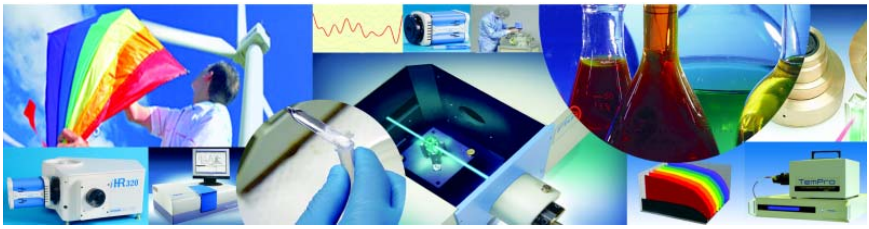
FluorEssence™

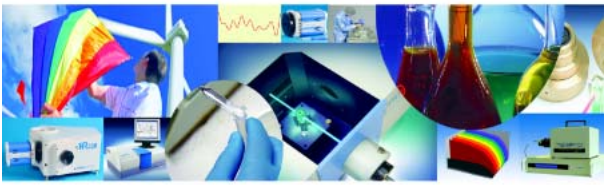


User's Guide for software version 3.0

with Multigroup software rev. 1.0

HORIBA Scientific

FluorEssence™ for Windows®



Fluorescence Multigroup Application
version 3.0.0.0

http://www.Horiba.com/Scientific

December 2008

revision 1.0

Part Number 810000

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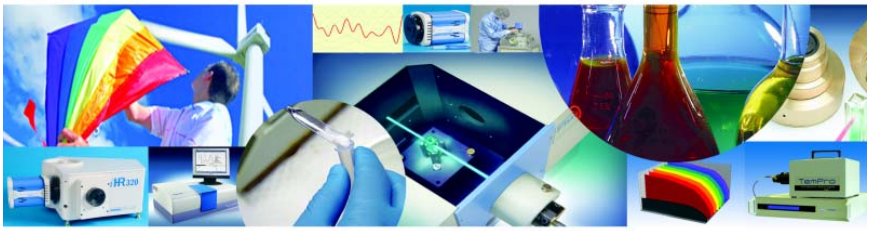
0: Introduction

HORIBA Scientific

FluorEssence™ for Windows®



Powered by ORIGIN 8 © OriginLab Corporation

About FluorEssence™

FluorEssence™ is the easiest data-acquisition software ever created by HORIBA Scientific. All aspects of spectrofluorometer control are available with only a few mouse-clicks or keystrokes, with a minimum of overlapping screens and windows. Data can be previewed while they are being recorded, and then immediately used with Origin® presentation and graphical analysis. FluorEssence™ runs using Windows® 2000 or XP Pro.

About Multigroup

Multigroup is a special data-acquistion software in which multiple steps can be automated. Repeating loops, delays, with multiple-wavelength acquisition are possible. Multigroup runs using Windows® 2000 or XP Pro.

Note: *Keep this and the other reference manuals near the system.*

Disclaimer

By setting-up or starting to use any HORIBA Jobin Yvon product, you are accepting the following terms: You are responsible for understanding the information contained in this document. You should not rely on this information as absolute or all-encompassing; there may be local issues (in your environment) not addressed in this document that you may need to address, and there may be issues or procedures discussed that may not apply to your situation. If you do not follow the instructions or procedures contained in this document, you are responsible for yourself and your actions and all resulting consequences. If you rely on the information contained in this document, you are responsible for:

- Adhering to safety procedures
- Following all precautions
- Referring to additional safety documentation, such as Material Safety Data Sheets (MSDS), when advised

As a condition of purchase, you agree to use safe operating proceduresin the use of all products supplied by HORIBA Jobin Yvon, including those specified in the MSDS provided with any chemicals and all warning and cautionary notices, and to use all safety devices and guards when operating equipment. You agree to indemnify and hold HORIBA Jobin Yvon harmless from any liability or obligation arising from your use or misuse of any such products, including, without limitation, to persons injured directly or indirectly in connection with your use or operation of the products. The foregoing indemnification shall in no event be deemed to have expanded HORIBA Jobin Yvon's liability for the products.

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environment. Any software manufactured by HORIBA Jobin Yvon is also under constant development and subject to change without notice.

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Symbols used in this manual

Certain symbols are used throughout the text for special conditions when operating the instruments:

General information is given concerning operation of the equipment.

1: FluorEssence™ Installation

Requirements

To successfully install FluorEssence™, your host computer needs the following:

Software

Windows® 2000, Windows® XP Pro, or Windows® Vista

Hardware

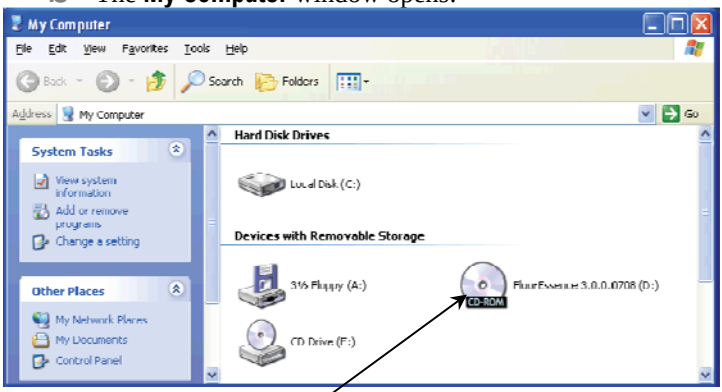
- Supports Windows® 2000, Windows® XP Pro, or Windows® Vista
- 128 MB RAM (256 MB recommended)
- 200 MB hard-disk space
- One available Ethernet Network Interface Card (NIC) connection (no hubs)
- One available USB port
- Video resolution of at least 1024 × 768

Installation instructions

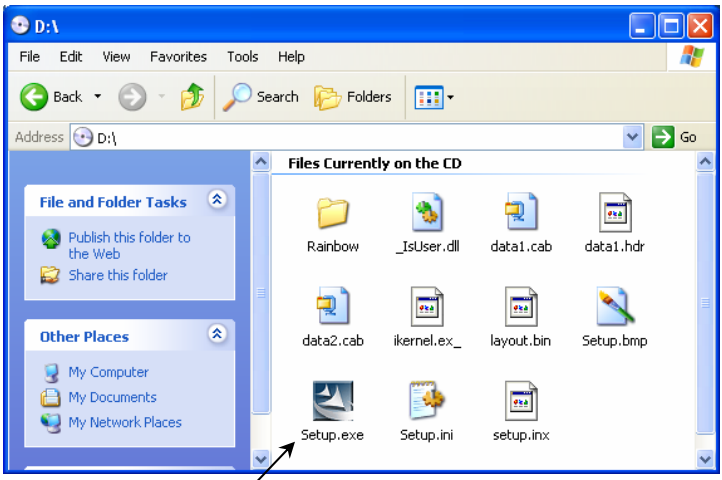
1. Remove any HORIBA USB software key (if inserted) from the host computer before starting the installation.
2. Insert the FluorEssence™ CD-ROM in the host computer's CD-ROM drive.
3. If Autorun is not operating, continue here:

   a. On the desktop, open the My Computer icon.

   b. The **My Computer** window opens:



   c. Click on the CD-ROM drive to open the FluorEssence™ CD-ROM:

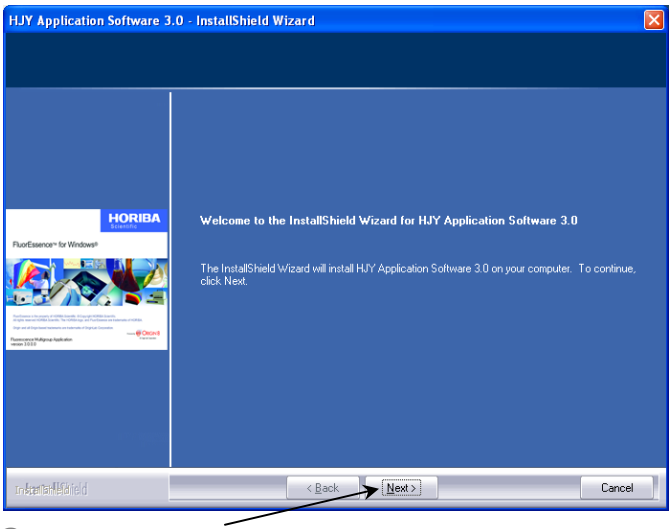


   d. Click the Setup.exe icon.

e Continue with step 4 below.

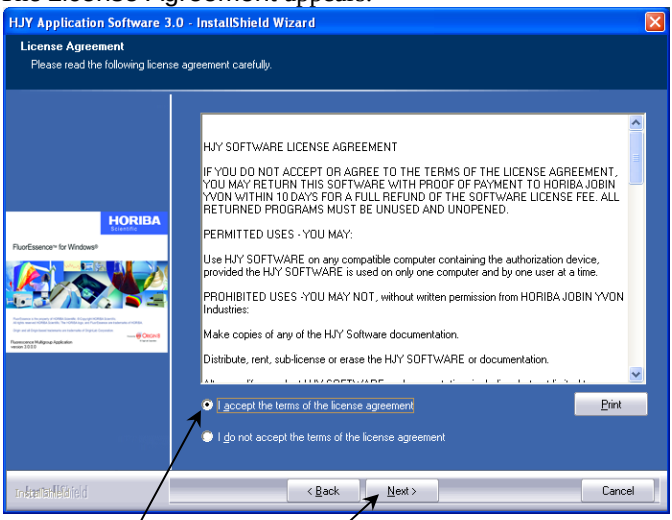
4 If Autorun is operating, continue here, to install FluorEssence™ software:

The InstallShield® Wizard starts.



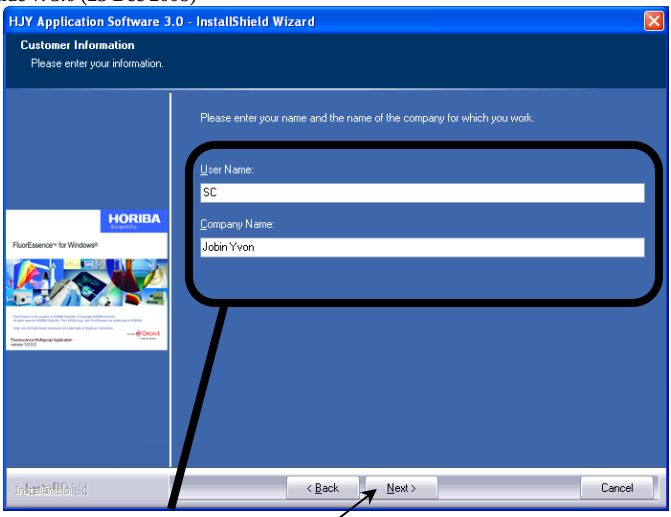
a Click the Next > button.

The License Agreement appears.



b Click I accept the terms of the license agreement radio button, then the Next > button.

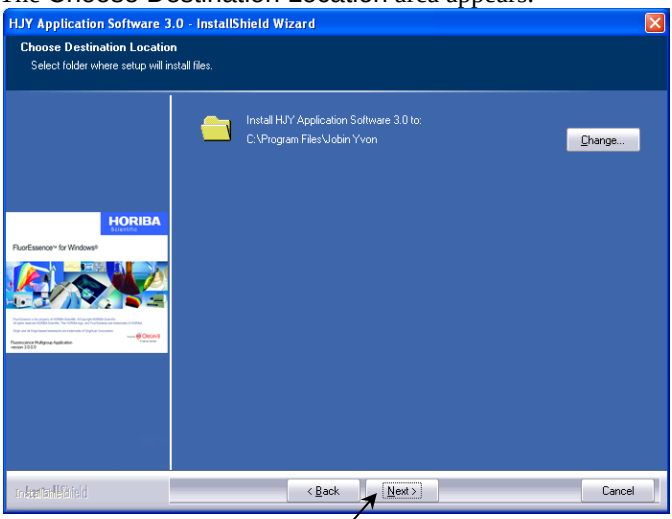
The Customer Information area appears.



Enter your User Name and Company Name. The Next > button activates.

c Click the Next > button.

The Choose Destination Location area appears.

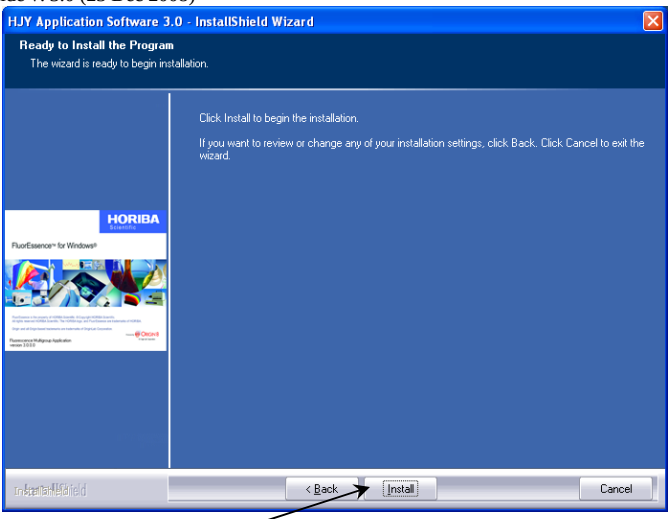


d Choose the location where FluorEssence™ is to be installed.

Most people prefer the default location. Click the Change button to find a different location.

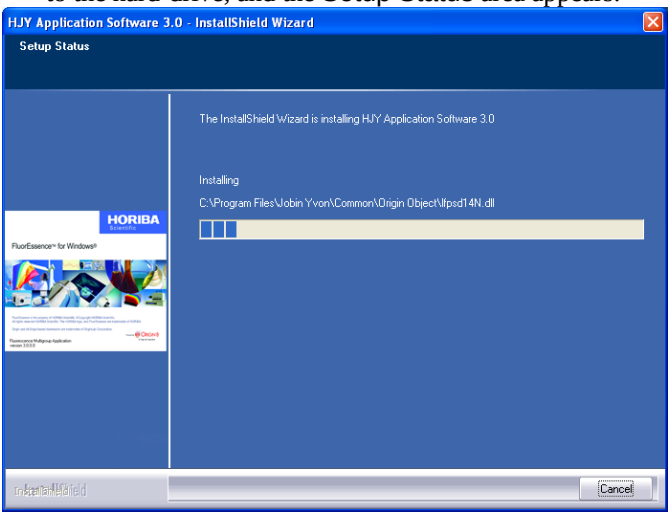
e Click the Next > button.

The Ready to Install the Program area appears:

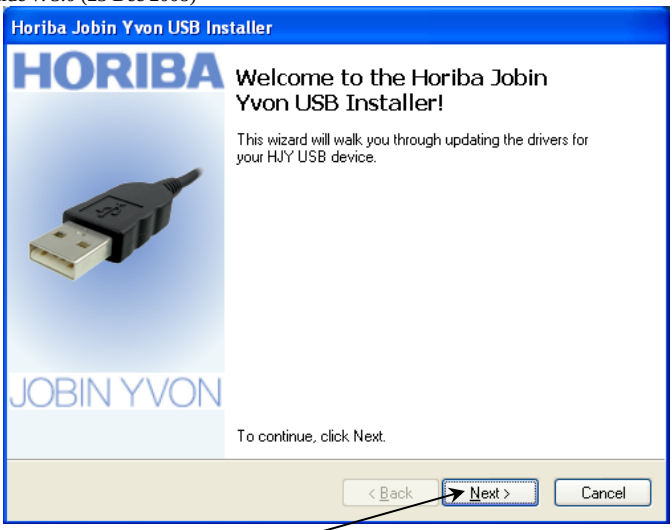


f Click the Install button.

g The computer starts copying the files from the CD-ROM to the hard-drive, and the Setup Status area appears:

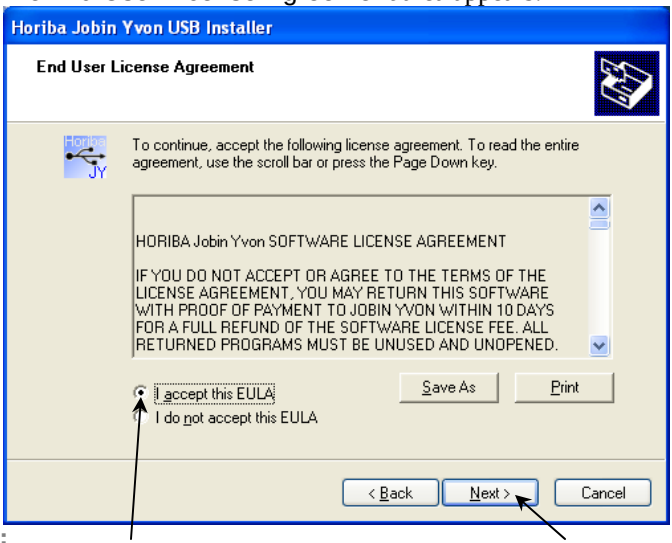


Eventually the **Horiba Jobin Yvon USB Installer** window appears:



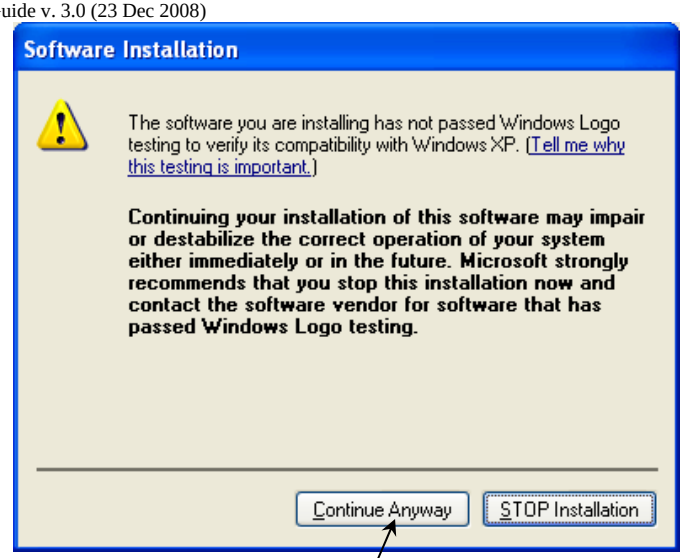
h Click the Next > button.

The End User License Agreement area appears:



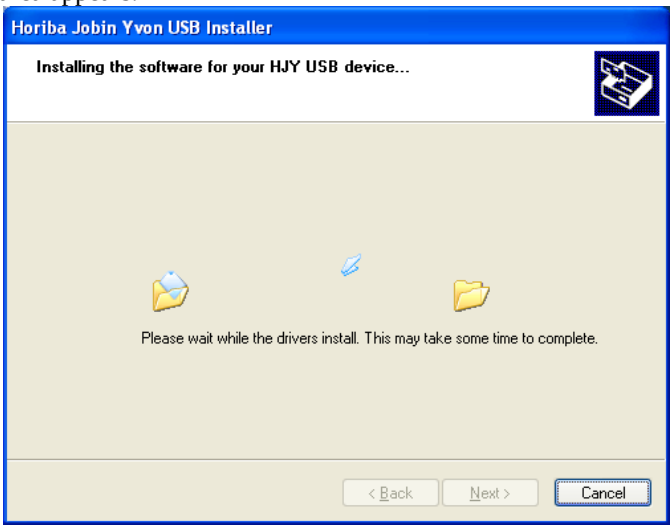
i Click the I accept this EULA radio button, then click the Next > button.

j A **Software Installation** warning window may appear:



k Click the Continue Anyway button.

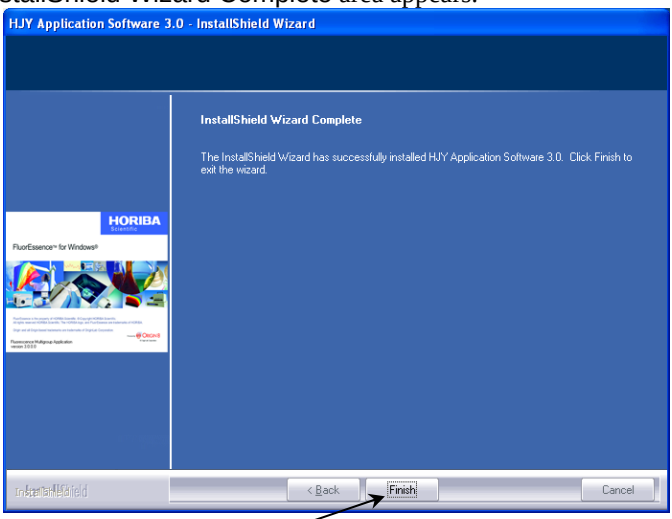
The Installing the software for your HJY USB device... area appears.



When complete, the Congratulations! You are finished installing your HJY USB device. area appears:

l Click the Finish button.

The **Horiba Jobin Yvon USB Installer** window closes. The InstallShield Wizard Complete area appears.



m Click the Finish button.

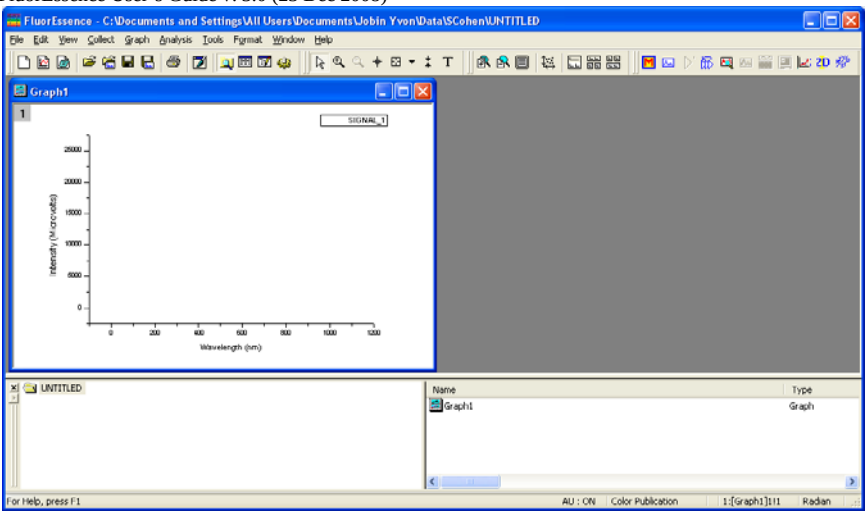
Installation of FluorEssence™ is complete.

n Plug in all HORIBA software keys. Remove the FluorEssence™ CD-ROM from the host computer.

5 Start FluorEssence™.

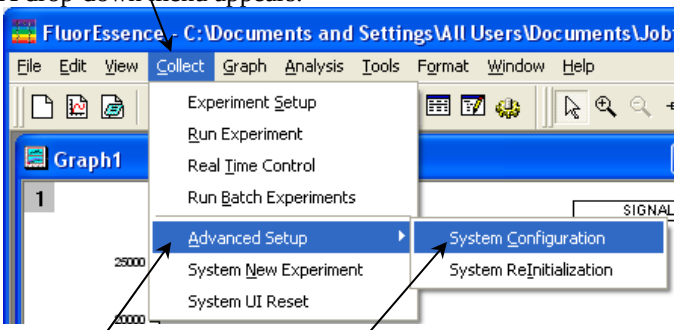
a On the desktop, double-click the FluorEssence V3 icon.

The **Fluorescence** window appears:



6 Choose a hardware configuration to run.

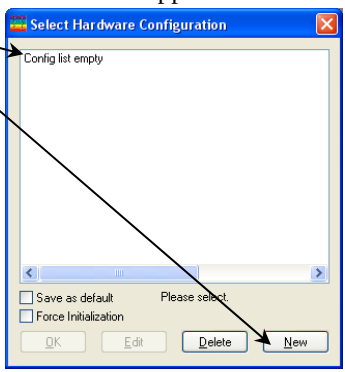
a Choose Collect.
A drop-down menu appears.

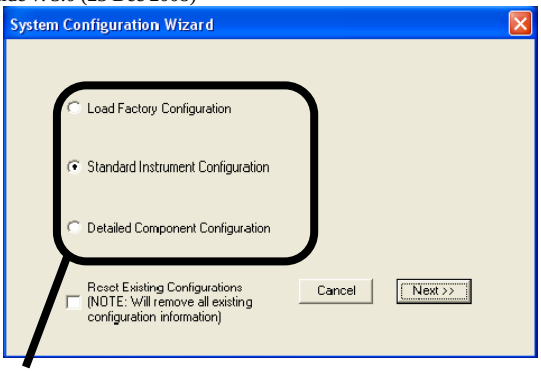


b Choose Advanced Setup.
A drop-down menu appears.

c Choose System Configuration.
The **Select Hardware Configuration** window appears.

d If Config list empty is shown, click the New button to create a new instrument configuration.
The **System Configuration Wizard** appears:





e Choose one possible hardware configuration that your system can run correctly. You may choose a radio button for:

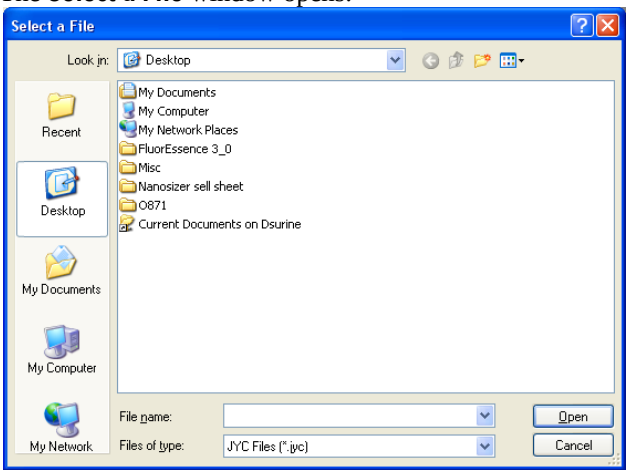
- Load Factory Configuration The exact hardware setup that HORIBA Scientific built for you.
- Standard Instrument Configuration A basic hardware configuration, for example, a typical FluoroMax®-4.
- Detailed Component Configuration Your own hardware setup in which every component can be tailored.

Note: *Most users do not choose the* Detailed Component Configuration.

f Click the Next >> button.

If you chose Load Factory Configuration:

The **Select a File** window opens.



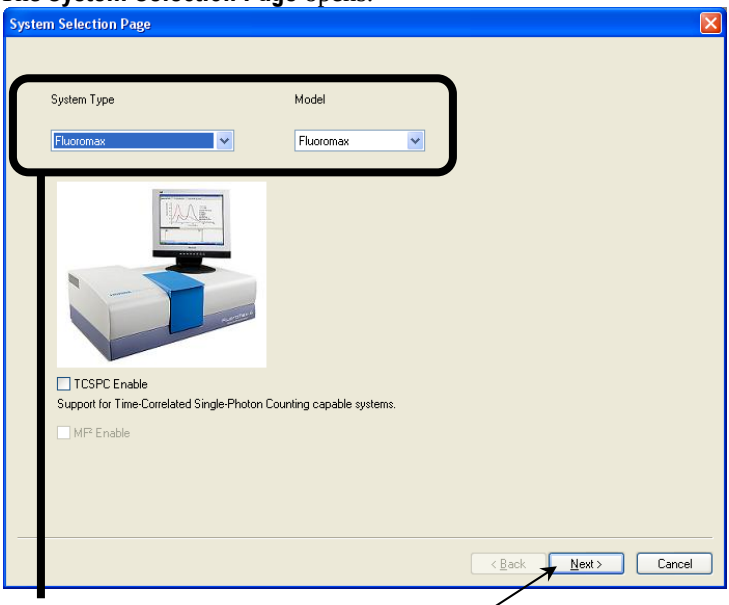
g Browse through the folders, and select the desired `.jyc` file.

The **InstallShield Wizard Complete** window opens.

h Continue with step 6 on page 21.

If you chose Standard Instrument Configuration:

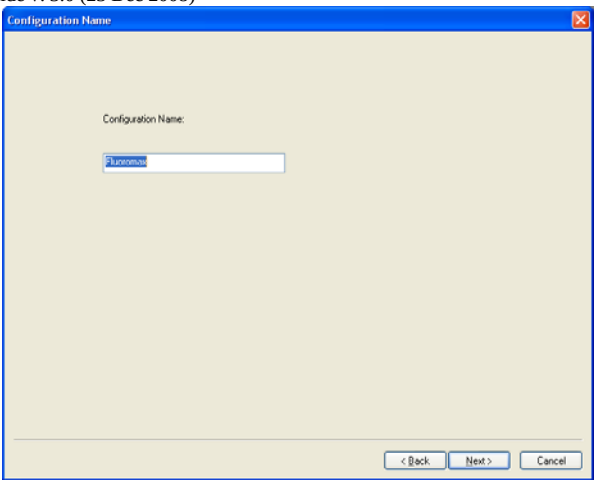
The **System Selection Page** opens.



a From the drop-down menus, choose the System Type and Model. If your particular instrument includes TCSPC, activate the TCSPC Enable checkbox.

b Click the Next > button.

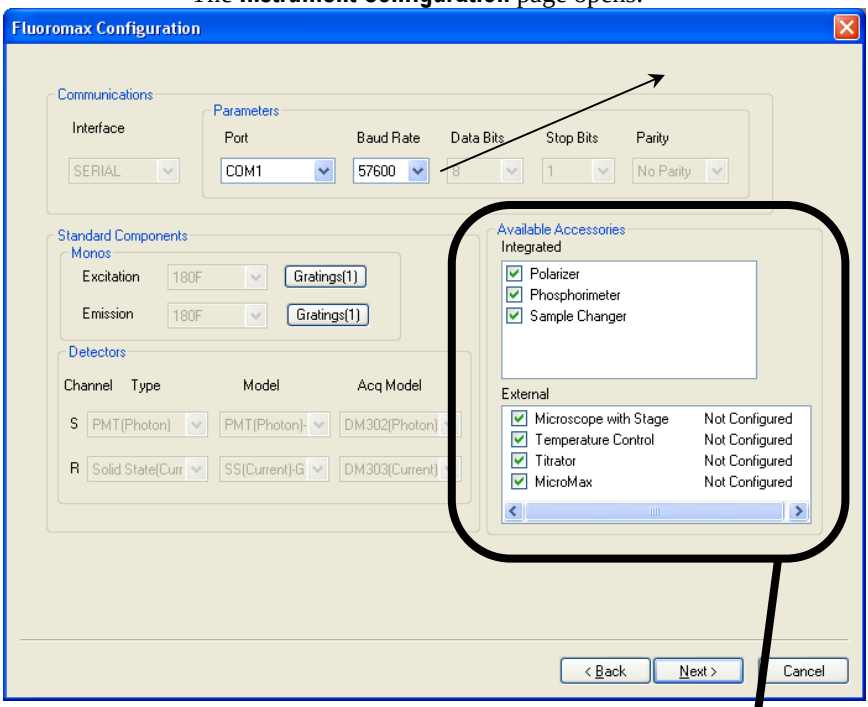
The **Configuration Name** page opens:



c Use the default name, or enter your own in the field.

d Click the Next > button.

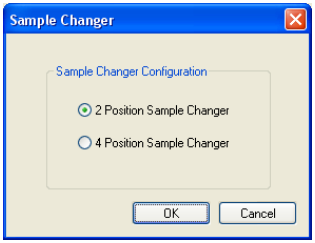
The **Instrument Configuration** page opens:



e Choose the appropriate settings, leave the defaults, or adjust as desired.

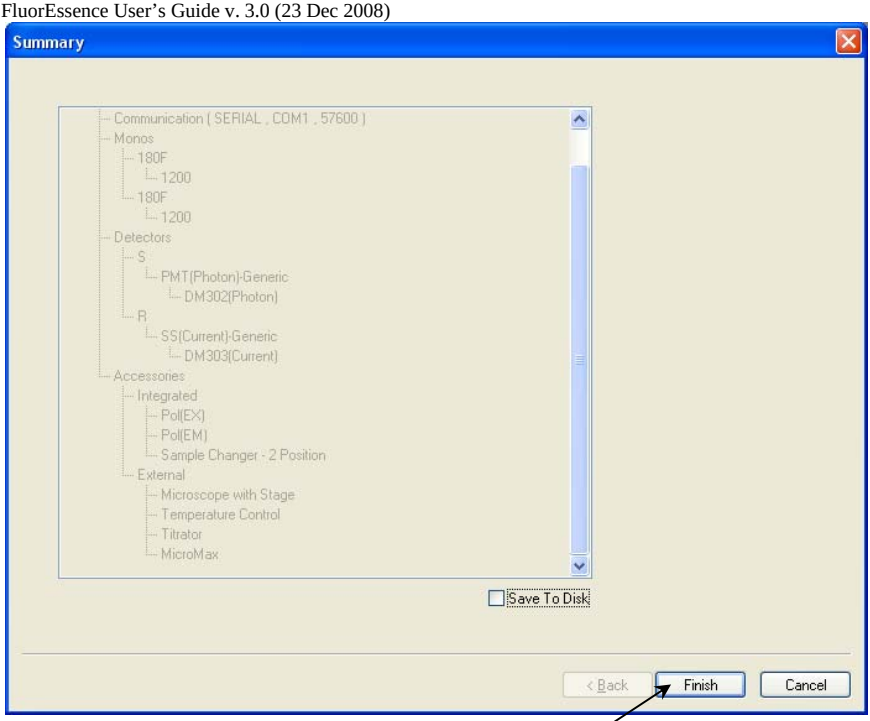
Click the checkboxes in the Available Accessories area to activate all desired and available accessories.

Note: *If you select a Sample Changer as an accessory, a window appears asking which Sample Changer: 2-position or 4-position. If you select a Temperature Controller or Microscope as an accessory, a window appears asking for its details: be sure to choose the correct Manufacturer.*



Note: *Some parameters are not available for certain systems (e.g., FluoroMax®), and thus are grayed-out automatically.*

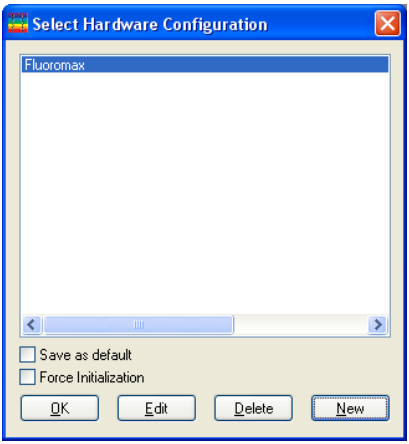
f Click the Next > button.
The **Summary** page opens.



g Examine the **Summary** page to be sure that your configuration is correct. To change the entries, click the < Back button.

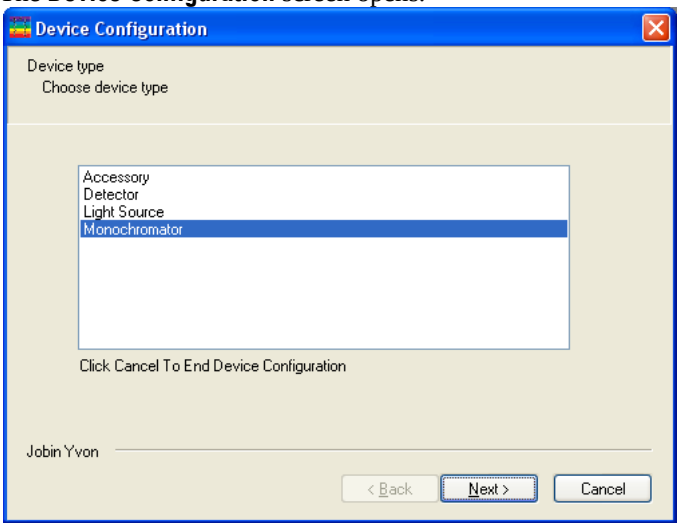
h Click the Finish button.

The **Select Hardware Configuration** window re-appears, with the newly created hardware configuration in the list.



If you chose Detailed Component Configuration:

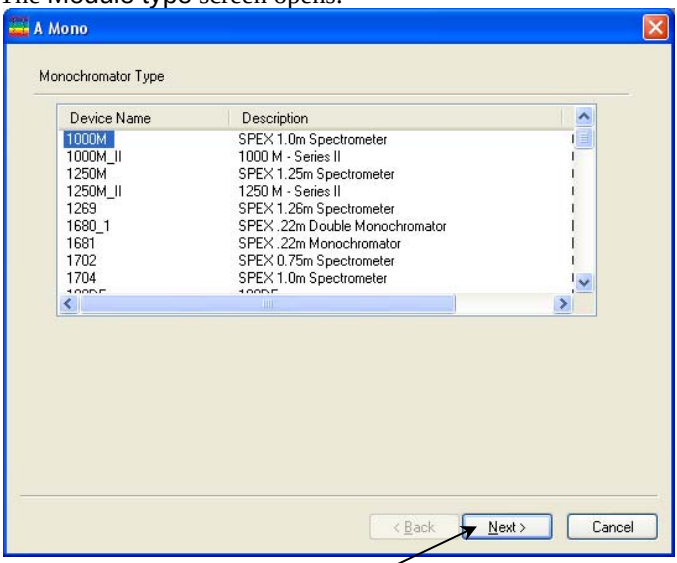
The **Device Configuration** screen opens.



a Choose a component of your instrument to add from the menu. In this case, a monochromator was selected.

b Click the Next > button.

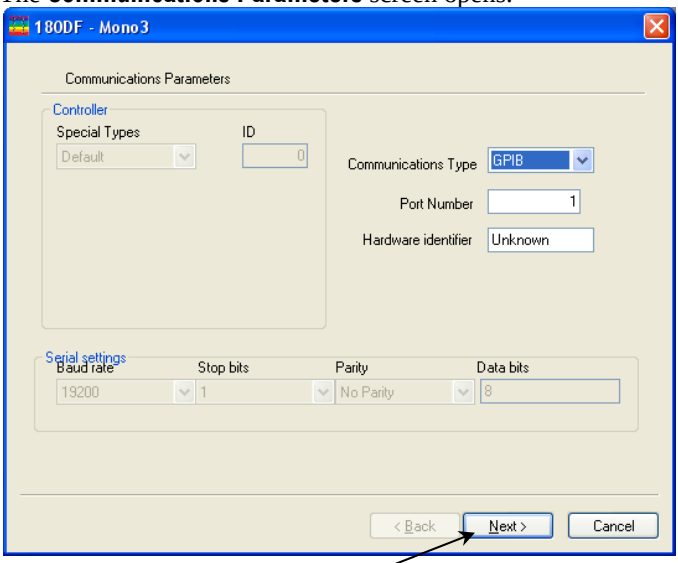
The Module type screen opens.



c Choose the particular model of the component from the menu.

d Click the Next > button.

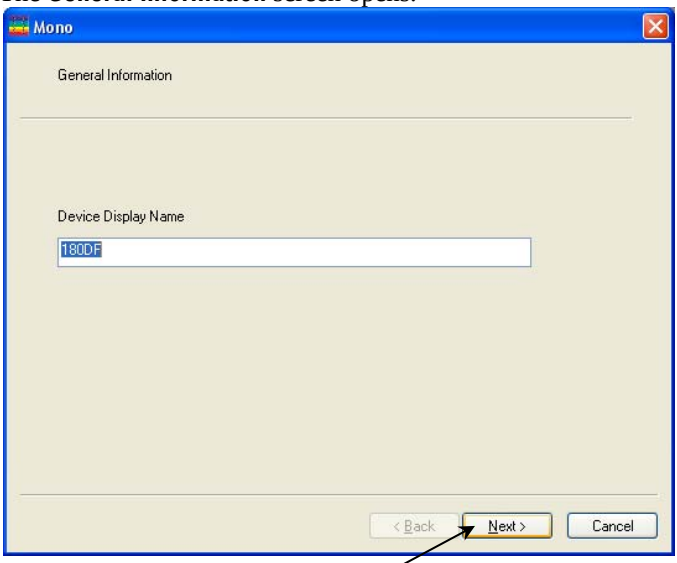
The **Communications Parameters** screen opens.



e Choose the parameters, or accept the default values.

f Click the Next > button.

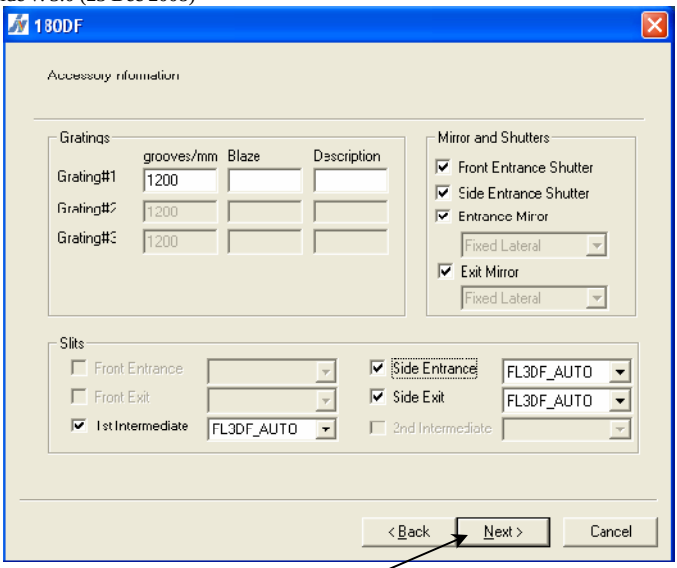
The **General information** screen opens.



g Enter a name or description for the new component, or use the default provided.

h Click the Next > button.

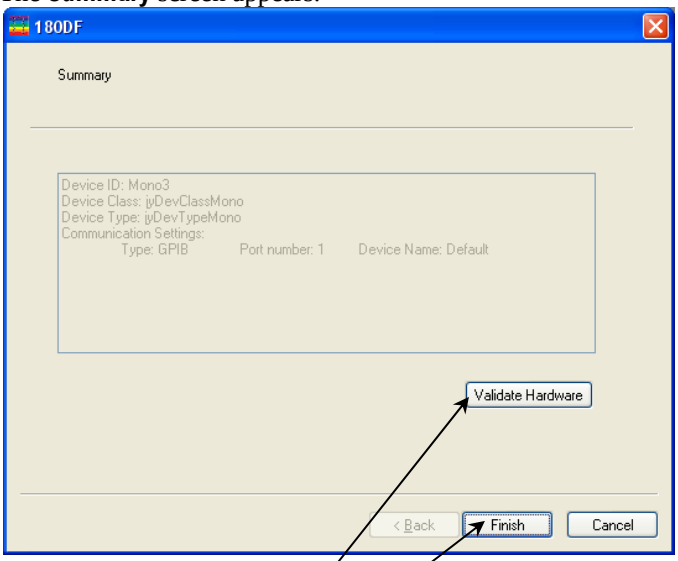
The **Accessory information** screen opens:



i Choose the parameters, or accept the default values.

j Click the Next > button.

The **Summary** screen appears.

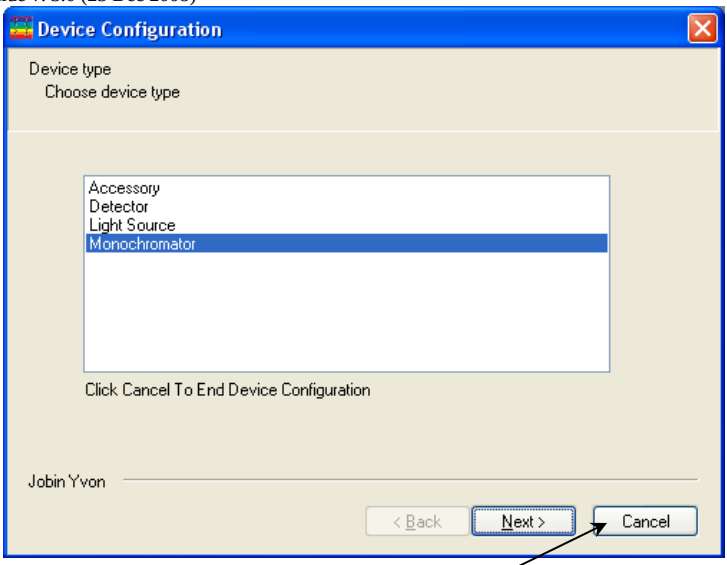


k Review for correctness.

l Click the Validate Hardware button to verify that the host computer communicates with the hardware.

m Click the Finish button.

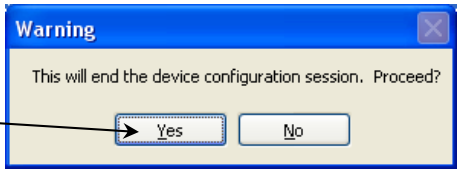
The **Device Configuration** window reappears.



n Continue to add new components until the system configuration is complete.

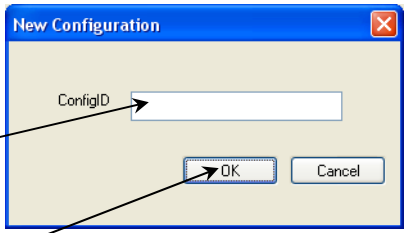
o When complete, choose the Cancel button.

A **Warning** window asks you to confirm cancellation.



p Click the Yes button to stop adding items to the configuration.

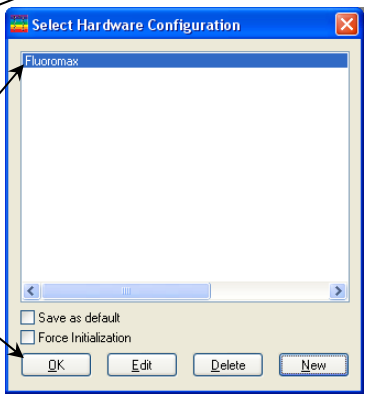
The **New Configuration** window appears.



q Enter a brief name for the hardware configuration in the ConfigID field.

r Click the OK button.

The **Select Hardware Configuration** window re-appears, with the newly created hardware configuration in the list.



7 Choose the desired hardware configuration, and click the OK button.

Loading correction-factor files

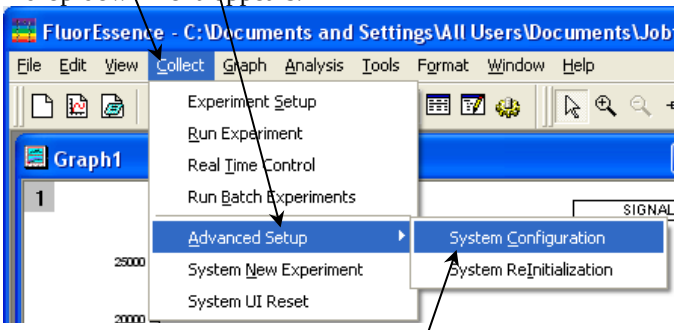
Correction-factor files adjust specific instruments for their optical responses.

1. In the main **FluorEssence** window, choose Collect.

   A drop-down menu appears.

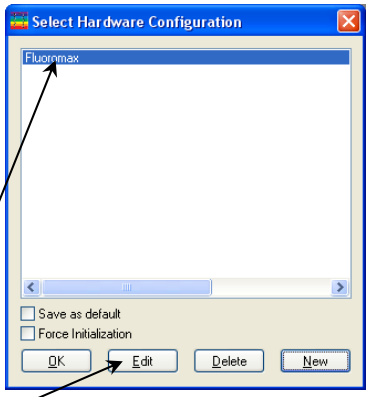
2. Choose Advanced Setup.

   A drop-down menu appears.

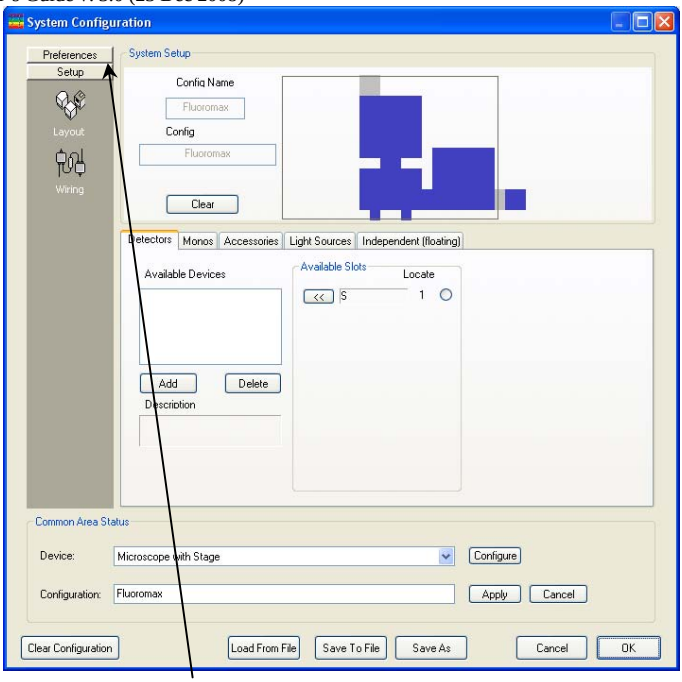


3. Choose System Configuration.

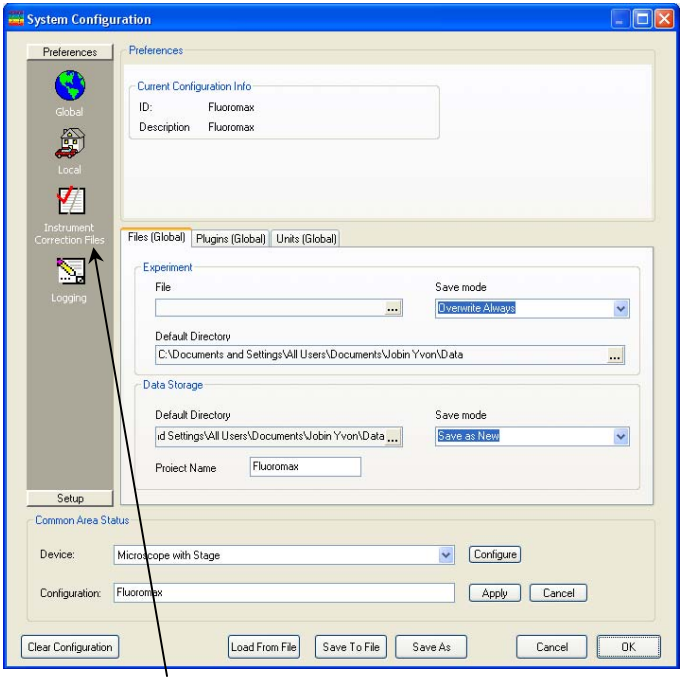
4. If there is more than one hardware configuration available, the **Select Hardware Configuration** menu appears. Choose the desired hardware configuration for the correction-factor file, then click the Edit button.



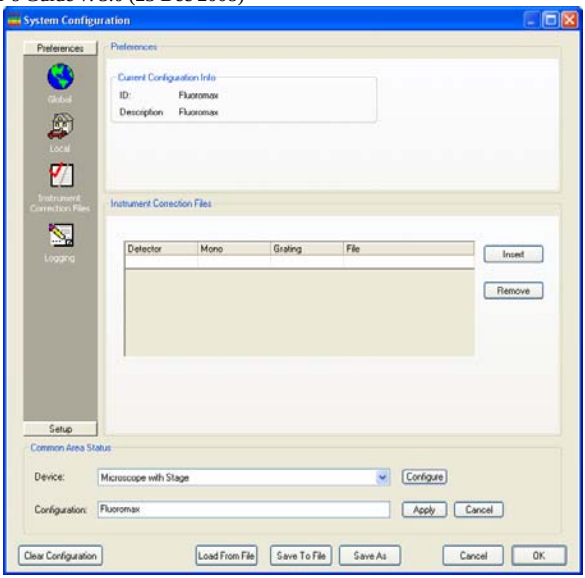
   The **System Configuration** window appears.



5 Click the Preferences button.

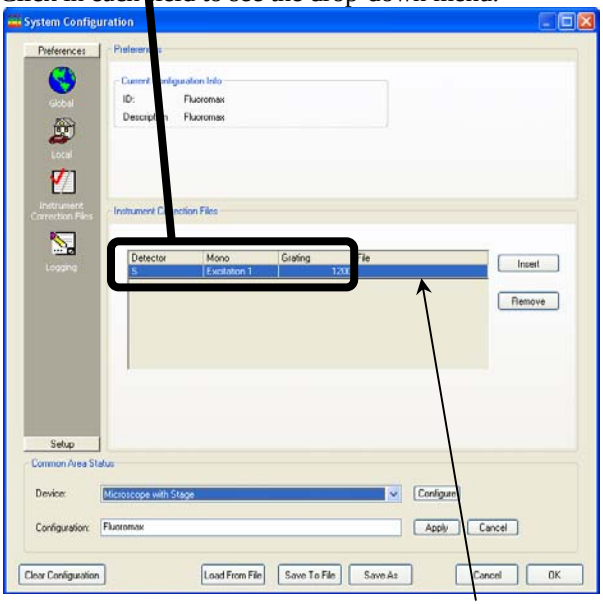


6 Click the Instrument Correction Files icon.



7 Choose the detector from its drop-down menu, the monochromator from its drop-down menu, and the grating from its drop-down menu:

Click in each field to see the drop-down menu.



8 Browse for the appropriate correction-factor file in the File field.

9 If you need an extra row in the table for additional combinations of detectors, monochromators, and gratings, click the Insert button.

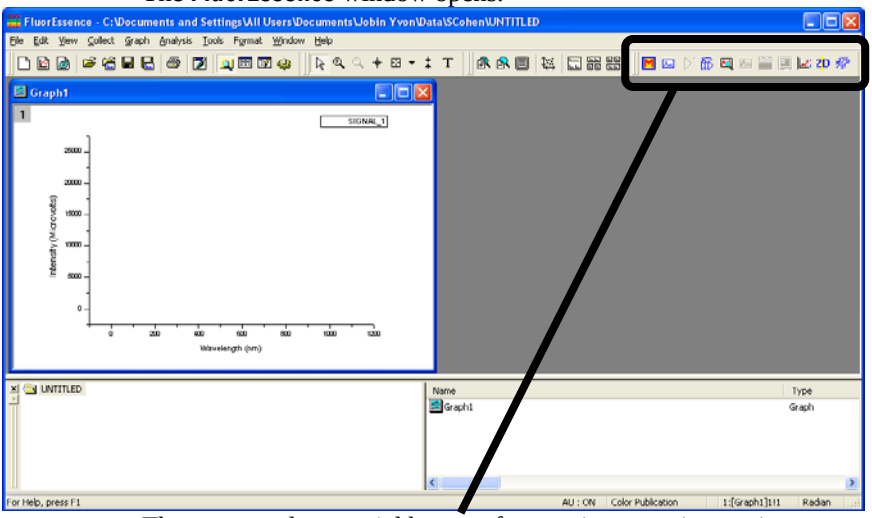
10 Click the OK button when you are finished.

Note: *You can have separate correction files for different gratings on the same monochromator.*

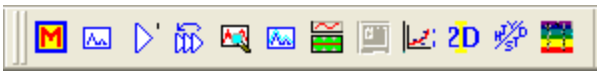
2: Quick Guide to Running a Scan

1. Turn on the host computer, and all instruments and accessories, as explained in their respective instruction manuals.
2. Click on the FluorEssence short-cut to start FluorEssence™.

The **FluorEssence** window opens.



There are twelve special buttons for running experiments in FluorEssence™:

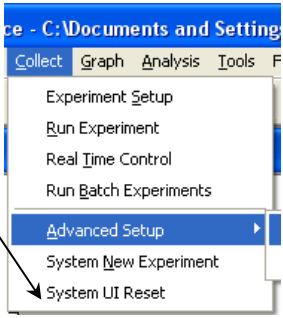


Experiment Menu button		Choose an overall type of experiment to run, such as Phosphorescence, general Spectra (e.g., emission or excitation), or Single Point.
Previous Experiment button		Modify slightly a previously set-up experiment, and run it.

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.
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.
Launch DataStation button		Close the FluorEssence™ software, and start DataStation software.
Create/Use Calibration Curve from CWA Data button		From Single Point experiments, create and use a calibration curve for analytical measurements.
2D Intensity Map button		Create a two-dimensional intensity map from microscope mapping data.
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.

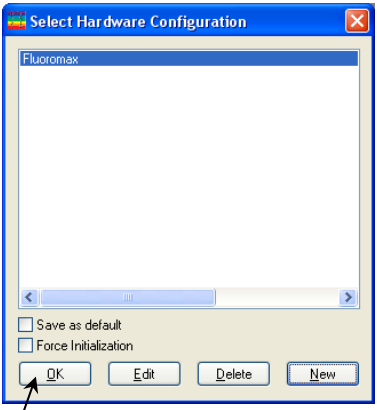
From many of these buttons, *upon initial start-up of the software*, you can choose a hardware configuration and experiment type. After a hardware configuration is loaded, each button has its own separate function.

The Collect menu near the top of the main window also has these functions, plus another important command, System UI Reset. In case the six special buttons are grayed out, choose the System UI Reset command. See *Chapter 7* for more details.



3 Click the Experiment Menu button.

The **Select Hardware Configuration** window opens. To force the appearance of the **Select Hardware Configuration** window:



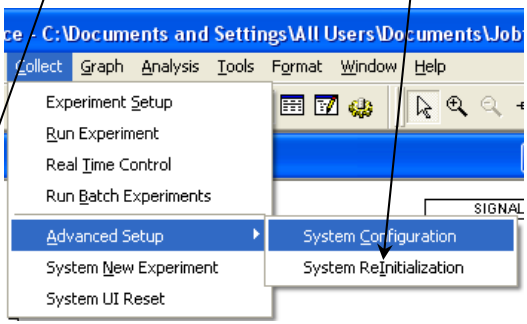
a Immediately upon opening FluorEssence™, press the F8 key and simultaneously click the Experiment Menu button  ,

Or

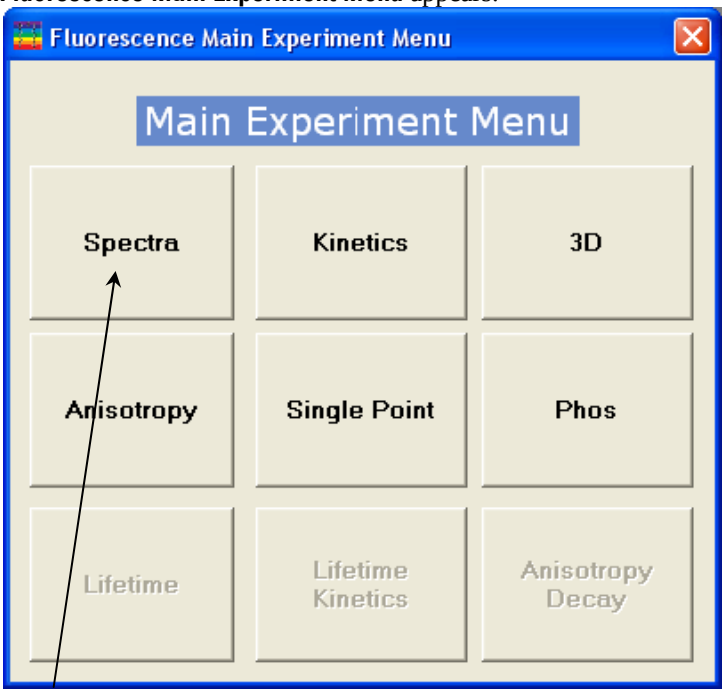
b At any time within FluorEssence™, press the F8 key while choosing the Collect Menu / Advanced Setup / System ReInitialization.

Note: *This window does not appear if you have only one hardware configuration installed. Skip to page 33.*

4 Choose a system configuration from the list, then click the OK button.



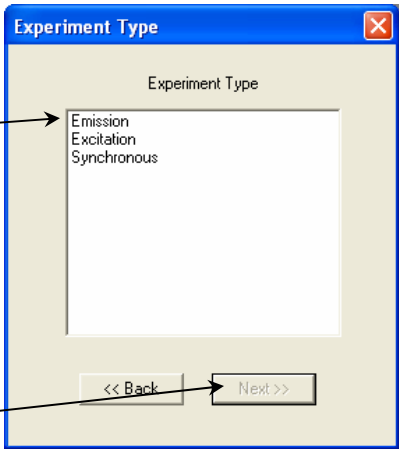
FluorEssence™ loads the chosen system configuration. The **Fluorescence Main Experiment Menu** appears:



5 Click an available scan-type button, say, Spectra.

The **Experiment Type** window appears.

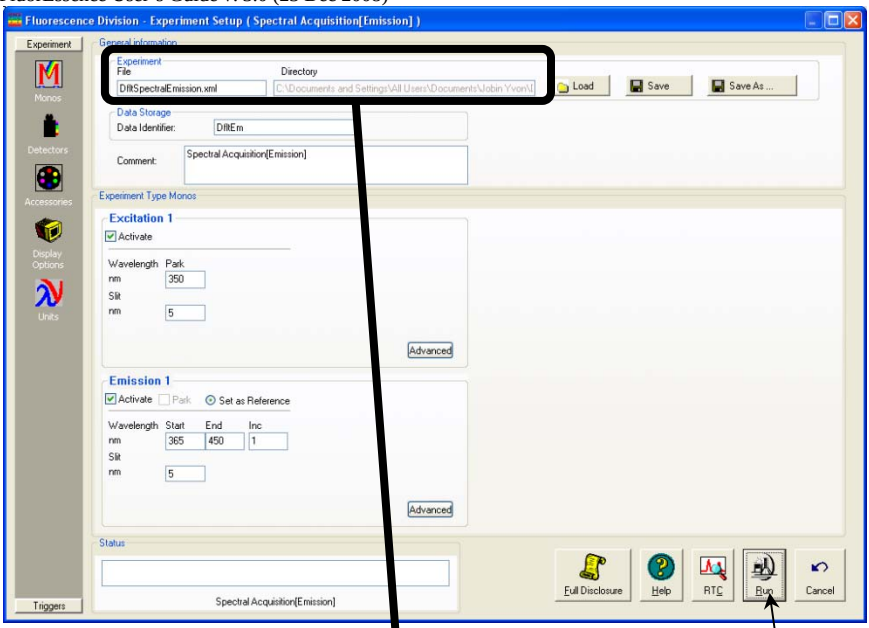
6 Choose an Experiment Type from the menu, say, Emission.



The parameters required for an emission scan are loaded into the **Experiment Setup** window, which appears. For an emission scan, the default parameters are a water-Raman scan.

7 Click the Next >> button.

The **Experiment Setup** window opens:



8 Click the Experiment File field, and enter a new file name, or select a previously saved file with the Load button.

9 Verify that the experimental parameters are correct.

Be certain to check all parameters under all icons in the lefthand column.

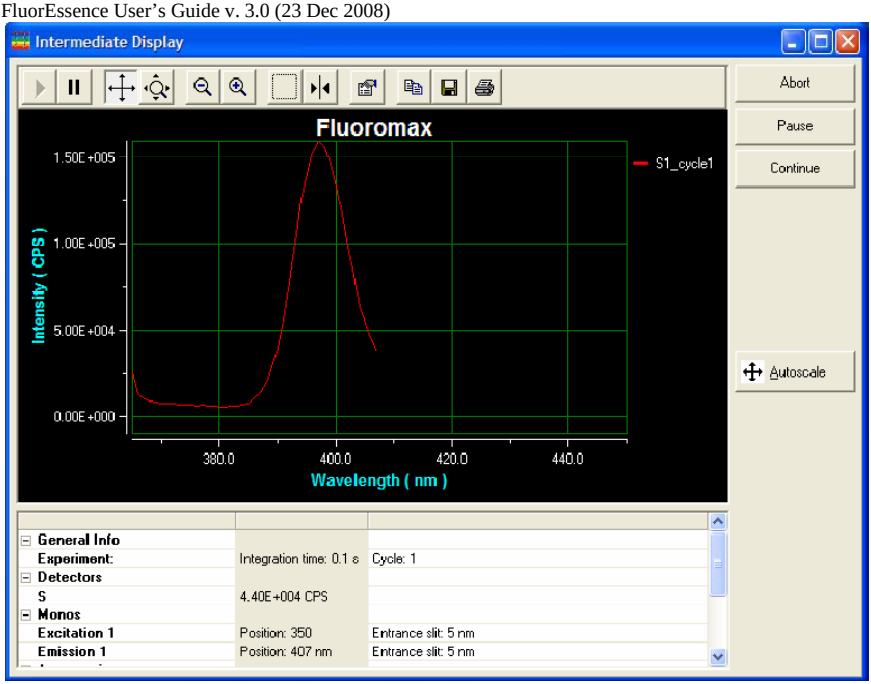
10 Insert the sample into the sample compartment, and close the cover of the sample compartment.

11 Click the Run button 

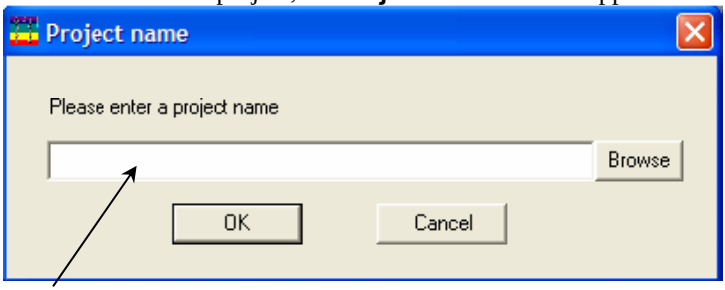
.

The collected spectrum is displayed on the **Intermediate Display** screen:

Note: *If the scan is extremely fast, the* ***Intermediate Display*** *may be only incompletely or rapidly displayed before the* ***Origin*** *window appears.*

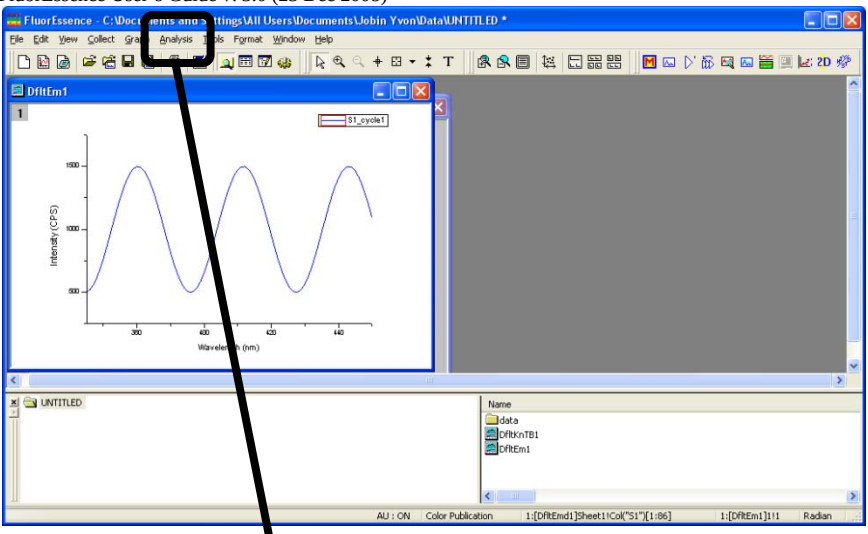


You can watch the incoming data in real time, along with how the positions of accessories vary. The scan may be paused, continued, or aborted. After all data are recorded, the **Intermediate Display** vanishes. For a new project, the **Project name** window appears:



12 Enter a name for the entire project, or browse for an existing project name with the Browse button, then click the OK button.

All data are moved to Origin®'s graph window:



13 Do post-processing as needed, using the Analysis menu.

3: FluorEssence™ Tips & Tricks

Calibration of your instrument

Excitation calibration

Monochromator parameters for the xenon-lamp scan:

Monochromator (1200 grooves/mm)	Initial wavelength	Final wavelength	Increment	Slits (bandpass)
Excitation	200 nm	600 nm	1 nm	1 nm
Emission	350 nm	--	--	1 nm

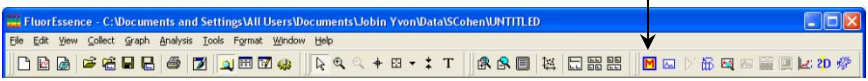
Detector parameters for the xenon-lamp scan:

Detector (Signal)	Integration time	Units
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA

1 Close the sample compartment's lid.

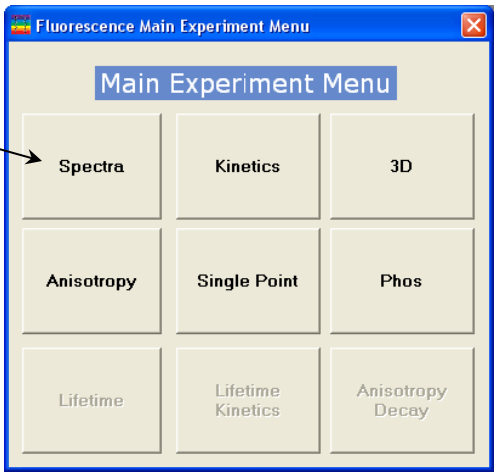
2 Start FluorEssence™.

3 In the main **FluorEssence** window, choose the Experiment Menu button  .



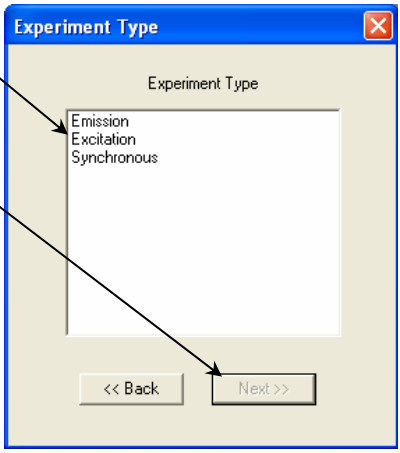
The **Fluorescence Main Experiment Menu** appears.

4 Choose the Spectra button.

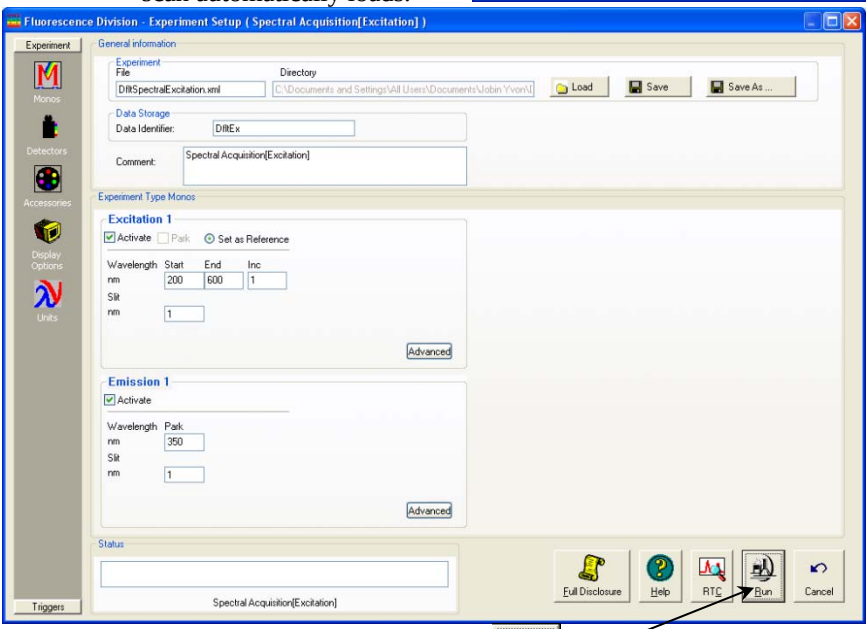


The **Experiment Type** window appears.

5 Choose Excitation, then click the Next >> button.



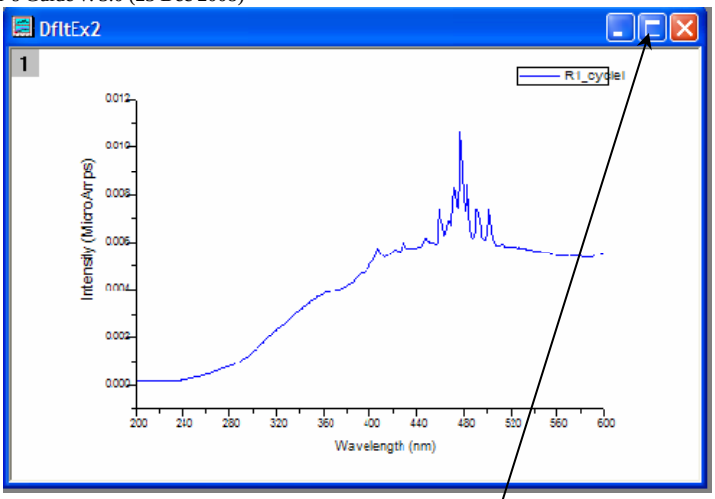
The **Experiment Setup** window for the xenon-lamp scan automatically loads.



6 Click the Run button 

.

The **Intermediate Display** opens. The xenon-lamp scan runs:

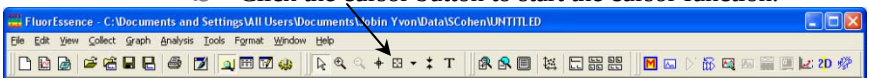


Above is an uncalibrated FluoroMax® lamp-scan. The main peak ought to be at 467 nm, but here appears near 480 nm.

7 Calibrate the excitation monochromator, if required.

a Expand the plot by clicking the Expand button.

b Click the cursor button to start the cursor function.

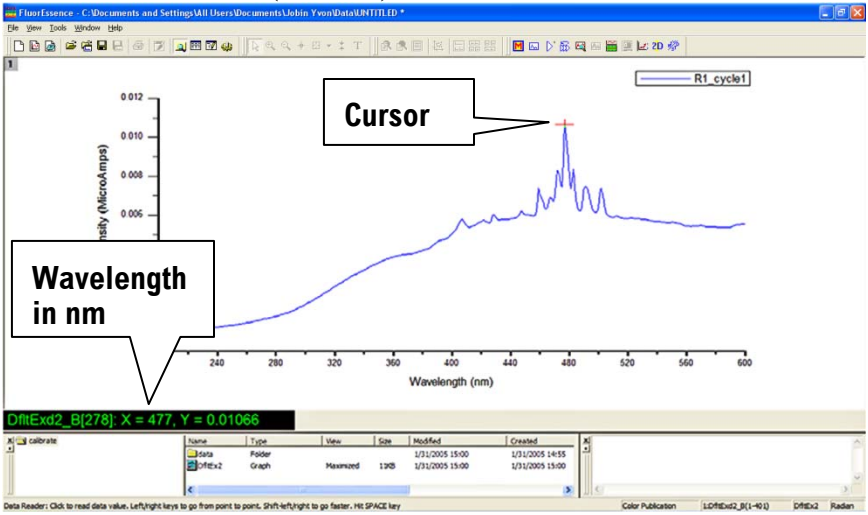


c Click on the graph near the peak, to place the cursor on the graph.

d Using the left and right arrows on the keyboard, move the cursor to the top of the peak.

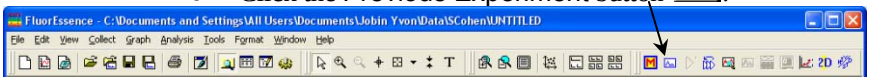
e Read the *x*-value of the plot: this is the wavelength of the peak:

Note: *Your lamp scan may appear different, depending on the instrument and its configuration.*

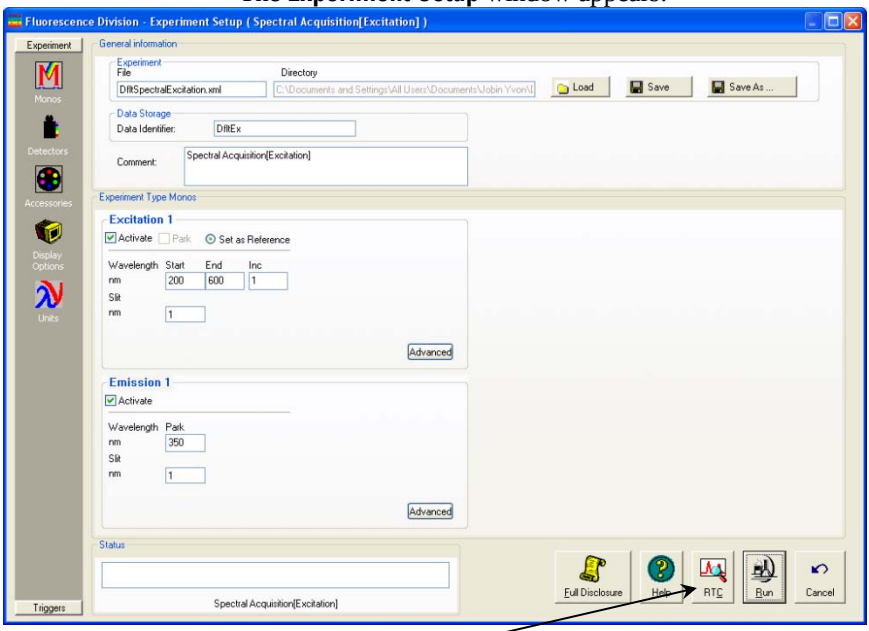


This example shows the peak at 477 nm, which is 10 nm too high. **Therefore we must calibrate the monochromator.**

f Click the Previous Experiment button  .

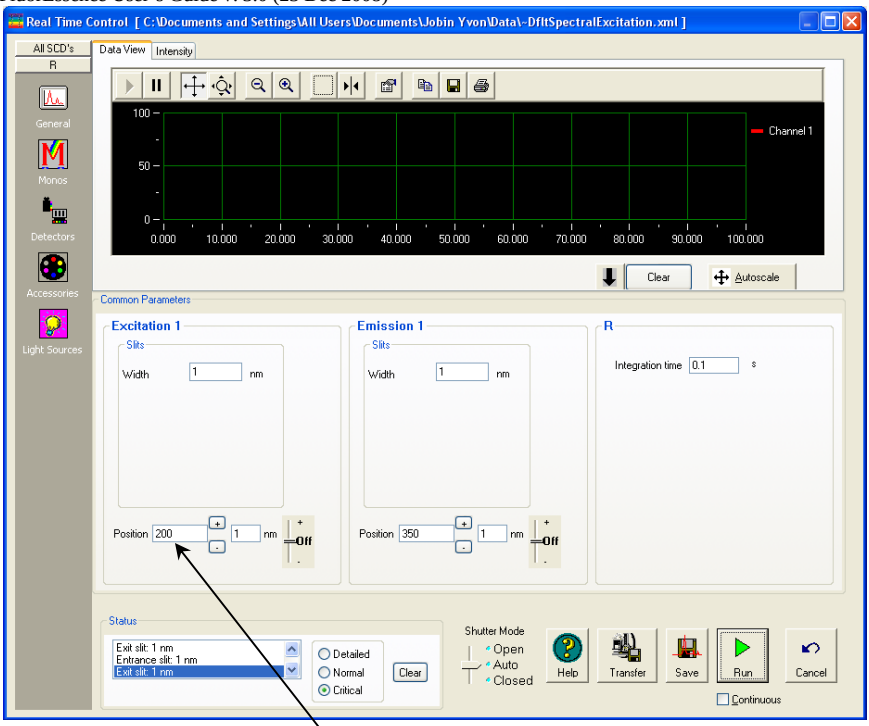


The **Experiment Setup** window appears:



g Click the RTC button.

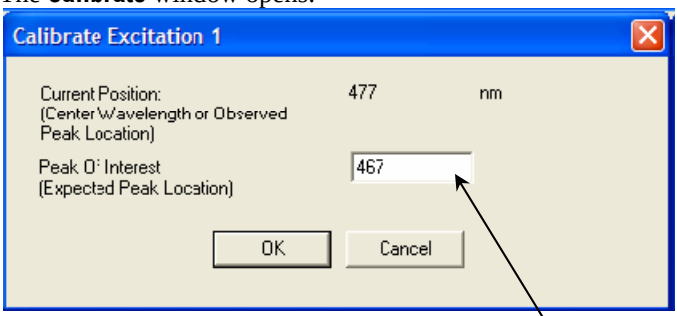
The **Real Time Control** window opens.



h Enter the current, observed position of the peak in the Position field (here, 477 nm).

i Click Calibrate Excitation 1.

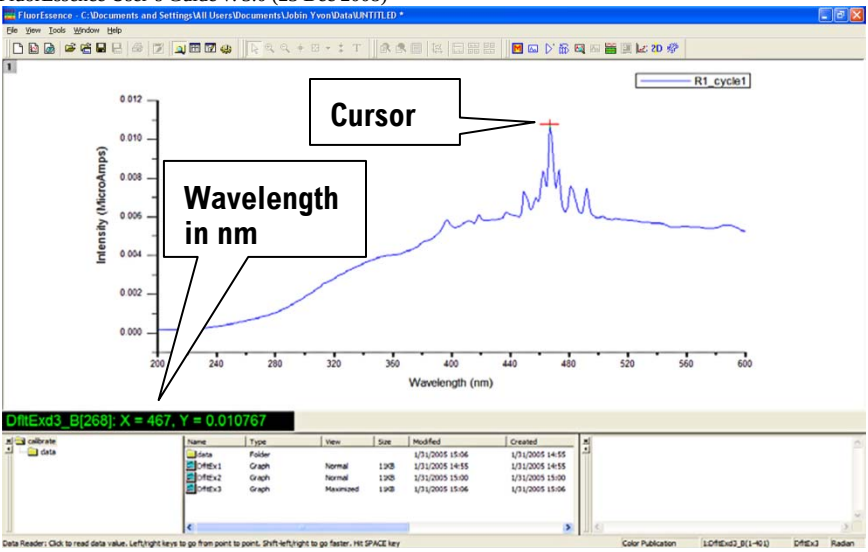
The **Calibrate** window opens:



j 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.

k At the bottom right of the **Real Time Control** window, click the Cancel button.

l In the **Experiment Setup** window, click the Run button to confirm the correct peak position. A correct scan is shown below (peak is at 467 nm):


Cursor
Wavelength in nm
R1_cycle1
Intensity (MicroAmps)
Wavelength (nm)
DfltExd3_B[268]: X = 467, Y = 0.010767

Emission calibration

Monochromator parameters for the water-Raman scan:

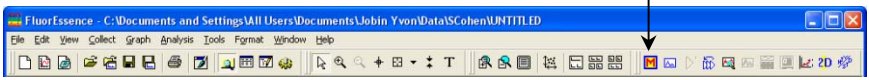
Monochromator (1200 grooves/mm)	Initial wavelength	Final wavelength	Increment	Slits (bandpass)
Excitation	350 nm	--	--	5 nm
Emission	365 nm	450 nm	1 nm	5 nm

Detector parameters for the water-Raman scan:

Detector (Signal)	Integration time	Units
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA

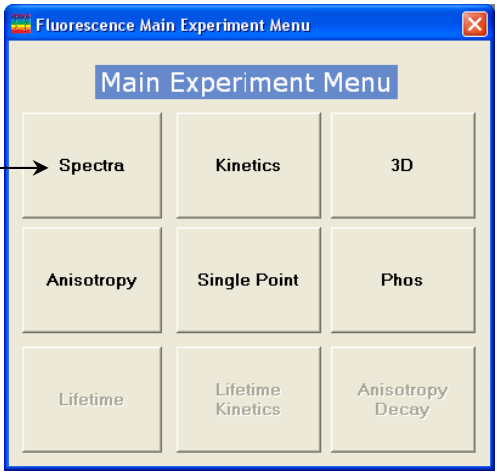
Note: *You can calibrate a T-side emission monochromator in this way also.*

1. Insert a cuvette with HPLC-grade, triple-distilled water in the sample compartment.
2. Close the sample compartment's lid.
3. Start FluorEssence™.
4. In the main **FluorEssence** window, choose the Experiment Menu button  .



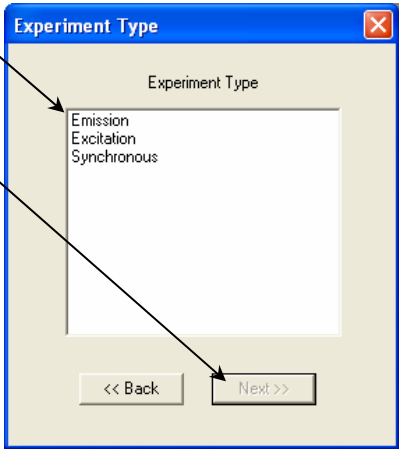
The **Fluorescence Main Experiment Menu** appears.

5. Choose the Spectra button.

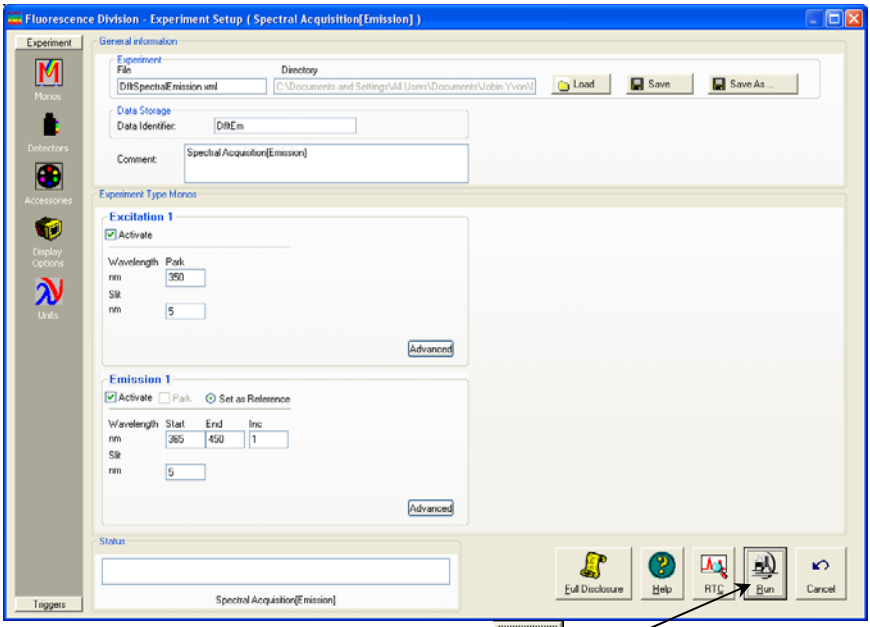


The **Experiment Type** window appears:

6 Choose Emission, then click the Next >> button.



The **Experiment Setup** window for an emission scan appears, with the water-Raman experiment parameters automatically loaded:



7 Click the Run button 

.

The **Intermediate Display** opens. The water-Raman scan runs.

8 If the water-Raman scan is not at 397 nm, calibrate the emission monochromator as shown on pages 39–42.

Using corrected signals

Introduction

Subtracting blanks, removing dark noise, and correcting for inhomogeneities in the instrument or detector response give more accurate spectra. Take special precautions to incorporate these functions properly into a FluorEssence™ experiment. If S is defined as the signal, correction follows the equation

$$S_{corrected} = (S_{measured} - S_{dark} - S_{blank}) \times \text{Correction-factor file}$$

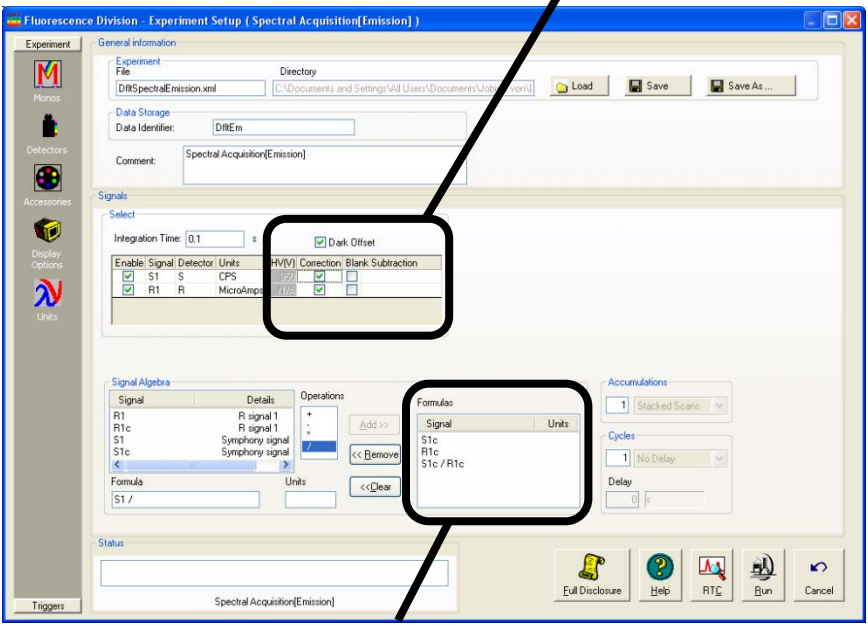
Method

Any corrected signal (with a subscript "c") or algebraic use of corrected signals must explicitly include all desired corrected signals in the Formulas list. Corrected signals include:

- Dark offset
- Blank subtraction
- Correction-factor file

Note: *All desired corrections must be activated in their respective checkboxes.*

Example



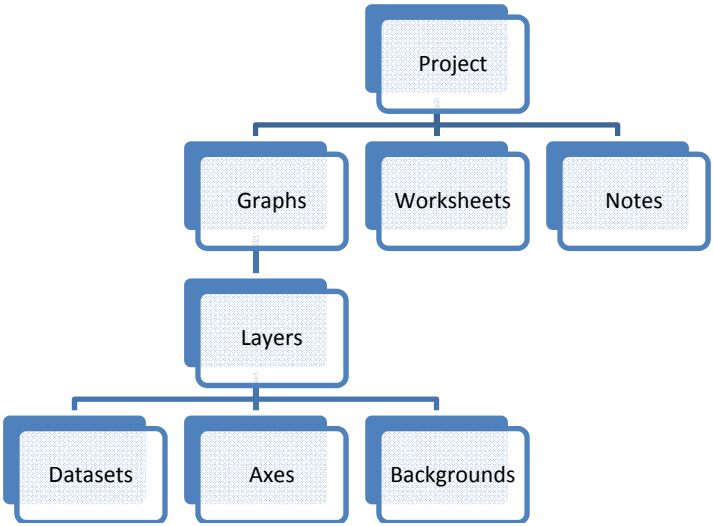
The corrected signal, S1c, and corrected reference, R1c, along with their ratio, S1c/R1c, all must be included in the Formulas list in the Signal Algebra area. If unchecked, $S_{dark} = 0$, $S_{blank} = 0$, and Correction-factor file = 1

Projects and files

What is a project?

A project is a collection of data that contains:

- Graphs (visual diagrams of the data)
- Worksheets (tables of data)
- Notes (comments about the data)



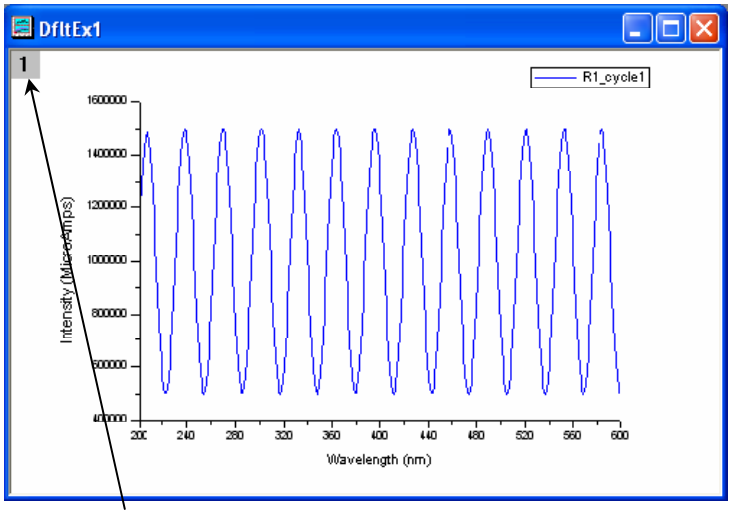
Graphs themselves may contain multiple kinds of information, including separate layers describing the data, the axes, the background colors, etc.

Concerning worksheets, a dataset must contain at least two columns, corresponding to *x*-*y* data pairs. Multiple *y* columns may correspond to a single *x* column.

Note: *For greater detail about projects, graphs, layers, and how to merge, combine, and separate them, see the Origin® on-line help files.*

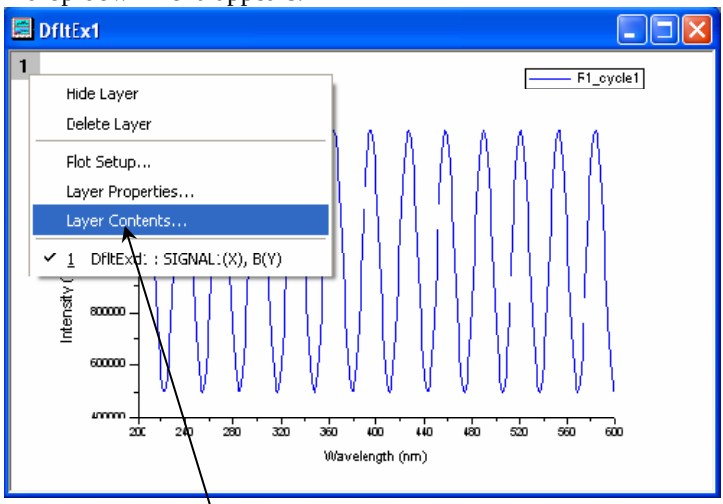
Combining two plots (datasets) into one graph

In its upper left corner, an open graph has a small box with a number in it.



1 Right-click on the numbered box in the upper left corner of the graph.

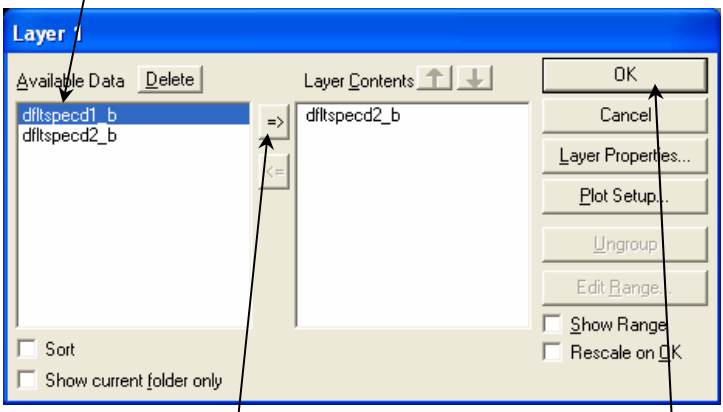
A drop-down menu appears.



2 Choose Layer Contents....

The **Layer Number** window opens. In the Available Data column, a list of *x*-*y* data available to plot is shown:

3 Click on the *x-y* dataset you wish to add to the plot.



Note: *For datasets with multiple* y *columns with one* x *column, the first y column plus the* x *column is listed as ***_a, and the second* y *column plus the x column is listed as *** b, etc.*

4 Choose the => button to add this dataset to the plot.

The dataset appears in the Layer Contents column.

5 Adjust its order (top to bottom) with the ↑ and ↓ buttons.

6 Click the OK button to cause the dataset to appear in the graph.

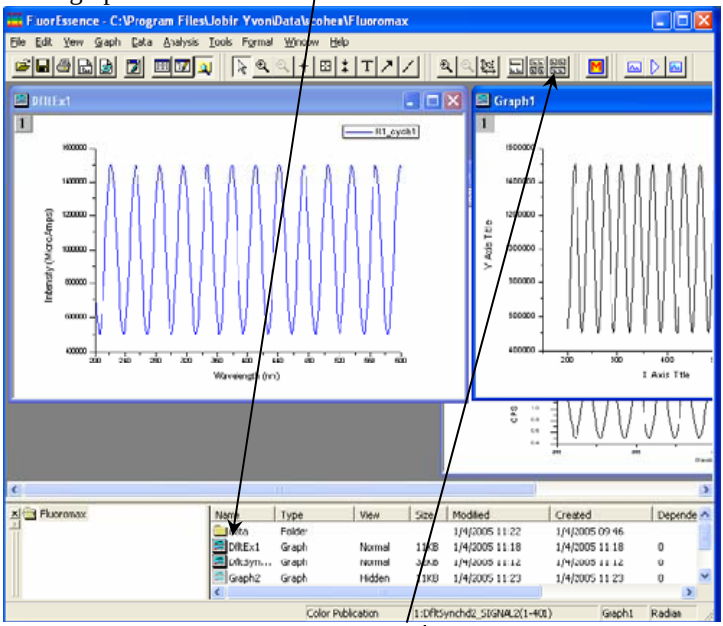
The **Layer Number** window closes.

Merging two or more graph windows

This puts all the open layers on one single page.

1 Close all graph windows you don't want to merge.

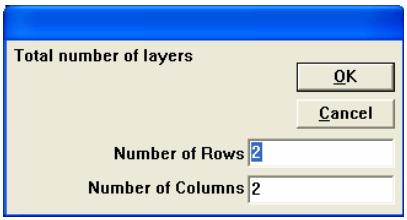
In the Project Explorer at the bottom of the main **Fluoresence** window, double-click on the names of the undesired open graphs. These graphs close.



2 Click the Merge button .

All the windows are combined onto one sheet of paper. This is called a "graph". To preserve the old graphs while creating a new, overlaid version, answer Yes to the question, "Do you wish to keep the old graphs?"

3 A window appears asking you for numbers of rows and columns.



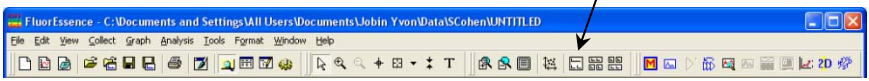
To exactly overlay the graphs, choose 1 row and 1 column.

Note: *Each layer has its own number in the upper left corner.*

Splitting two graphs by extraction

This extracts each plot to a separate layer in the graph.

1. Click on the desired plot to activate it.
2. In the toolbar, choose the Extract to Layers button .



Note: *Other buttons available using the Customize Toolbar command are the button for splitting each layer into a separate graph window, and the button for merging all open graph windows into one graph. See the Origin® on-line help for more information.*

Saving and recalling a file

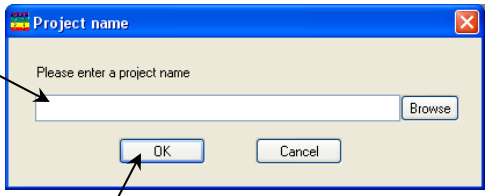
To save a project, when in a new, untitled project

Note: *To determine if you are in an untitled, new experiment, examine the path shown at the top of the main* ***FluorEssence*** *window. It should show the word "UNTITLED" at the end of the path.*

1 Run an experiment.

When the experiment is complete, the **Intermediate Display** disappears. The **Project Name** window appears.

2 Enter a new name for the project, or browse for an existing one.



Note: *If you are using an existing project name, the software will allow you to overwrite existing data, or append the new data to the project.*

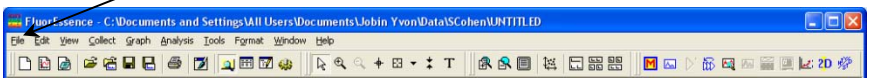
3 Click the OK button.

The path of the project appears at the top of the main **FluorEssence** window. The data are now saved.

To save data into a new project when another project is already open

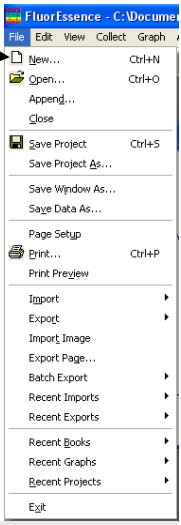
1 Run the experiment.

2 Choose File.

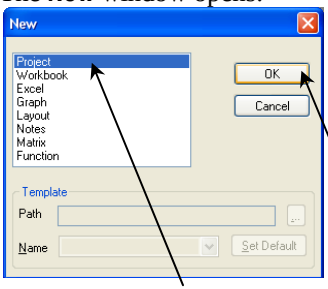


The File menu opens:

3 Choose New....



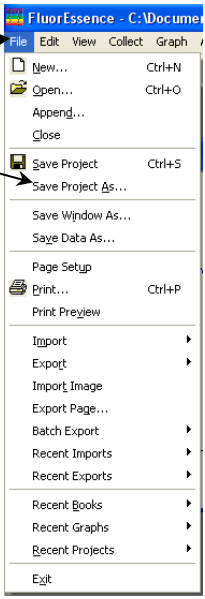
The **New** window opens:



4 Choose Project from the list of objects to create, then click the OK button.

Note: *Only a Graph or a Matrix lets you pick a new Path and Name.*

The data are now in an untitled project. Next you must create the name for the file.

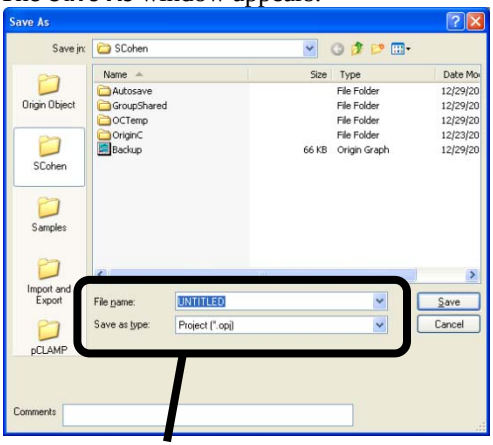


5 Choose File again.

The File menu opens.

6 Choose Save Project As....

The **Save As** window appears:



7 In the File name field, enter a name. In the Save as type field, choose Project (*.opj) from the list.

8 Click the Save button.

Now the project has a new name.

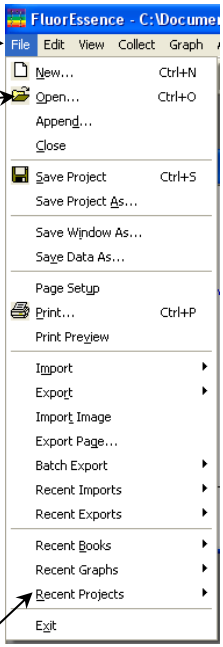
To recall and open an existing project

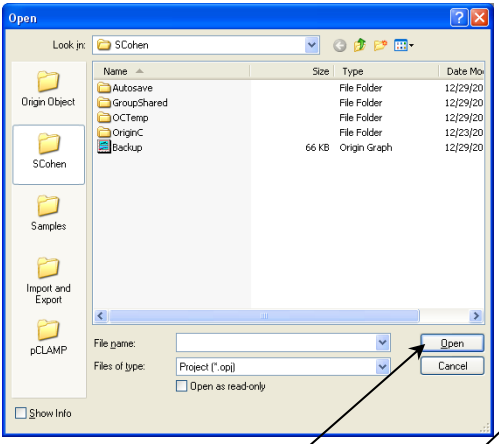
1 Click File.

The File menu opens.

2 Choose Open....

The **Open** window appears:





3 Browse for the desired project, or examine the Recent Projects list.

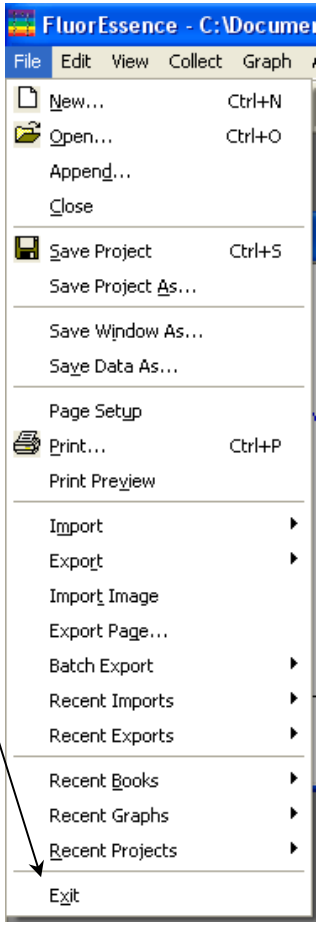
4 Click the Open button.

The project opens.

4: Shutting Down FluorEssence™

1. Save experiment files (and data files, if created).
2. In the **Experiment Setup** window, click the Close button or the Cancel button 

.
3. Close the **FluorEssence** window, using either the Close button, or, in the File drop-down menu, Exit.

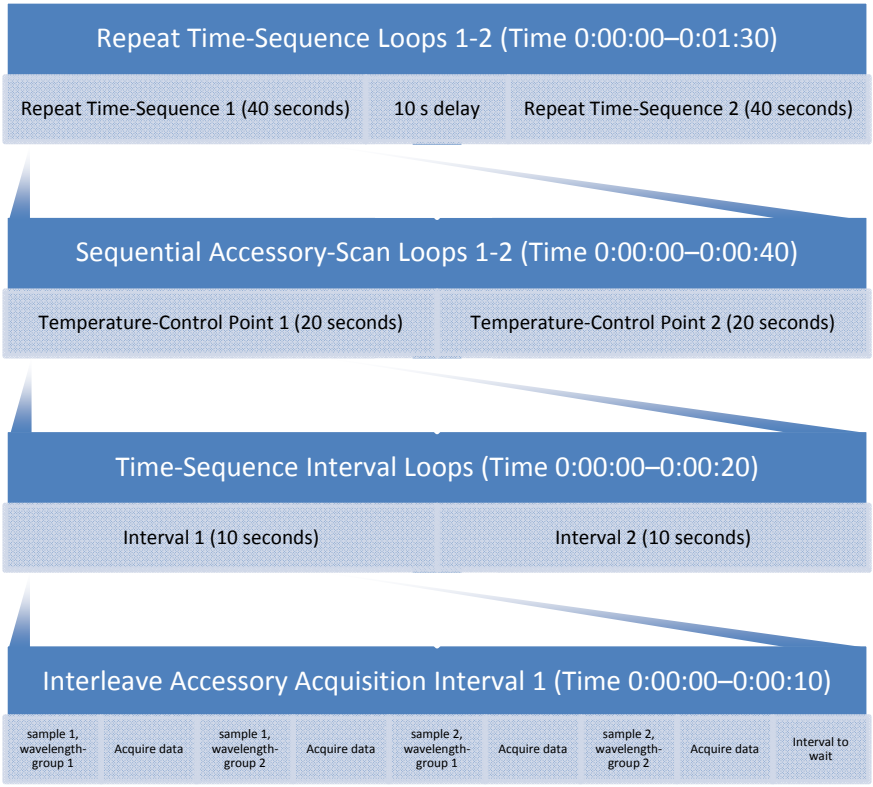


5: Multigroup Software

About Multigroup

Multigroup runs sequential and repeated fluorescence experiments. Delays, temperature ramps, and multiple samples and wavelength-groups are all included within Multigroup. You can sequentially excite a sample with different wavelengths, then plot the emission data on one view. This method is useful for energy-transfer studies, and dual-wavelength experiments with fluorescent probes to examine ion-transport.

Below is a schematic of how the levels of multigroup looping and repeat system can be set up, with two samples at two wavelength-groups, plus a temperature-control accessory.



Requirements

To successfully install Multigroup, your host computer needs the following:

Software

- Windows® 2000, Windows® XP Pro, or Windows® Vista
- Microsoft® .NET Framework 3.5.

Note: *If the host computer does not have Microsoft® .NET Framework 3.5 installed, then the Multigroup Installer attempts to access the internet to download .NET Framework 3.5. If there is no internet connection to the host computer, contact HORIBA Scientific for advice.*

- Same version of FluorEssence™ (but 2.5.2 or higher) as Multigroup

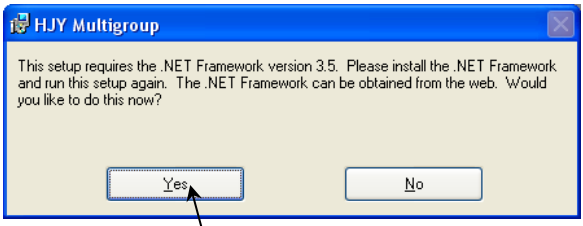
Hardware

- Supports Windows® 2000, Windows® XP Pro, or Windows® Vista
- 128 MB RAM (256 MB recommended)
- 200 MB hard-disk space
- One available Ethernet Network Interface Card (NIC) connection (no hubs)
- One available USB port
- Video resolution of at least 1024 × 768

Installation

1. From the Multigroup CD-ROM, run the installer.

   If your host computer does not have Microsoft® `.NET Framework 3.5`, the **HJY Multigroup** window appears.

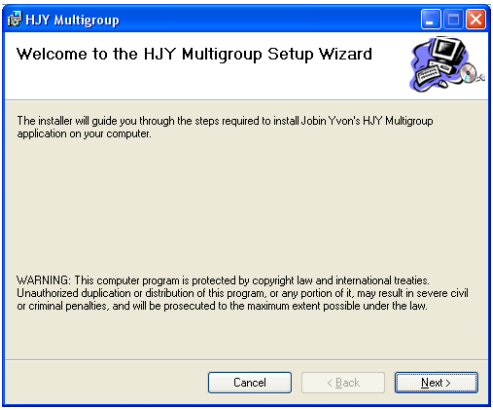


2. Click the Yes button to download the software.
3. Follow the instructions on the Microsoft® website for installing .NET Framework.

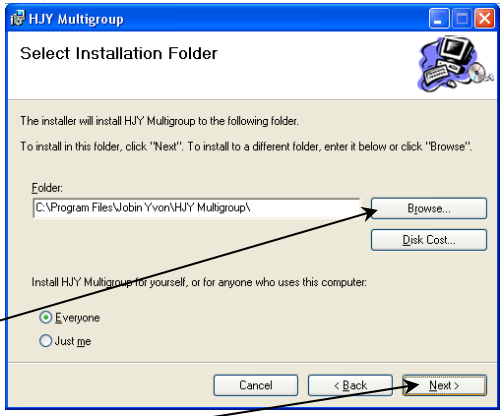
   The program is large; the download and installation may take some time.

4. Continue with the installer.
5. Click the Next > button on the **HJY Multigroup** window.

   The Select Installation Folder area appears:

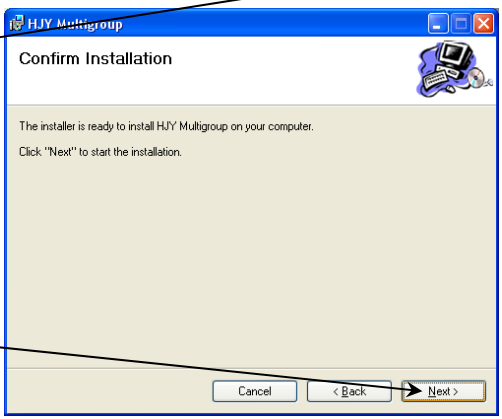


6 Leave the default folder, or choose a different folder using the Browse... button.



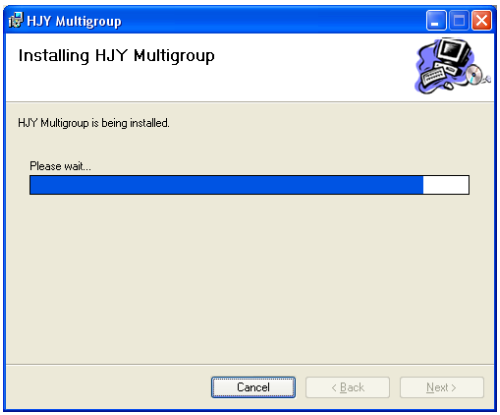
7 Click the Next > button.

The Confirm Installation area appears.



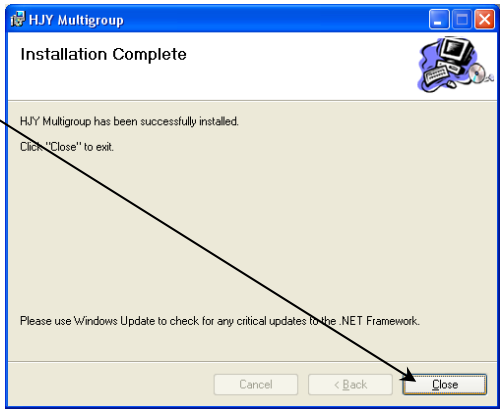
8 Click the Next > button.

The Installing HJY Multigroup area appears, with a progress bar.



When installation is complete, the Installation Complete area appears:

9 Click the Close button to exit the **HJY Multigroup** window.

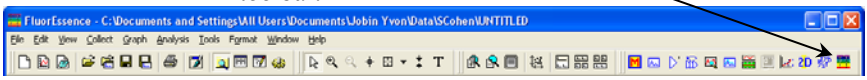


An HJYMultigroup icon has been placed on the computer's desktop.

Running Multigroup

1 Start the software in one of three ways.

a Click the Multigroup button in the FluorEssence™ toolbar.



FluorEssence™ automatically closes.

or

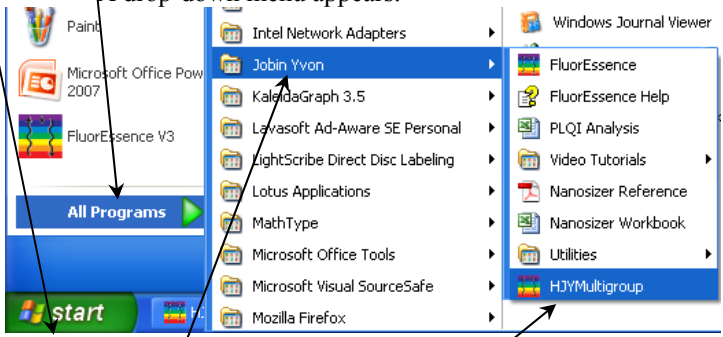
b On the desktop, double-click the HJYMultigroup icon,

Be sure to exit from all Origin® or FluorEssence™ software.

Note: *FluorEssence™ and Origin® run in the background as a shared resource while Multigroup is active.*

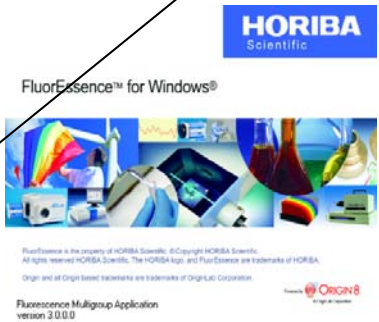
or

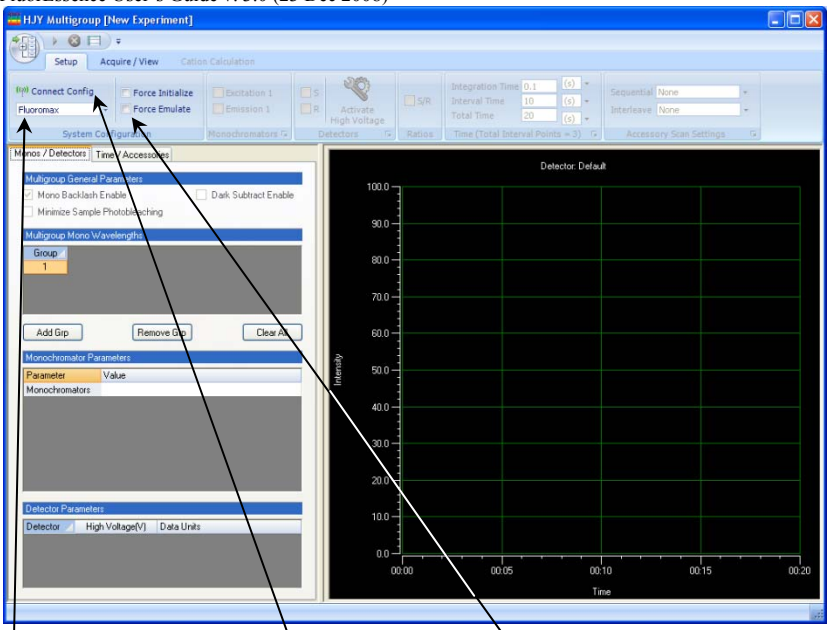
c In the Windows® Start menu, choose All Programs. A drop-down menu appears.



d Choose Jobin Yvon. A drop-down menu appears.

e Choose HJYMultigroup. The **HJY Multigroup** window appears after the splashscreen:





2 Set up the experiment.

a In the System Configuration tab, choose the instrument configuration from the drop-down menu.

b Click the Connect Config button to connect to the instrument.
Multigroup attempts to gain access to the desired instrument configuration in FluorEssence™. If unsuccessful, a **Devices Not Found** window appears, asking you to emulate. Choose the Yes button if you want to emulate. If you want to force Multigroup to emulate an instrument, activate the Force Emulate checkbox.

The instrument configuration automatically activates experimental parameters, which you can change manually.

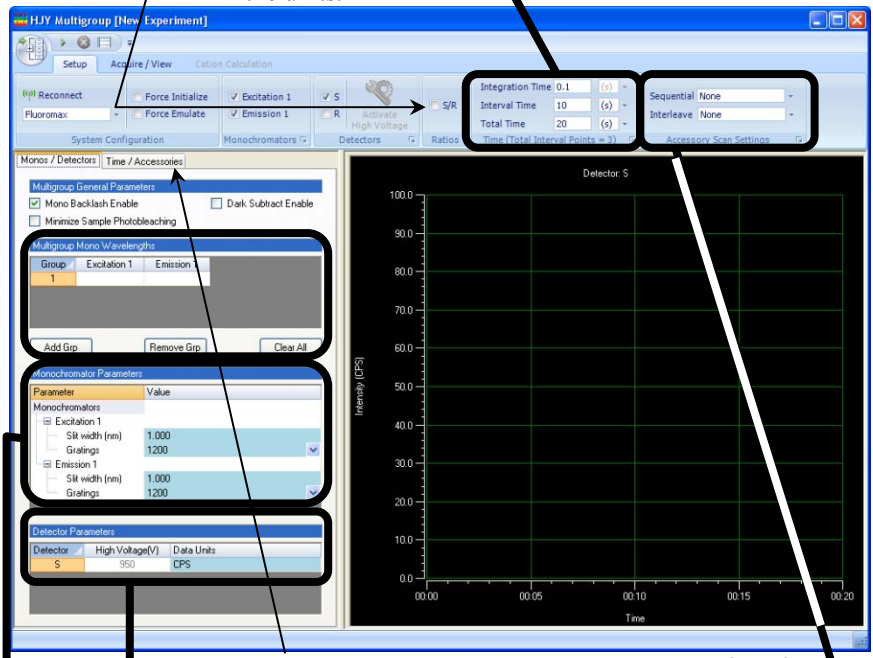
c In the Monochromators tab, activate the excitation and emission monochromators' checkboxes, if necessary.

d In the Detectors tab, activate the desired detector checkboxes, if necessary.

To apply voltage to the detectors, click the Activate High Voltage button.

e In the Ratios tab, to record the corrected output using the reference detector, activate the S/R button.

f In the Time tab, enter an Integration Time, and choose from the drop-down menu the units. Enter an Interval Time and choose from the drop-down menu the units. Enter a Total Time and choose from the drop-down menu the units.



You also can adjust these parameters in the Time / Accessories tab.

g In the Accessory Scan Settings tab, choose the accessory for Sequential or Interleaving scans, using the drop-down menus.

h Click the Monos / Detectors tab to see the monochromator and detector parameters.

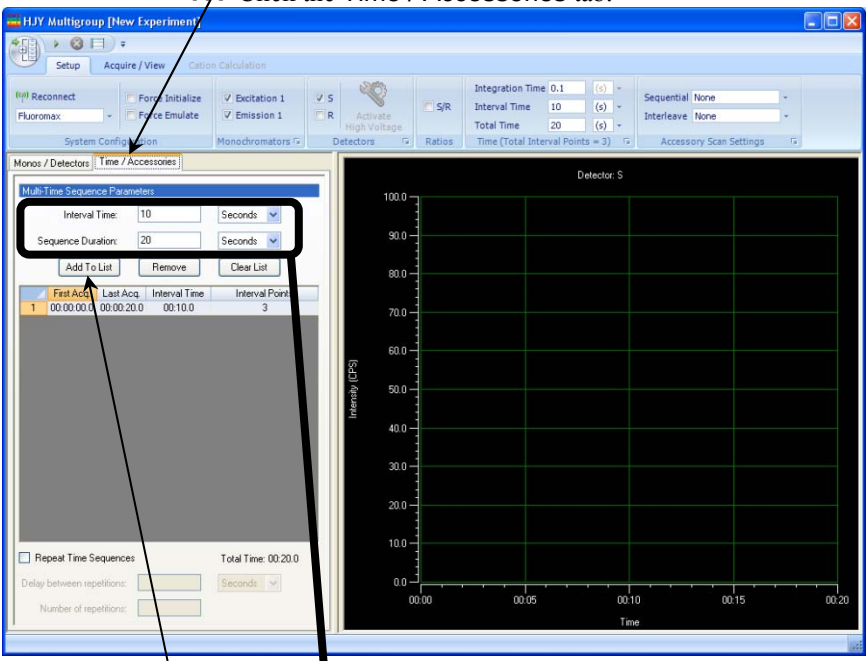
i Enter an excitation wavelength and emission wavelength in row 1 of the Multigroup Mono Wavelengths table.

j Add another row using the Add Grp button.

k Change the Monochromator Parameters if necessary, by entering a new value next to each parameter, or choosing the parameter from a drop-down menu.

l Change the High Voltage or Data Units of each detector by entering a new value if necessary.

m Click the Time / Accessories tab.

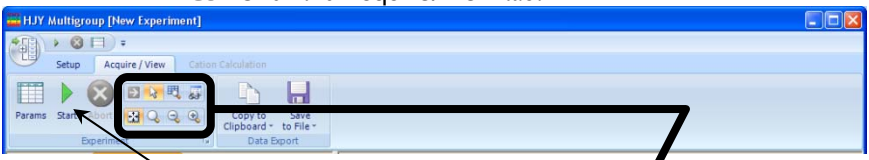


n Enter the Interval Time and Sequence Duration, and choose their units from the drop-down menus.

o To add another sequence row, click the Add to List button.

3 Run the experiment.

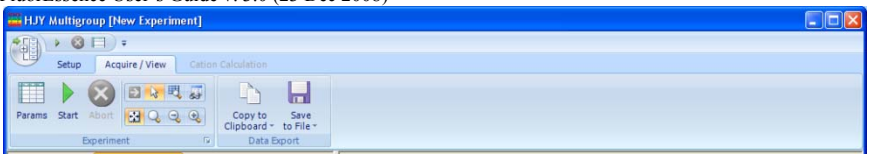
a Click the Acquire/View tab.



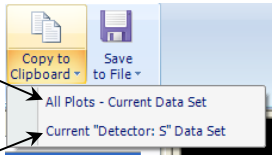
b Click the Start button.

c Use the various buttons to zoom in on and track the data as they are recorded.

4 When finished, save the data.

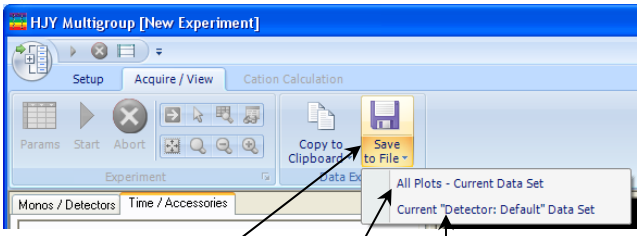


a To copy the data to another program, choose Copy to Clipboard.
A drop-down menu appears:



b Select All Plots – Current Data Set, to save all the plots on the graph.

c Select Current "Detector: S" Data Set to save only the currently selected plot.



d To save the data in a file, choose Save to File, and select the appropriate data-set.

e Select All Plots – Current Data Set, to save all the plots on the graph.

f Select Current "Detector: Default" Data Set to save only the currently selected plot.

Working with experiments and data

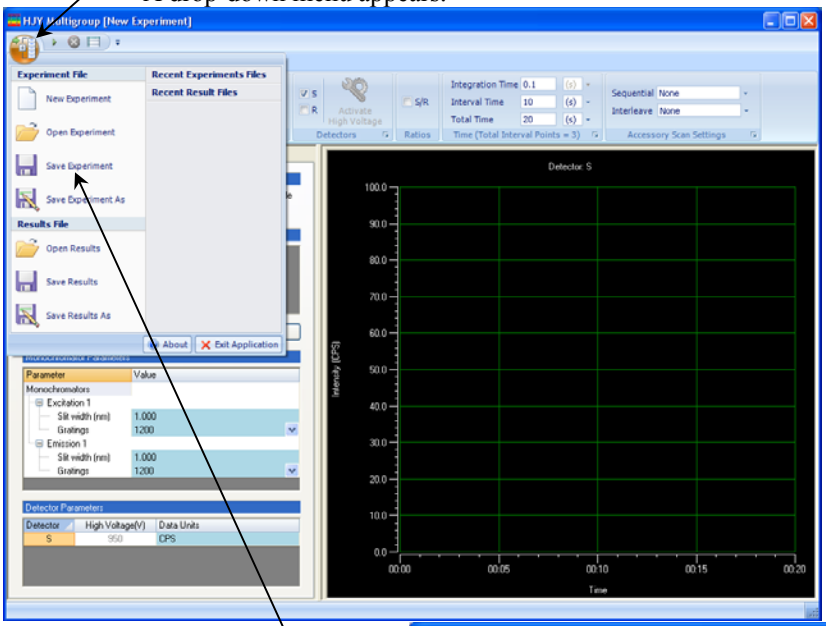
You can save existing experimental parameters (an "experiment") as well as data ("experimental results) to recall later for future use and reference. An "experiment" file contains only the experimental parameters, but no results. A "results" file contains both experimental parameters ***and*** data recorded.

To save an experiment

1 Set up experimental parameters.

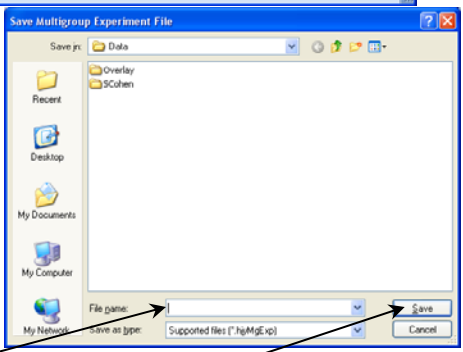
2 Click the Multigroup button.

A drop-down menu appears.



3 Choose Save Experiment.

The **Save Multigroup Experiment Files** window appears.



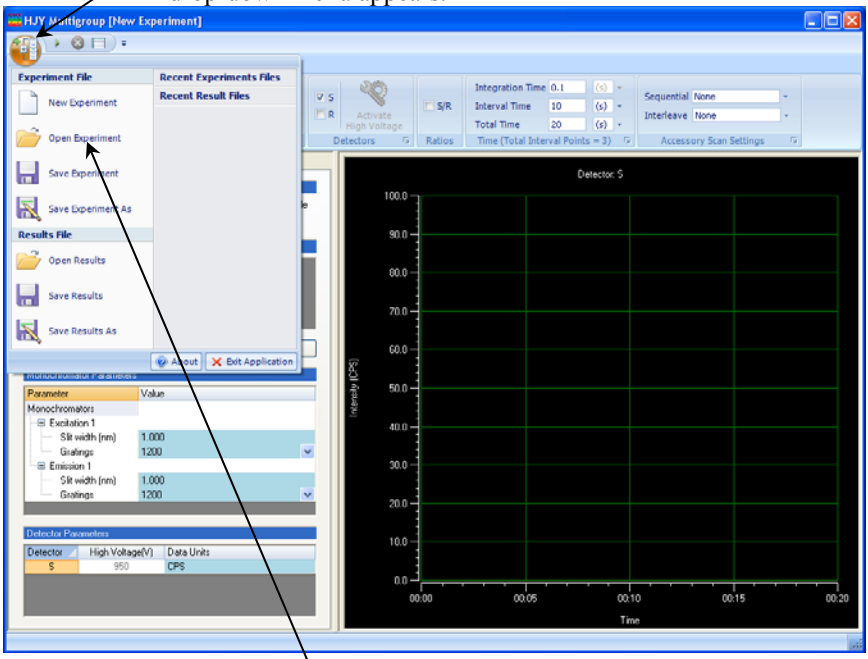
4 Enter a file name and browse for the desired folder.

5 Click the Save button.

To recall an existing experiment

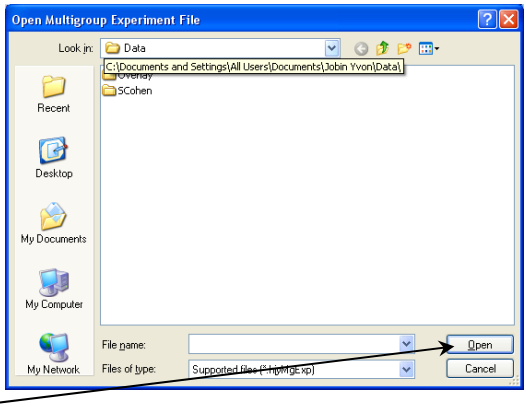
1 Click the Multigroup button  .

A drop-down menu appears.



2 Choose Open Experiment.

The **Open Multigroup Experiment File** window appears. You may also choose an experiment from the Recent Experiments Files list.

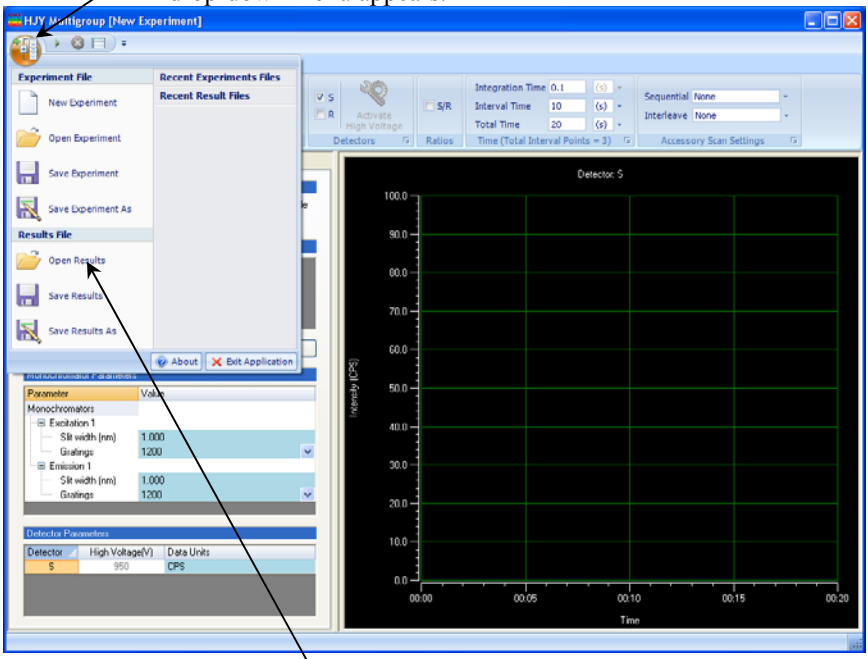


3 Browse for the desired file and click the Open button.

To recall existing data (experimental results)

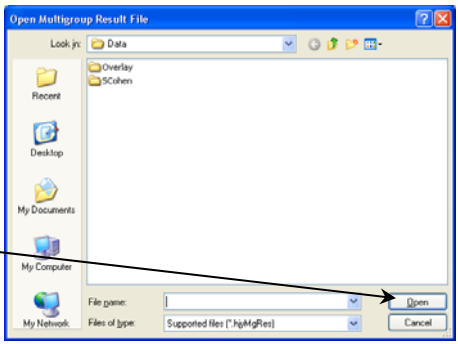
1 Click the Multigroup button  .

A drop-down menu appears.



2 Choose Open Results.

The **Open Multigroup Result File** window appears. You may also choose data from the Recent Result Files list.



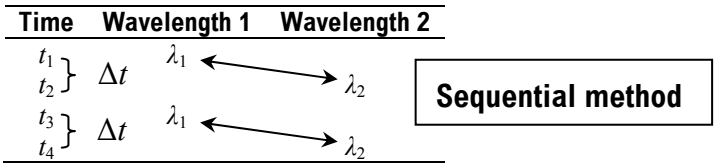
3 Browse for the desired data, and click the Open button.

Interleaved and sequential data

The choice of interleaved or sequential data is possible in Multigroup. Imagine an experiment over time examining a pair of wavelengths, λ_1 and λ_2.

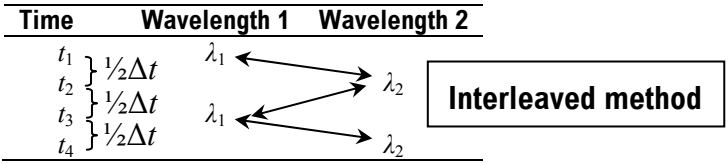
Sequential data-acquisition

Sequential data-acquisition compares the first two data-points, then the next two, then the next two, and so on. Direct comparison between one λ_1 and the next λ_1 is only possible over a time interval Δt:

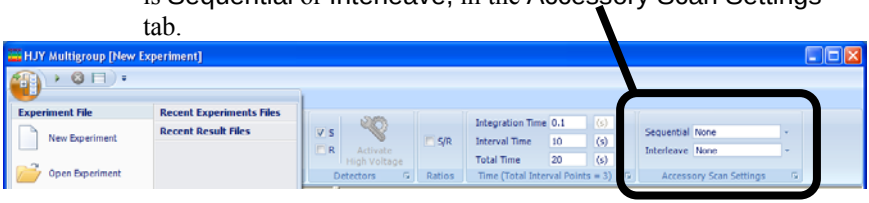


Interleaved data-acquisition

Interleaved data-acquisition compares the first two data-points, then the second with the third, then the third with the fourth, and so on. Each wavelength measurement is used twice, once with the one before it, and again with the one after it. Comparison between one λ_1 and the next λ_1 is possible over a time interval $\frac{1}{2}\Delta t$, half that of sequential data-acquisition (see table below). This technique is better for weak fluorescence or quicker analyses.



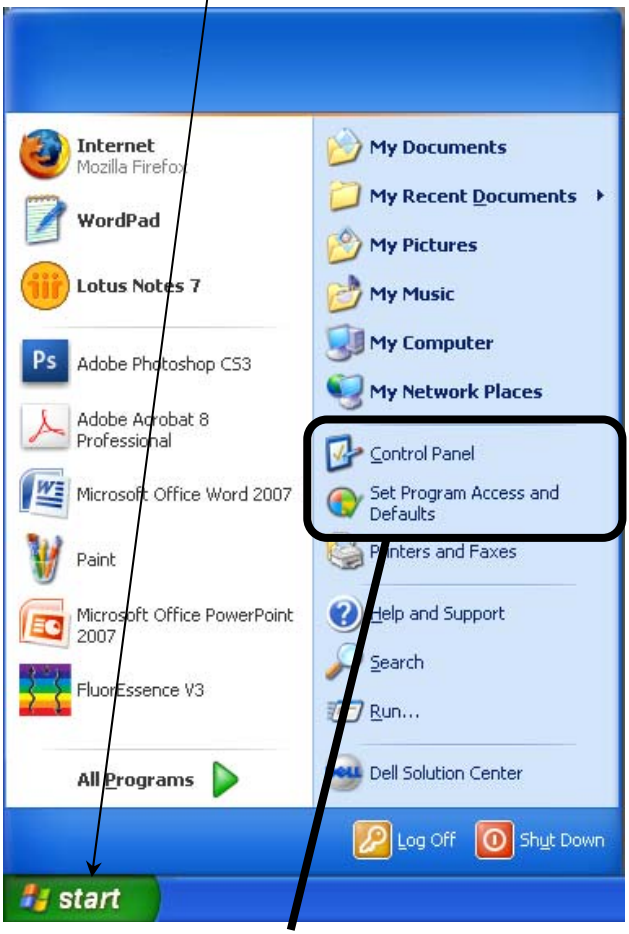
Choose the accessory, and whether its method of data-acquisition is Sequential or Interleave, in the Accessory Scan Settings tab.



6: Un-Installation

FluorEssence™

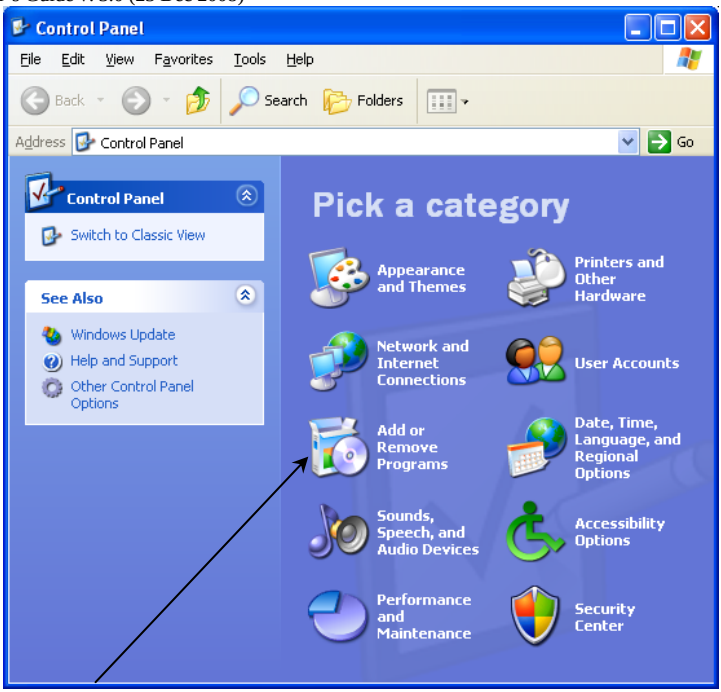
1. Close FluorEssence™.
2. Click the Start button to open the Start menu.



3. There are two ways to continue:

   a. Choose Set Program Access and Defaults, or...

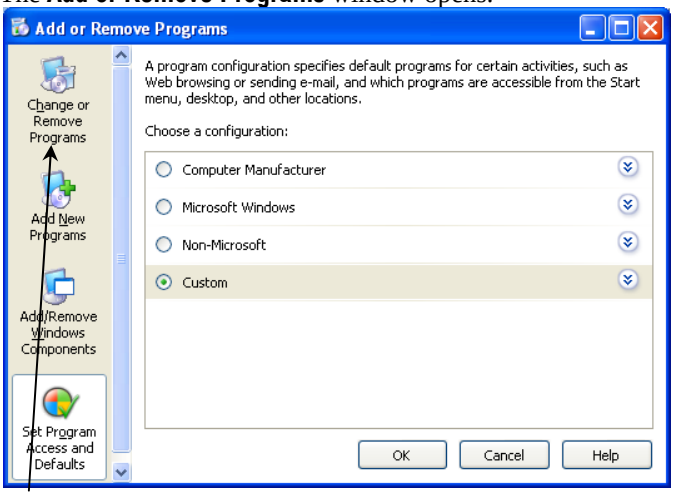
   b. Choose Control Panel.
   The **Control Panel** opens:



Click Add or Remove Programs.

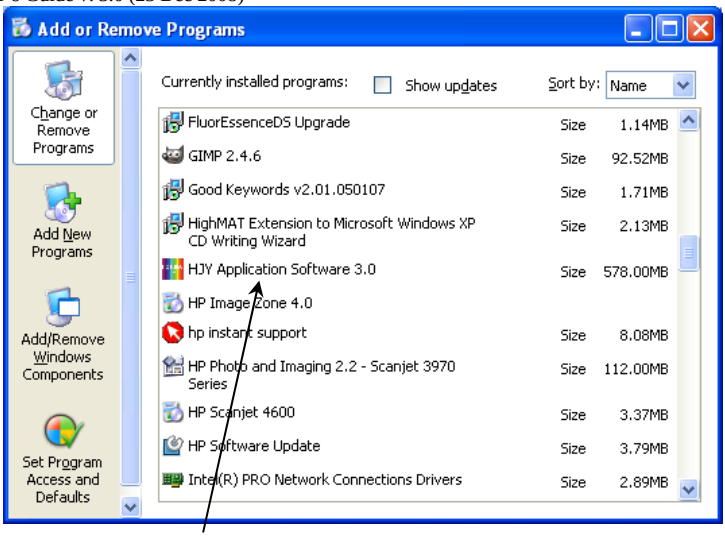
4 In both cases, continue here.

The **Add or Remove Programs** window opens.

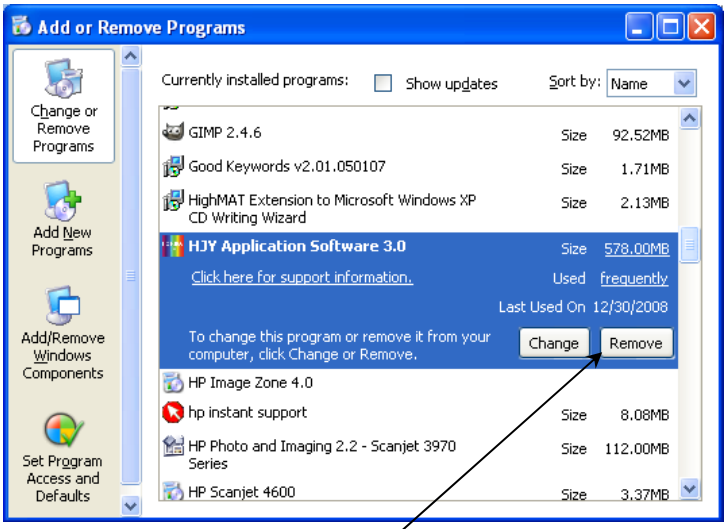


5 Click the Change or Remove Programs icon.

A list of currently installed programs on the host computer appears:



6 Click HJY Application Software 3.0, which becomes active:



7 Click the Remove button.

8 Follow the instructions to remove FluorEssence™.

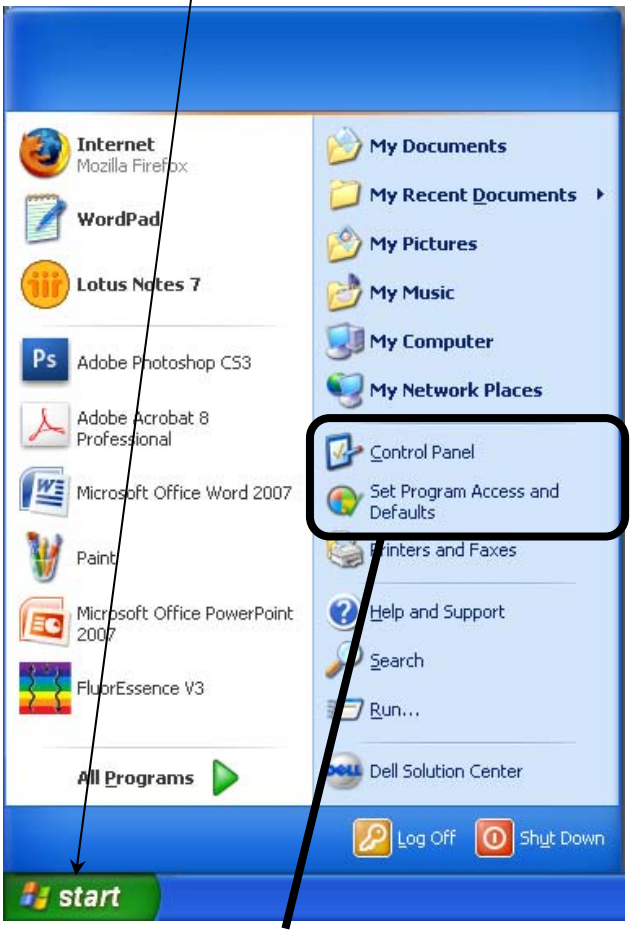
9 You may need to reboot the host computer.

FluorEssence™ is removed from the host computer.

10 Remove the USB key from the USB port.

Multigroup

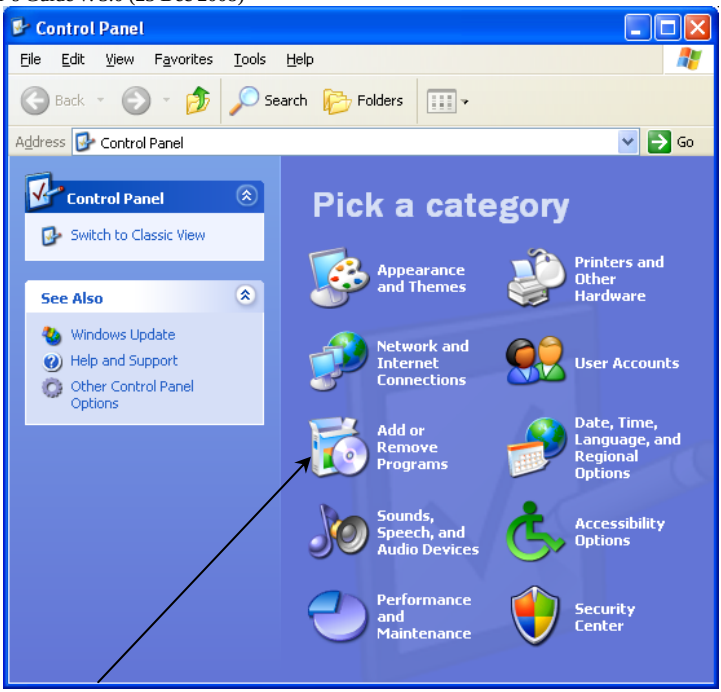
1. Close Multigroup.
2. Click the Start button to open the Start menu.



3. There are two ways to continue:

   a. Choose Set Program Access and Defaults, or...

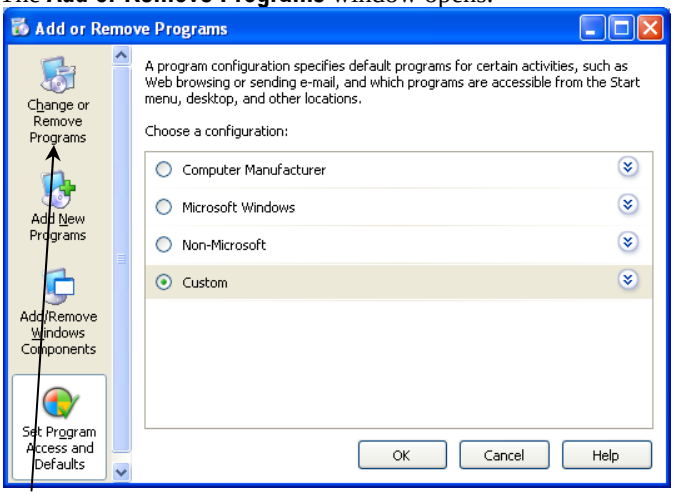
   b. Choose Control Panel.
   The **Control Panel** opens:



Click Add or Remove Programs.

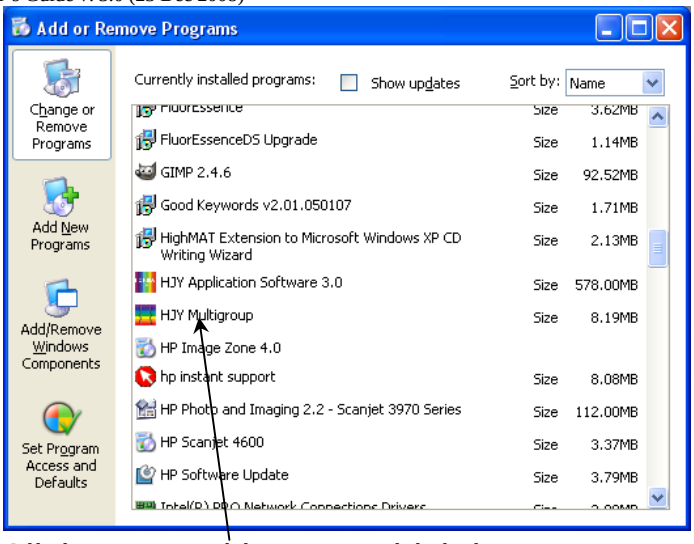
4 In both cases, continue here.

The **Add or Remove Programs** window opens.

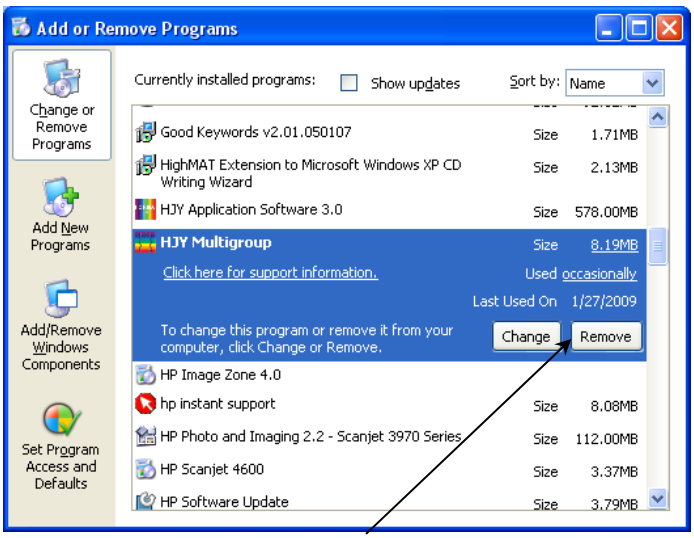


5 Click the Change or Remove Programs icon.

A list of currently installed programs on the host computer appears:



6 Click HJY Multigroup, which becomes active:



7 Click the Remove button.

8 Follow the instructions to remove Multigroup.

9 You may need to reboot the host computer.

Multigroup is removed from the host computer.

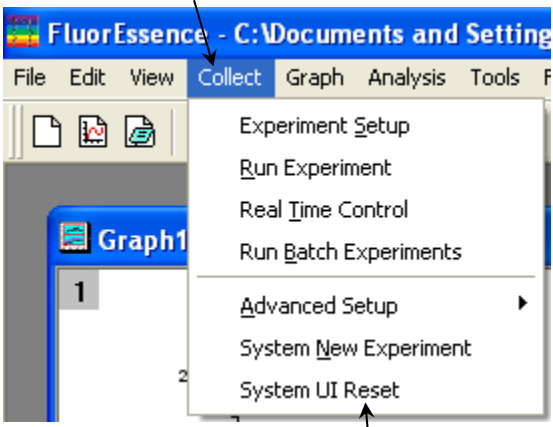
10 Remove the USB key from the USB port.

7: FluorEssence™ Troubleshooting & Technical Support

Troubleshooting

If the twelve special buttons are gray,

1 Choose Collect.



A drop-down menu appears.

2 Choose System UI Reset.

The twelve buttons should become active again.

On-line help files

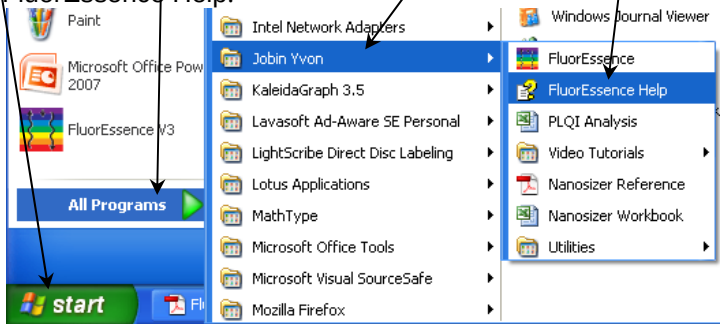
Access from the Windows® Start menu:

1 Click the Windows® Start button.

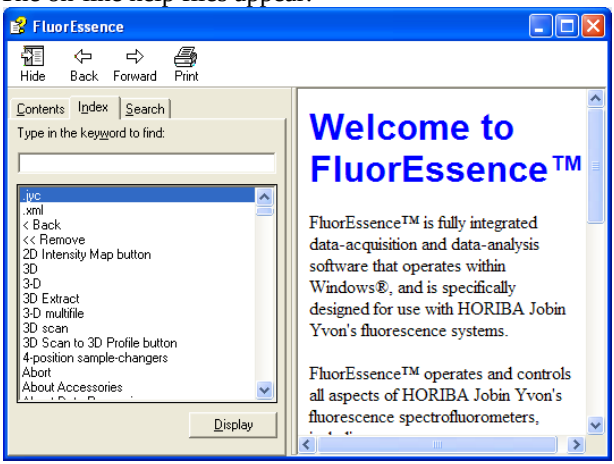
A drop-down menu appears.

2 Choose All Programs.

From the drop-down list, select the Jobin Yvon group, then FluorEssence Help.



The on-line help files appear:



Resize the window to your liking.

Access from the **Experiment Setup** or **Real Time Control** window:

1 Click the Help button 

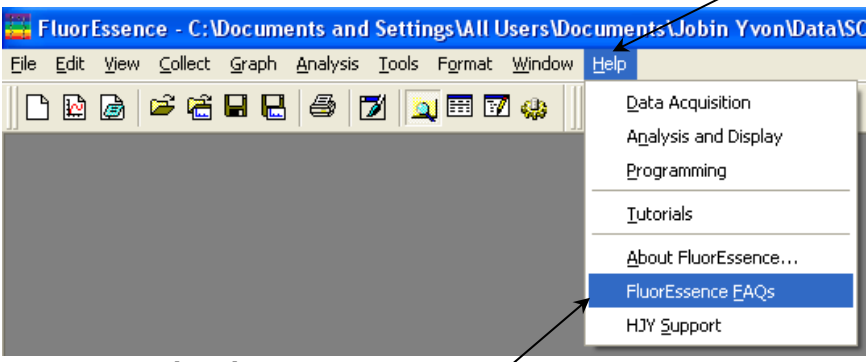
or the F1 key.

Context-sensitive on-line help files appear. Resize the window to your liking.

Frequently-asked questions about FluorEssence™

Many frequently-asked questions (FAQs) about FluorEssence™ may be found on the HORIBA Scientific website.

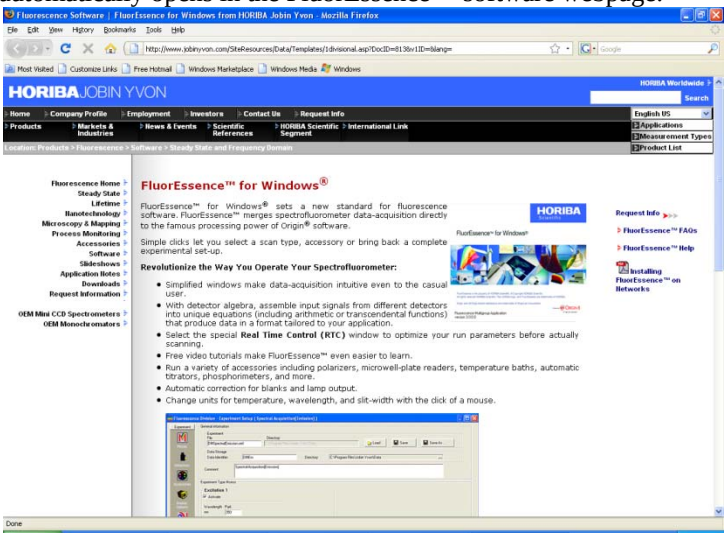
1 In the **FluorEssence** toolbar, choose Help.



A drop-down menu appears.

2 Choose FluorEssence FAQs.

If your computer is connected to the internet, your web browser automatically opens in the FluorEssence™ software webpage:



Video tutorials

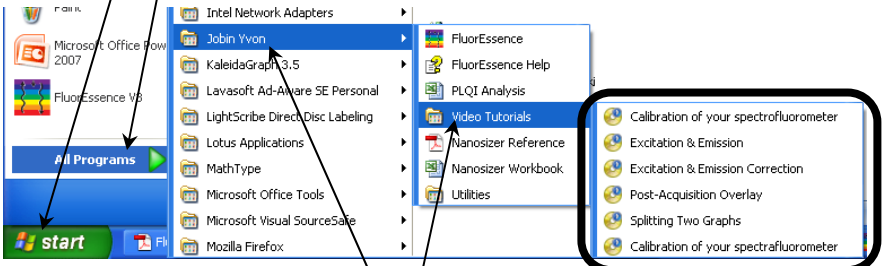
For some common procedures, video tutorials are available to guide you. The videos are `.avi` files, which can be played by software such as RealPlayer®, Windows Media Player, etc.

Access to video tutorials

1 Click the Windows® Start button.

The Start menu appears.

2 Choose All Programs.

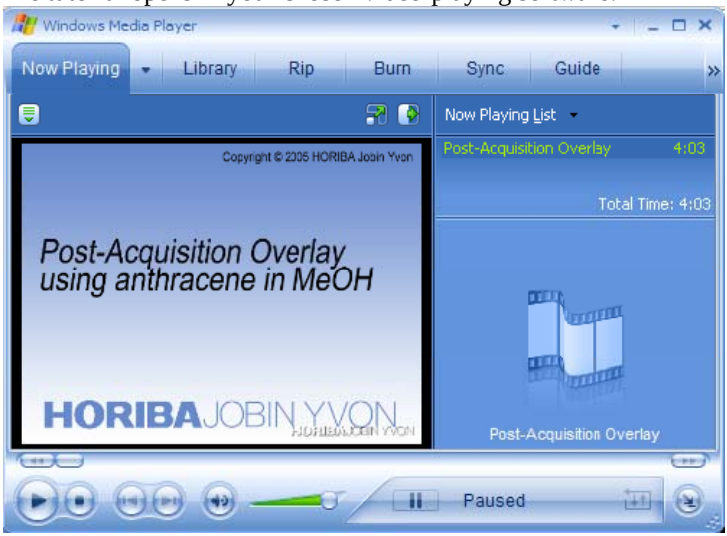


3 Choose the Jobin Yvon group.

4 Choose the Video Tutorials subgroup.

5 Click on the desired tutorial.

The tutorial opens in your chosen video-playing software.

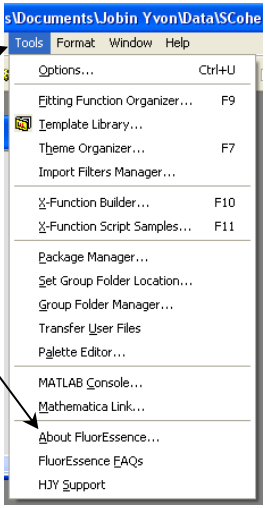


If you have a technical problem,

1. Please consult the FluorEssence™ help files and this User's Guide, as well as all other manuals supplied with the system.

If you are unable to solve the problem,

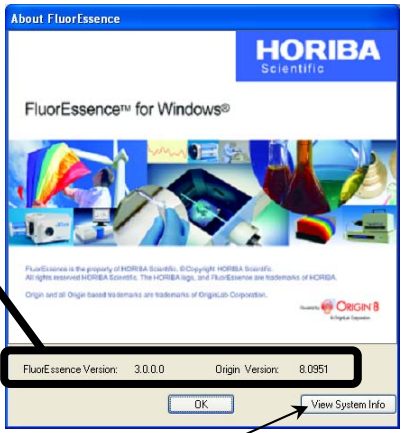
2. Note the problem and any accompanying error messages.
3. Determine FluorEssence™'s version number.



a Choose the Tools menu.

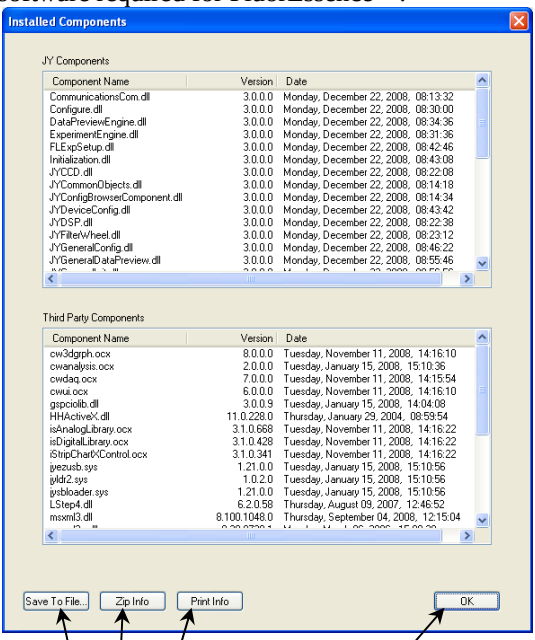
b Choose About FluorEssence....

The **About FluorEssence** window opens. Near the bottom are the FluorEssence™ and Origin® version numbers.



c Click the View System Info button.

The **Installed Components** window appears, displaying all the software required for FluorEssence™.



d Record the information by clicking the:

- Save To File... button, which saves the information to a file;
- Zip Info button, which compresses the information while saving it;
- Print Info button, which prints out the software information.

e Click the OK button to close the **Installed Components** window.

f Click the OK button to close the **About FluorEssence** window.

4 Write down the software's version numbers, along with the purchase dates, model numbers, system configuration, and serial numbers of the instrument and its accessories.

5 Please contact a HORIBA Scientific Fluorescence Service Department listed below.

Be prepared to describe the malfunction and the attempts, if any, to correct it. Note any error messages observed, and have any relevant spectra (sample, polarization ratio, xenon-lamp scan, water Raman scan, etc.) and system information ready for us to assist you.

Contact information

Via the internet:

World-Wide Web	www.horiba.com/scientific
E-mail	info.sci@horiba.com

In North America:

Telephone	1-732-494-8660
Fax	1-732-549-5125

In France:

Telephone	+33 (0) 1 64 54 13 00
Fax	+33 (0) 1 69 09 93 19

Worldwide:

China	+86 (0) 10 6849 2216
Germany	+49 (0) 89 462317-15
Italy	+39 (0) 2 57603050
Japan	+81 (0) 3 58230141
UK	+44 (0) 20 8204 8142

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Arial font	command, menu choice, or data-entry field
Arial Condensed Bold font	window
`Courier New` font	file name or expression

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