Standard Operating Procedure: Fluorolog

Personal Protective Equipment

Wear gloves, goggles and a lab coat when preparing samples. No special PPE is required for operating the fluorimeter.

Hazards

Aside from the risks when handling chemicals and glass, the main hazard is electric shock. Probably the most dangerous step of this SOP is turning on the white light; unplug everything else before doing this to avoid blowing a fuse.

Procedure

Before turning anything on make sure everything is off (the fluorimeter, the tower, and CPU) and the UV-Vis is unplugged. Make sure the tower and CPU are unplugged: only the fluorimeter should be plugged into the outlet before beginning.

Turn on the bottom switch on the fluorimeter (main power). Wait until the fan is on and sounds stable. This should talk about 5-10 seconds

Turn on the top switch on the fluorimeter (the lamp). Let the lamp warm up for at least 1 hour before doing anything else.

It is important to have only the fluorolog plugged in so a fuse doesn't blow while turning on the lamp.

Turn on the tower (SpectrAcq) by pushing the button on the right side in and making sure the button does not get stuck pressed in. Wait until the green light on the tower stops flashing. Startup is complete when the green light is no longer lit.

Turn on the CPU, there is no password (hit enter).

Open the software called Instrument Control Center and chose 1 detector. When asked if the hardware should be brought to the last position, it doesn't matter what you choose.

Click on Run Experiment (the Left button)

Place the sample in by removing the cover and lightly pulling back the metal sample holder so the cell does not scratch it. Turn off the lights, or if the cover can fit over the sample place it back on.

Make sure no light from external sources hits the detector. Otherwise, you might fry it.

Chose Collect experiment.

Choose data file, the name of the file, and where you want to save it on the computer. NOTE: *this is an old software, so you can't use more than 7 characters for your filenames.*

Pick and appropriate integration time (typically 1 s is fine but it depends on what you are doing).

Choose an excitation wavelength.

Make sure the slits are at 1.5 nm (if you must change the slits due to a low signal change the excitation/entrance slits), the detector (HV) 900V, and the signals are R, S, S/R.

*** Do not change the emission/exit slits or the high voltage. The correction factors have been generated using 1.5 nm and 900 V, so changing either of these renders the correction factors unusable.***

Once all of the settings are correct hit run and make sure the intensity of the signal does not go over 750,000 counts (this is monitored at the bottom of the screen). *If you think the counts will go too high stop the experiment by clicking Collect and chose Halt Experiment.* Then close the excitation slits enough to get the counts within an acceptable range.

The instrument saves three files as your file's name ending with an uppercase letter. A = signal (raw); B = noise; C = signal/noise. File C is the one you should work with.

The spectra that was just collected needs to be corrected by subtracting a solvent spectra and then a dark counts and then corrected by correction factors that correct the sensitivity of the detector.

The dark counts are collected once, even if you have multiple samples. To do this, turn off the Xe lamp, set the solvent cuvette in the sample holder and collect an emission spectrum with the same settings you used for your sample(s). You should do the dark counts last, because you must not turn on the Xe lamp again if the instrument is running and everything is plugged in.

To do this chose Arithmetic at the top, Functions

- *To subtract the solvent spectra and dark counts:*

  - make sure the function is "subtract", the operation is Term File*k.

  - set k = 1 and chose the file you want to subtract (the solvent file ending with C).

  - click apply, OK, and add new, this will open the subtracted spectra as a new file so you can save this as a separate file. Once you save the new spectra Close All Open Slots, (if too many spectra are open it will give an error while correcting new data).

  - repeat this for the dark counts

- *To correct the spectra with correction factors:*

– Select the function to multiply, the operation is Term File*k.

– set k = 1 and chose the correction file which is in a folder called EMCORR and the file is named with the most recent date.

– click apply, OK, and add new, this will open the corrected spectra as a new file so you can save this as a separate file. Once you save the new spectra Close All Open Slots, (if too many spectra are open it will give an error while correcting new data).

– right click on the spectra and copy to clipboard and this can be pasted into a notepad file and then transferred to a graphing program.

If you haven't done so yet, turn off the Xe lamp *before turning off anything else*. You'll have to keep the fluorimeter on until the air coming out of the fans is cool (i.e., you should not turn off the fluorimeter without cooling down the Xe lamp).

Once you are done, close the software, turn off the PC, then the tower and finally the main power switch.

Last Updated: December 2016 – MW & DAR