STANDARD OPERATING PROCEDURE:

HORIBA SPECTROFLUOROMETER_STEADY STATE

Purpose of this Instrument: Essential tool for characterizing the relationship between absorbed and emitted photos at specified wavelengths.
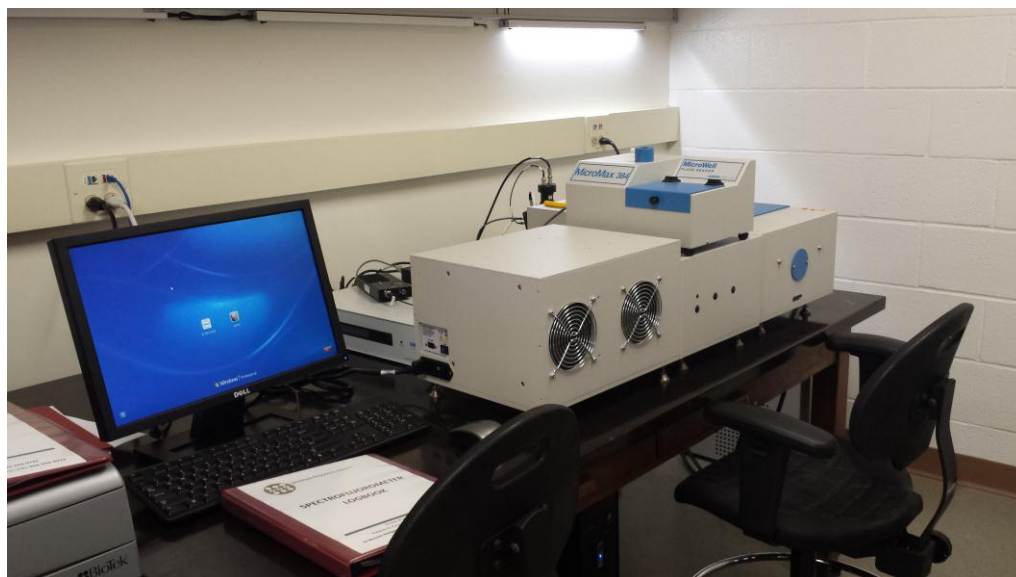

Figure 1. Horiba Fluorolog-3 spectrofluorometer in 381 CRL

Location: WVU – Chemistry Research Laboratory Building – Room: 381

Primary Staff Contact: Dr. Huiyuan Li   (304) 293-0747

The Shared Research Facilities are operated for the benefit of all researchers. If you encounter any problems with this piece of equipment, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.

1) INITIAL CHECK

This text is a guide to operating the Horiba spectrofluorometer. A more detailed and thorough account of these procedures can be found in the Horiba Fluorolog-3 spectrofluorometer Operation Manual.

1. Log in your "BNRF CRL Spectrofluorometer" session on the CORES. (https://sso.wvu.edu/cas/login?service=https://cores-wvu.mis.vanderbilt.edu/login.cfm)

2. Check log sheet to see the notes of previous users. This will inform you about anything unusual that occurred before.

3. Write down your name, date, start/end time, sample type and experiment type information on the log sheet.

> *IMPORTANT: Please log in/out CORES correctly. We need record the exact Xenon lamp usage time, because the Xenon lamp only has 1800-2000 hour lifetime.*
>
> *IMPORTANT: This instrument is used in conjunction with ultraviolet light. Exposure to these radiations, even reflected or diffused, can result in serious, and sometimes irreversible, eye and skin injuries.*
>
> *Overexposure to ultraviolet rays threatens human health by causing:*
>
> *• Immediate painful sunburn*
>
> *• Skin cancer*
>
> *• Eye damage*
>
> *• Immune-system suppression*
>
> *• Premature aging Do not aim the UV light at anyone.*
>
> *Warning: UV exposures are not immediately felt. The user may not realize the hazard until it is too late and the damage is done.*
>
> **Do not look directly into the light.**
>
> **Always wear protective goggles, skin protection clothing and gloves when using the light source**

2) START-UP

1. Start the lamp. The lamp must be turned on prior to the Fluorolog-3 accessories, and peripheral equipment. Turn on the **POWER** and wait the fan to start. After 5 min, turn on the **MAIN LAMP** switch.
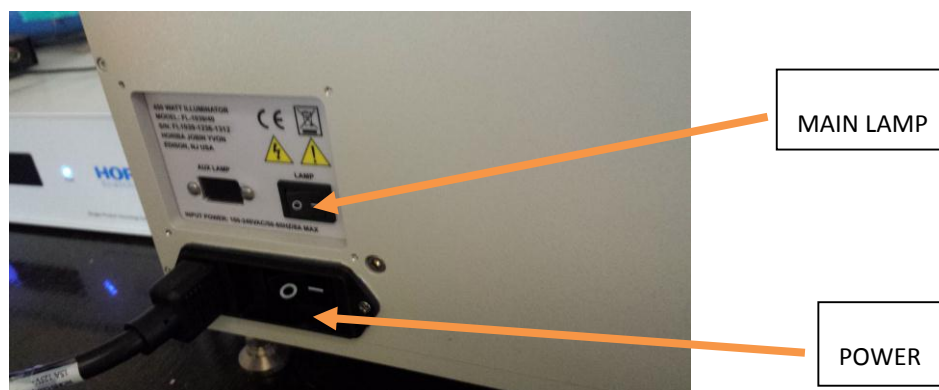
Figure 2. Power switch and main lamp switch

IMPORTANT: *Warning: To avoid explosion from lamp failure, do not allow xenon lamp to exceed rated lifetime. One clue to imminent failure may be extremely low water-Raman intensity. Please note the hour the xenon lamp has been used.*

2. Turn on the single photocounting controller.

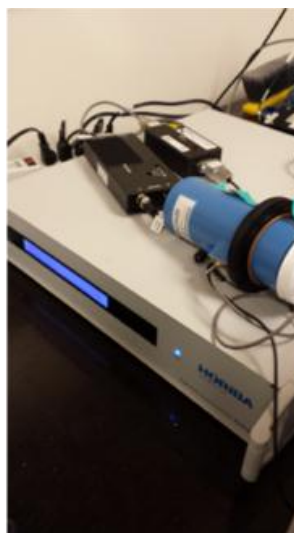*Figure 3. Turn on the single photocounting controller from the back panel.*

3. Start the fluorometer accessories. If you need temperature control, or plate reader, you can turn on the relative accessories.

4. Start up the computer. Double click on the FluorEssence icon on desktop.

5. The instrument initialized, then the fluorEssence window appears.
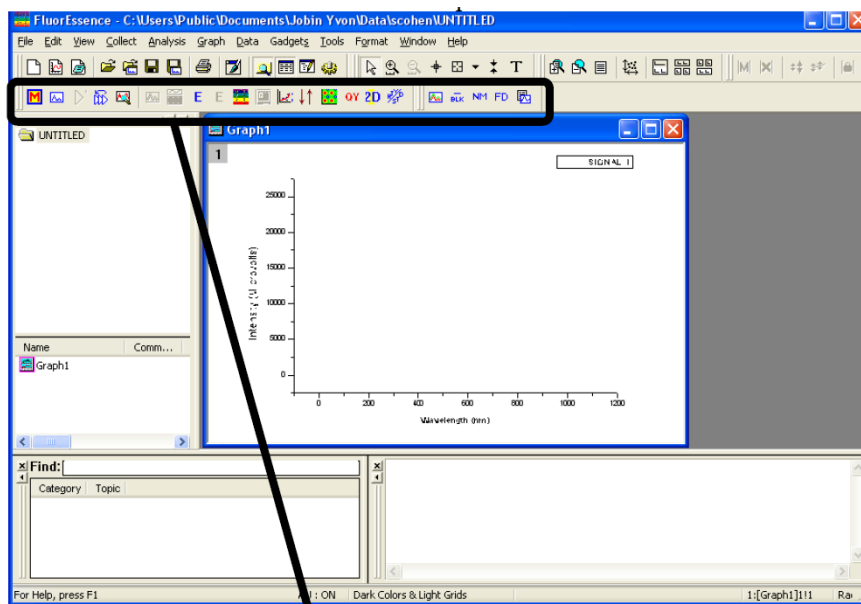
Figure 4. FluorEssence window

3) CALIBRATION

Excitation calibration
1. Fill 2 mL MilliQ water in cuvette and place it in sample compartment.
2. Close the sample compartment's lid.
3. Start FluorEssence.

4. In the main FluorEssence window, choose the experiment menu button .
5. Choose the spectra button.
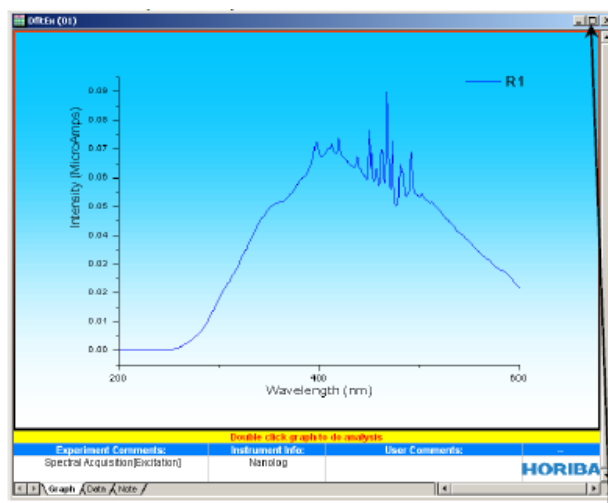6. Choose excitation, then click the Next >> button.
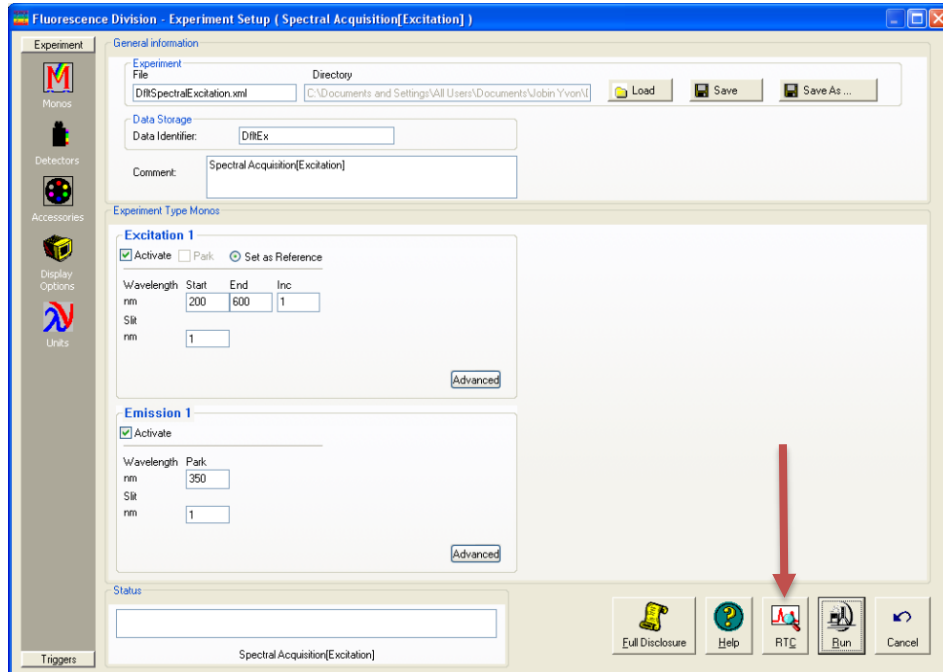7. Click the Run button.

Figure 5. The water exciation spectrum

8. Double click on the graph to un-embed it from the workbook.
9. Expand the plot by clicking the expand button.

10. Click the cursor button to start the cursor function .
11. Click on the graph near the peak, to place the cursor on the graph.
12. Using the left and right arrows on the keyboard, move the cursor to the top of the peak.
13. Read the x-value of the plot: this is the wavelength of the peak. The main peak ought to be at 467 nm. If it is not, calibrate it.
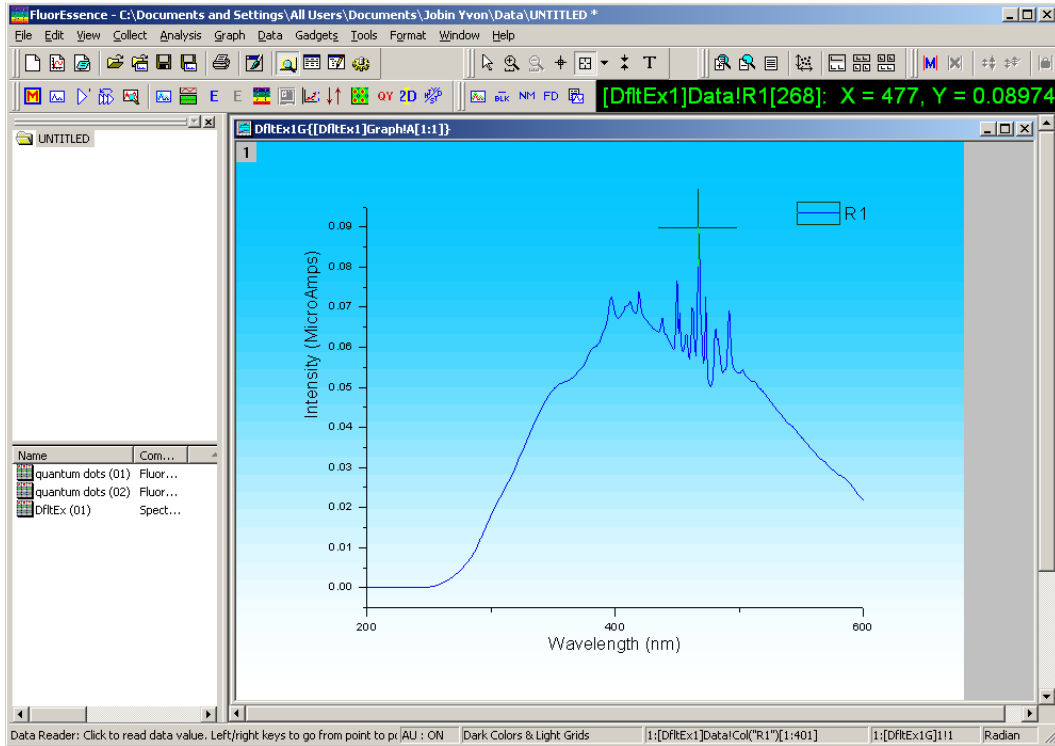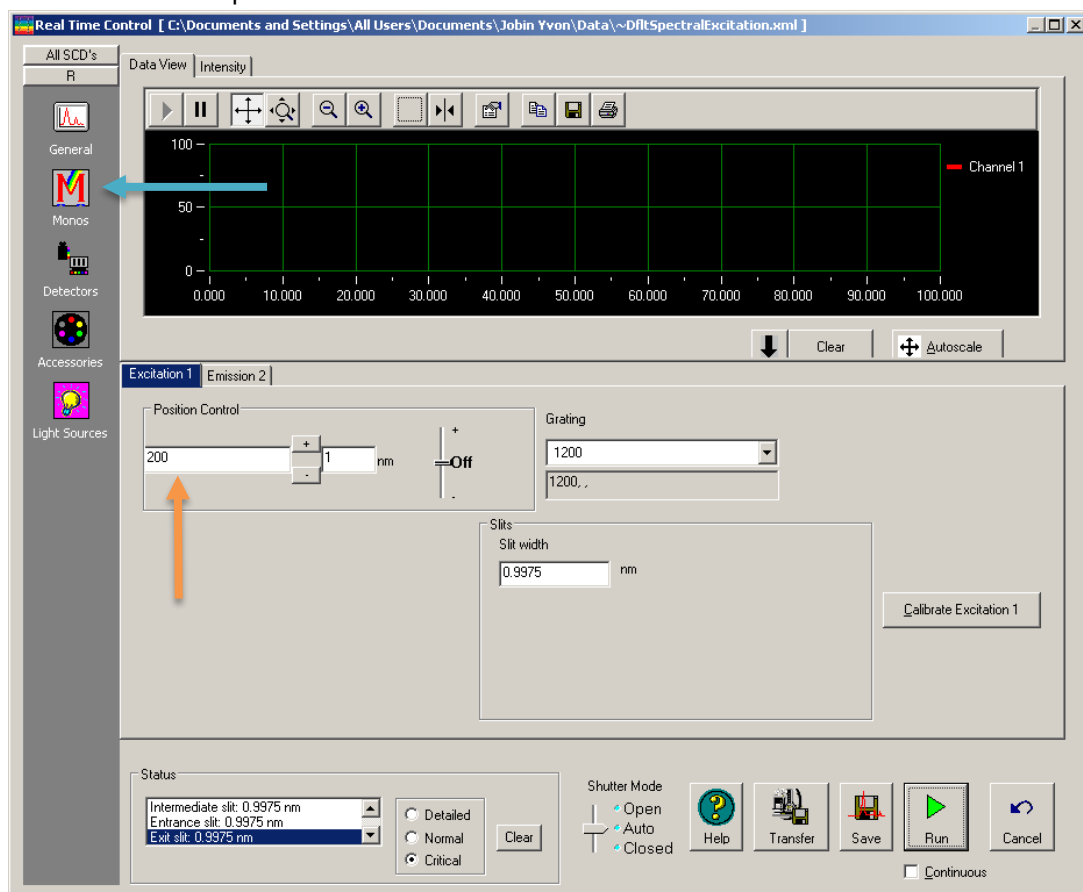
Calibration

1. Click the previous experiment button .
2. Click the RTC button.

3. The RTC window opens. Click the Monos icon in the left column ![Monos] .

4. Enter the current observed position of the peak in the position control field.
5. Click the calibrate excitation 1 button. The calibration window opens.
6. In the peak of interest field, enter the actual or expected position of the peak (it ought to be 467 nm), then click the OK button.
7. At the bottom right of the real time control window, click the Cancel button.
8. In the experiment window, click RUN again to confirm the correct peak position.

Emission calibration

1. In the main FluorEssence toolbar, choose the experiment Menu button. ![M]
2. Choose Spectra.
3. Choose Emission, then click Next.
4. Use the default parameters.
5. Click RUN.

6. If the water-Raman peak is not 397 nm, calibrate the emission monchromator as shown excitation calibration describe above.

3) START YOUR EXPERIMENT

1. Click , a main experiment menu will show.
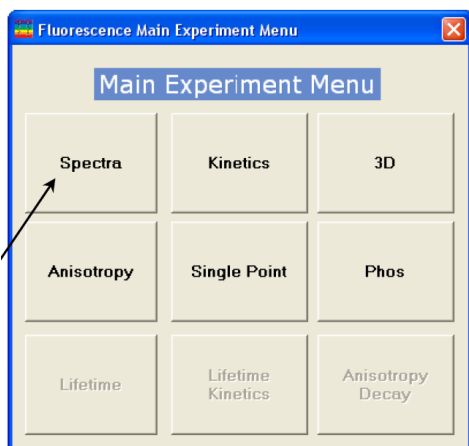
2. Select the experiment type you wish to use.

4) EXAMPLE : SPECTRA

1. Click on Spectra.

2. Select an experiment type form the menu, for example, excitation.

3. Click the Next >> button.

4. Set up parameter. Click the experiment file field and enter a new file name, or select a previously saved file with the load button.

5. Check the Detector from the left column. "Enable" check S1 and disable A1. (S1, sample; R1, reference; A1, near infrared, which is not installed.)

6. Enable correction for S1 and R1.

7. Insert the sample into the sample compartment and close the cover.
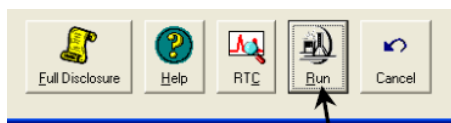
8. Click the RUN button.

9. The data collection real-time window will show.

10. Enter a name for the entire project. Then click the OK button.
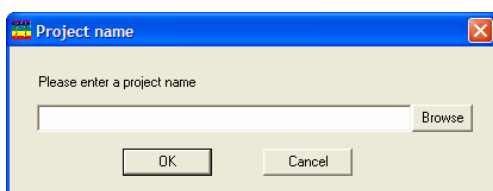
11. All data are moved to Origin's workbook window.
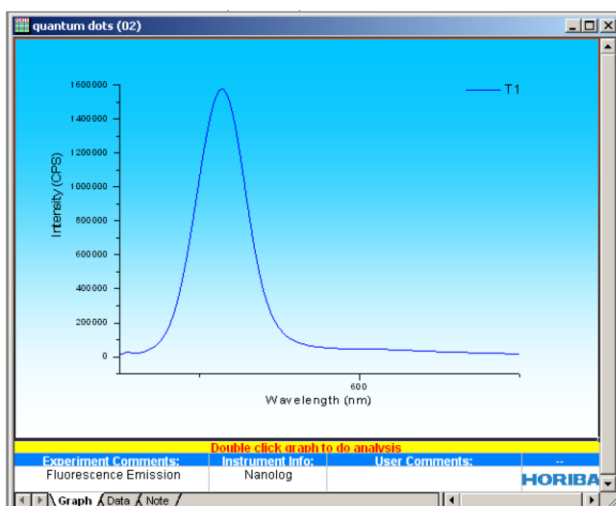
12. Do post-processing as needed, using the analysis menu in the toolbar.

5) SHUT DOWN PROCEDURE

1. Save experiment files (and data files, if created).

2. Close the FluorEssence window, using either the close button, or in the file dropdown window menu, Exit.

3. Turn off all the accessories. And then MAIN LAMP, then POWER.

4. Logoff your FOM section. And note down the instrument status in the BNRF logbook.

Video tutorials:

On the instrument control computer, find the video following the path:

Windows> all programs> Jobin Yvon > Video tutorials

Reference:

1. FluorEssence User's guide for software version 3.5.
2. Fluorolog-3 spectrofluorometer. Operation manual.

EMERGENCY OPERATING PROCEDURES

If you have any questions, even if you are just slightly unsure, ASK someone who knows and can help. There are no penalties for asking for help but there may be for not reporting damage to the equipment that may delay or prevent others from working.

If, at any time, you need to contact someone for help, call or locate the following staff of the Shared Research Facility (SRF):

Huiyuan Li   Office: 381 CRL   Phone: (304) 293-0747   Cell: (304) 906-5368

If none of the above-listed individuals is available, the user must:

- Turn the light source OFF.
- Turn the instrument OFF.

If possible, the user should stay with the spectrofluorometer while trying to contact the above individuals. If it becomes necessary to leave the instrument then the user should leave a large, legible note on both the instrument and at least one of the above individuals' offices, stating:

- The problem (describe what happened and steps taken)
- When it occurred (date and time)
- User name and phone number

If a dangerous situation is evident (smoke, fire, sparks, etc), ONLY if it is safe to do so, the user should turn OFF power to the entire spectrofluorometer and notify the proper emergency personnel. In any case, the user should leave the facility and contact emergency personnel as soon as possible from a safe place.

Huiyuan Li   Office: 381 CRL   Phone: (304) 293-0747   Cell: (304) 906-5368

Marcela Redigolo   Office: ESB G75D   Phone: (304) 293-9973   Cell: (304) 680-3007

Experiment Menu button

Choose an overall type of experiment to run, such as Phosphorescence, general Spectra (e.g., emission or excitation), or Single Point.

Previous Experiment button

Modify slightly a previously set-up experiment, and run it.

Auto Run Previous Experiment button		Run a previously set-up experiment without modification.
Run JY Batch Experiments button		Run an automated series of experiments, including adjustable repeats and delays between experiments.
Real Time Control button		Open the **Real Time Control** window directly, to adjust experimental parameters in real time.
Make Overlay File button		With an existing graph selected, create an .SPC file for use as an overlay file. The existing graph should contain a single spectrum.
Create/Use Calibration Curve from CWA Data button		From Single Point experiments, create and use a calibration curve for analytical measurements.
3D Scan to 3D Profile button		Extract excitation and emission profiles from an excitation-emission matrix. The active file must be such a data matrix.
2D Intensity Map button		Create a two-dimensional intensity map from microscope mapping data.
Show Events button		The left E button reveals hardware triggering events recorded during a kinetics scan, for example, using a hand-held pushbutton. A red line appears at each event.
Hide Events button		The right E button hides red-line-denoted hardware triggering events recorded during a kinetics scan.
Switch menu between HJY Software Application and Origin Std. button		Switches the menus at the top of the main **FluorEssence** window between FluorEssence™ and Origin® functions.
Multigroup button		Close FluorEssence™ software, and open Multigroup software.
Launch DataStation button		Close the FluorEssence™ software, and start DataStation software.

Quantum Yield calculator button	QY	Opens the quantum-yield calculator spreadsheet to calculate the quantum yield of a sample and chromaticity.
Overlay graph(s) button		Overlays one plot on top of another.
Blank Subtraction button	BLK	Automatically subtracts a blank (solvent) set of data from the sample data.
Normalize Data button	NM	Automatically normalizes data to a minimum intensity, a maximum intensity, or a user-defined constant.
View Experiment Settings button	FD	Lets you see all the parameters for the experiment in a single window.
Extract Experiment file from Data (Notes) button		Extracts the experimental parameters from Notes in a data file, and creates an experiment file from them.
Rescale Y button	↓↑	Rescales the y-axis on a graph to fit data on-scale.
Convert XYY data to Contour Plot button		Converts a table of data with multiple y-columns into a contour plot .