NanoLog Spec Fluorimeter Instructions

THE PMT RUNS AT 1450V, which is different from the default value. Check it!

General note

The pmt usually saturates at $2x10^6$ counts per second, so we want to be safe and *work at $1x10^5$ to $1x10^6$ counts per second*. This is controlled by adjusting the slits in a proper manner. The ratio between excitation (lamp side) and emission (detector side) is *ideally 1:1, but you can go to a 1:3 ratio*.
For emission spectra the slit at the detector side should be small (1 nm) to get high resolution, and the slit at the lamp should be 1 to 3 nm. This should be adjusted to get 1 10^5 counts per second at the maximum. If the sample is poorly emitting increase the slits to keep within the 1:1 to 1:3 range of ratios (for example excitation slit 5nm, detection slit 2nm).
For excitation spectra the lamp side should have a small slit, and the detector side a bigger slit (if necessary). In this case you want high resolution at the lamp side. All of the above applies to the choice of slits for excitation spectra as well (swapping excitation and detection in the above).

Emission spectra

Choose an excitation wavelength. The emission must be scanned *staying at least 10 nm away from λexc and $2\lambda exc$*. For example, when exciting at 400 nm scan from 410 to 790 nm. This to avoid the excitation light and its second harmonic, which might overload the detector.
Increment 1 nm
Integration 1s/nm. Lower integration times give faster scans, with lower signal to noise ratios. (0.1s/nm is good to have a quick look for example, don't waste your own or other peoples time)
Check collected signals: S and Sc

Excitation spectra

Choose an emission wavelength. The excitation source must be scanned *staying at least 10 nm away from λexc and $2\lambda exc$*. For example, when detecting at 500 nm scan from 260 to 490 nm. This to avoid the excitation light and its second harmonic, which might overload the detector.
Increment 1 nm
Integration 1s/nm. Lower integration times give faster scans, with lower signal to noise ratios. (0.1s/nm is good to have a quick look for example, don't waste your own or other peoples time)
Check collected signals: S/Rc, S, R

Step by step Standard Operating Procedure (SOP)

1. Turn on the mail power supply that turns on the water circulation system for PMT
2. switch on the fan to cool the lamp
3. switch on the lamp
4. turn on the PMT
5. start the spectraacq computer
6. wait until it copletes reading the floppy diskette
7. boot up the PC that runs datamax pragram
8. start datamax software
9. choose Steady-State operation of the system (system initializes)
10. there are 4 icons within the software, click first icon on the left
    i.e. Run Experiment
11. go to collect experiment
12. choose experiment type (emission or excitation)
13. choose data file, point the path to your folder on the hard disk (required)
14. set up parameters (see attached description with screen shots)
15. **follow general guidelines on page 1 to handle the instrument properly**
16. optional: create an experiment file after entering all your parameters
17. Data management:
    a. store files under your directory on the hard disk
    b. the lab pc is not a backup system. You are responsible for copying your files ASAP and keeping backup copies elsewhere
    c. the lab PC is not a data analysis station. Plot and print data from your office computer
    d. files are stored with the .spc extension. These can be read into datamax on your office computer. In addition, you can convert your data to ascii format for use in origin, excel, …
    e. copy your files and take them to your office computer

<u>Shutdown procedure:</u>

Leave on the PC that runs datamax
Switch off spectraacq computer
Switch off PMT and cooling water
Leave the fan on for 15’ but switch off lamp