

## Fluorimeter Cheat Sheet – Basic Operation

*This is intended as a reminder to experienced users. You **MUST** be trained on this instrument by someone who knows what they're doing before you use it.*

### Logging Lamp Time

It is important that the lamp is changed after about 500-750 h of use to prevent damage to lamp housing. *Always log the number of hours the lamp is in use!*

### Starting the Fluorimeter

Power on the lamp and wait for ignition (the power readout will jump from about 5W to 70W). The lamp requires 10-15 min of warm-up time, after which it should settle at 75W. Adjust the power as necessary to get to 75W. (NB: If the lamp does not ignite after 10 min, turn the power off and get help. It's probably time to replace the lamp.)

Turn on the Peltier controller, the stirrer/motor drive and the shutter control. The titrator and stirring accessory can be left on all the time. There should be no need to adjust the PMT voltages for most work.

Open the Norton Antivirus software (shield icon in the system tray) and unload the services. NAV can interfere with the operation of the monochromator. Start the Felix program on the PC.

### Monochromator Positions and Slit Widths

Go to *Configure... Hardware...* Click *Initialize*. The instrument will move the monochrometers and report where it thinks they are. It's always wrong. Read the actual value on the source, channel A and channel B monochrometers and type these values in. Click OK and close the Hardware dialog box.

It is a good idea to set all the slit widths initially to 2 nm. All the way clockwise is closed, and each 180° turn counterclockwise opens the slits 1 nm. Note that there are a total of six slits to adjust, one at the entrance and exit of the source and each channel.

### Acquiring a Spectrum

For either an emission or excitation scan, the parameters are similar. For an emission scan, set the excitation wavelength and the emission range for each channel. You can set the step size (in nm, usually 1), the integration time (usually 0.1-1 sec) and the number of times to average (usually 1, unless you think your sample is fluctuating with time).

Click *More...* Enable the real-time correction. Click on the RT correction calibration button. Set the wavelength to the excitation wavelength (clicking Go and waiting for the monochromator). Open the shutter and adjust the gain sidebar (on the left) until it reads 3.0V or as high as possible below 3.0V. Close the shutter and press OK. (For excitation scans, check both of the extrema and set it so that the voltage will not exceed 3.0V over the excitation range.)

Click the temperature control box and set the temperature.

Change the shutter control to Automatic. (*This is important; the shutter will not open and close with your acquisitions if you don't do this!*)

Close the More dialog box.

When your sample is ready, click Acquire/Prep and wait for the Peltier device to set the temperature. Click Acquire.

### **Viewing and Saving Data**

Each scan will produce raw curves for each channel, corrected curves for each channel and a temperature curve. You can select curves by clicking on them (or Ctrl-clicking multiple curves, or Shift-clicking a range) and turn their display on and off with the display-curves button at the top. Typically, only the Corrected A spectrum will be of value for normal experiments.

Save the data as a Felix file for future analysis in the Felix program. For most purposes, you will want to export your data as text so that it can be manipulated in a graphing program or Excel. Check only the relevant spectra (the Corrected A, usually) and save these as a text file with the *Save Checked Spectra Only* box selected. You can then copy/paste these data into Excel.

### **Powering Down**

If you don't plan to use the machine for more than one hour, power the system down (especially the lamp). Leave the PC on at all times, and *be sure to restart the Norton Antivirus services*.

We ask that the fluorimeter be left in the same condition after each use. Remove polarizers if you used them, and return all the slits to 2 nm.

*Be sure to record how long the lamp was on!*