator tab to whatever is suitable for your experiment. This is not crucial for PMT and InGaAs measurements (beyond affecting signal intensity), but is of course important when using CCD. Excitation grating is fixed, and cannot be changed even though the software leads you to believe so.
- Select excitation/emission wavelength, scan range, and spectral bandwidth in the Monochromator tab
- Select detector (e.g. PMT) and integration time in the Detectors tab
    - Also do lamp-intensity correction by using the signal algebra section here. You want to output raw signal (e.g. "S2") divided by lamp intensity ("R") – dependent on detector but will look like "S2/R" for PMT. This is good to do for emission spectra, and strictly necessary for excitation spectra
- Select polarizers if needed in the Accessories tab
- Start the measurement!

- The monochromator calibrations needs to be checked regularly!
- Excitation monochromator does not usually change, but it can be checked by measuring the lamp spectrum on the reference diode:
    - Set up excitation spectrum measurement to scan over the visible range. Measure only lamp spectrum ("R"). The most intense line should be found at 467 nm.
- Emission monochromator DOES lose calibration frequently, and should be checked before each measurement, and definitely if you change grating. This calibration is done in the emission spectrum experiment.
    - Put a cuvette with pure water in the sample compartment and measure the Raman spectrum. The appropriate settings for this experiment is the default values (350 nm excitation, 5x5 nm slits etc)
    - With 350 nm excitation the Raman peak should be at 397 nm
- Adjustments to the monochromators are done in the Real-Time Control section (RTC button next to the Start Experiment button)
- If you do not know how to do these calibrations, please ask someone who knows (or read the manual!) rather than playing around with parameters!