
EasyLife X User's Manual



OPTICAL BUILDING BLOCKS



CORPORATION

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This warranty is in lieu of all other warranties, expressed or implied, including, without limitation, the implied warranties of merchantability and fitness for a particular purpose. OBB shall not be responsible for any liability, loss or damages, caused or alleged to be caused, by the instrument, as a result of use or operation including, without limitation, consequential damages and loss of profit.

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- 2) The original manufacturer's warranty will be maintained for instrument components not manufactured by OBB (e.g. computers and components thereof).
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- 7) Damage or loss caused by shipping is not covered by the warranty.
- 8) Damage caused by improper operation of the instrument will void the warranty.
- 9) Damage caused by equipment not purchased from OBB that is attached to the instrument is not covered by the warranty.
- 10) Warranty is valid only in the state, province or country of the original purchase.
- 11) Software upgrades performed on an OBB supplied computer workstation (e.g., adding word processors, image editors, etc.) not authorized by OBB will void the warranty on the computer.
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The customer will bear all shipping charges for warranty repairs. All service rendered by OBB will be performed in a professional manner by qualified personnel.

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INTRODUCTION

WELCOME TO THE FAMILY

Congratulations on your purchase of a fluorescence system from OBB. To help you get the most out of it and to safeguard your investment, please take the time to learn about your new instrument.

ABOUT THIS MANUAL

What it does

This guide provides the following information:

1. Hardware and software installation.
2. Basic operation.
3. Basic theory of fluorescence decay.
4. Data acquisition for typical fluorescence techniques.
5. Data analysis functions for fluorescence data.
6. Saving and retrieving data.
7. Basic troubleshooting.

What it does not do

Describe using Windows.

INSTRUMENT OVERVIEW

The EasyLife X is a dedicated and highly capable fluorescence lifetime system, which is extremely easy to operate with minimum intervention from the user.

The fluorometer contains a detachable and interchangeable nanosecond pulsed LED excitation source, a sample compartment, a lifetime detector, all necessary electronics to control and operate the LED source and detector and an optional magnetic stirrer.

OBB offers the EL series of nanosecond pulsed LEDs designed for the EasyLife X with excitation wavelengths ranging from 260 nm to 650 nm. An LED can be quickly and conveniently mounted or interchanged using a snap on/off mount on the backside of the fluorometer chassis.

The sample compartment contains a thermostatable single cuvette holder, which can accommodate 10 x 10 mm cuvettes and a variety of micro- and semi-microcuvettes with 10 x 10 mm adapters. The optical height of the light beam is 15 mm above the bottom of the cuvette holder. An optional Peltier cuvette holder is available for rapid control of the sample temperature.

There are 2 filter/polarizer holders on the excitation and emission sides of the sample compartment. Each holder can accept either a 2" x 2" square filter or a 1" diameter round filter with a filter adapter (2 provided). One set of band pass and three sets of long pass (cut-off) filters for UV-VIS, VIS and VIS-NIR ranges are optionally available from OBB. Another highly recommended optional accessory is a set of neutral density filters to control the light intensity reaching the detector.

There is only one manual adjustment for the EasyLife X – a micrometer-adjustable slit that controls the amount of emitted light reaching the detector. The micrometer adjustment dial is accessible on the back panel and controls the slit opening continuously from 0 to 6 mm.

The EasyLife X with an optional magnetic stirrer will also have a manual adjustment knob on the bottom of the front panel to control the speed of the stirring motor.

The computer communicates with the EasyLife X via a serial RS232 and a USB connection under the control of the program EasyLife X. This program also performs the post-acquisition data manipulation. Once the sample is placed in the sample holder, a proper filter placed in the filter holder and the emission slit is set, the instrument is operated entirely from a laptop or desktop computer.

There is no electronic adjustment or maintenance required for the EasyLife X!

SPECIFICATIONS

Lifetime range:	From 100 ps to 3 μ s
Sensitivity:	400 pM fluorescein or better
Excitation:	PTI proprietary pulsed nanosecond LEDs
Optical pulse width:	1.5 ns (typical)
Excitation range available:	280 - 670 nm
Temperature range:	10 – 70 °C
Temperature Ramp Rate:	Up to 20 °C/min
Emission range:	200 - 650 nm (optional to 900 nm)
Wavelength selection:	By filters
Detection:	PTI patented lifetime detector
Typical acquisition time:	20 s (sample dependent)
Timescale (menu selectable):	Linear, arithmetic and logarithmic
Acquisition mode:	Sequential or random
Software:	EasyLife X
Lifetime analysis:	Complete package: 1-to-4 exp, global, non-exponential, micelle kinetics, lifetime distribution (ESM, MEM), anisotropy, FRET calculator
QuickStart DVD:	Included
Sample holder:	Single 1 x 1 cm cuvette
Width:	15 inches (381 mm)
Depth:	13 inches (333 mm)
Height:	8.8 inches (223 mm)
Weight:	25 lb (11.4 kg)
Electrical supply:	100 – 240 VAC, 50 / 60 Hz
Fuse	1.5 A S/B

INSTALLATION

DAMAGE DURING SHIPPING

Your OBB instrument was shipped in specially prepared cartons and crates. The original manufacturer's cartons are used for the computer equipment. In almost all cases the equipment arrives without damage. However, on occasion, shipping damage does occur. In these cases it is vital that the shipper's responsibility be recognized.

Note Carefully examine all crates and cartons as soon as they are received for signs of damage. If there is any sign of damage, make sure to file a claim with the shipper and to contact OBB immediately. OBB is not responsible for damages incurred during FOB shipments unless special shipping arrangements are made. If damage occurs during shipping, OBB will assess the damage at installation time and issue an invoice for the extra charges required to bring the instrument to an operational condition.

Components

Make sure you have the following components:

1. EasyLife X unit
2. One or more EL LED sources
3. 15" CPC (Circular Plastic Connector) cable
4. 15" BNC cable
5. Serial cable
6. PL2303 USB-to-Serial adapter cable
7. Two USB cables with A and B ends
8. Two power cords
9. 10 x 10 mm quartz cuvette
10. Two filter adapters
11. Computer (may be included with some instruments)
12. EasyLife X Installation CD
13. EasyLife X manual

With optional Peltier temperature stage and controller:

14. Temperature Controller
15. DB-15 cable
16. Water circulator
17. Two hoses (5 ft / 1.5 m).
18. Magnetic flea

SITE REQUIREMENTS

The EasyLife X module occupies the following space (measurements are approximate):

15" (W) x 13" (D) x 9" (H) or 38 (W) cm x 33 (D) cm x 23 (H) cm.

Add 4" (10 cm) to the depth for connected cables and 5" (12 cm) to the height when the lid is open.

Space is also required for a laptop or desktop computer, and the temperature controller.

Notes There will be a need for three 120/220V electrical outlets. These can be supplied from a power strip since the total current demand is less than 5 amps. A surge suppressor is recommended to isolate the computer from electrical transients. For countries with 220V electrical supply, the system is appropriately configured for the required voltage. Voltage stabilizers may be required for sites that are known to have large fluctuations in the line voltage.

COMPUTER REQUIREMENTS

Microsoft Windows operating system: 32-bit, Windows XP SP2 to Windows 7

USB port (USB 2.0 recommended)

Microsoft .NET Framework 2.0 or later (required for graphics operation) (included in installation)

Intel Pentium III class processor, 500 MHz or higher

Video display: 800 x 600, 256 colors or greater

Minimum of 256 MB of RAM (512 MB or higher recommended)

Minimum of 125 MB of free hard disk space

Microsoft-compatible mouse

Microsoft Windows Installer version 3.1 (KB893803) or later (included in installation)

DOCUMENTATION

This manual provides documentation and operating instructions for the EasyLife X system.

HARDWARE INSTALLATION

The installation of the EasyLife X system requires a few very simple steps. Please follow the instructions below.

1. Set the EasyLife module on a bench.
2. Make sure the power switch on the EasyLife is in the OFF position.
3. Hold the LED unit vertically (the peak wavelength label on top) and position it above the LED clip mount on backside of the EasyLife, so the 3 pins on the LED unit are just above the clips. Press the LED unit down until it snaps in.
4. Connect one end of the 15” BNC cable to the LED unit and the other end to the ‘PULSED SOURCE CONTROL’ BNC connector on the backside of EasyLife.
5. Connect one end of the 15” CPC cable to the LED unit and the other end to the ‘PULSED SOURCE CONTROL’ CPC connector on the backside of EasyLife. Both ends have keyed connectors and you must rotate the connector until you feel the connector move into the socket, then rotate the collar until it is in its locked position.
6. Connect the USB cable ‘A’ end to a free USB port on your computer and the ‘B’ end to the back of the EasyLife.
7. RS-232 connector:
 - a. If you have an RS-232 port on your computer, connect the serial cable to the RS-232 port on the backside of the EasyLife and to the RS-232 port on the back of your laptop.
 - b. If your computer does not have an RS-232 port, then connect the serial cable to the RS-232 port on the backside of the EasyLife. Connect the PL-2303 USB-to-Serial adapter cable to the RS-232 cable.
 - i. If the EasyLife Installation CD software has not been installed on your computer do not connect the USB end of the USB-to-Serial adapter cable to the computer at this time – wait until step 6 in the Software Installation below (after all the software has been installed).
 - ii. If the EasyLife Installation CD software has been installed on your computer then connect the USB end of the USB-to-Serial adapter cable to a free USB port on your computer.
8. Connect each water hose to the EasyLife by pushing down on the metal tab on the metal port and inserting the white plastic connector into the metal port.

Warning: Do not disconnect the USB-to-Serial adapter cable while the computer is powered ON. This will cause Windows to increment the number of COM ports and change the one used for the EasyLife. You will then have to reconfigure the COM port used by the Delay gate Generator.

9. Follow the directions in the water circulator manual to fill the water circulator with water and prime the pump. Connect the brass fittings on the water hoses to the in and out connectors on the water circulator. Turn on the water circulator to check for water leaks and continuous water flow. Turn off the water circulator.
10. Use the DB-15 cable to connect the Temperature Controller to the EasyLife X unit.
11. Connect the USB cable 'A' end to a free USB port on your computer and the 'B' end to the back of the Temperature Controller.
12. Attach a power cable to the EasyLife and plug it into the power outlet.

EXCHANGING LEDs

1. Turn the EasyLife power switch to the OFF position.
2. Disconnect the 15" CPC cable from the LED unit.
3. Disconnect the 15" BNC cable from the LED unit.
4. Grasp the LED unit and lift it straight up until the 3 pins are free of the clips on the backside of the EasyLife.
5. Hold the new LED unit vertically (the peak wavelength label on top) and position it above the LED clip mount on backside of the EasyLife, so the 3 pins on the LED unit are just above the clips. Press the LED unit down until it snaps in.
6. Connect the 15" BNC cable from the LED unit.
7. Connect the 15" BNC cable from the LED unit. The cable end is a keyed connector and you must rotate the connector until you feel it move into the socket, then rotate the collar until it is in its locked position.

OPTIONAL ACCESSORY: COLD FINGER DEWAR

The Cold Finger Dewar accessory is used to refrigerate samples to liquid nitrogen temperatures, or with the use of organic solvent slushes, to a temperature up to room temperature.

Components

Figure 1 shows the components of the Cold Finger Dewar. In order to use this accessory, the sample compartment lid must remain open and the top hat lid placed over the sample compartment.

- A. Dewar holder for the cuvette holder
- B. Quartz cold finger dewar (accepts 5 mm tubes)
- C. NMR or EPR tubes (not provided)
- D. Foam lid for the dewar

Description

The dewar has a Suprasil quartz cold finger which passes light down to about 200 nm. 5 mm outside diameter NMR (visible) and 5 mm outside diameter EPR tubes (UV) are suitable containers for most samples. Liquid nitrogen placed in the dewar will typically last several hours.

Precautions

1. If a sample tube breaks while in the cold finger, do not attempt to remove it while cold. Instead, remove the liquid nitrogen or slush and let the dewar warm up, and then remove the spilled sample and broken tube. Attempting to remove a broken tube while frozen may break the cold finger.
2. It is generally better to freeze the sample first before placing it into the cold finger dewar.
3. Room air may condense into a sample tube and form a plug if it is open while frozen. The tube may then burst or spray sample solution when the tube is allowed to warm up. It is recommended that a cap be placed over the tube before freezing to reduce this risk. Always use caution when handling frozen tubes.
4. ***Do not drop the sample tube into the dewar.*** A drop of even 1 inch (25 mm) may break the inner cold finger. Place the sample tube into the dewar and very gently lower it all of the way to the bottom of the cold finger. Hint: a little ball of glass wool placed at the bottom of the cold finger will help prevent breakage. However, if the ball is too large, the sample may be moved above the excitation beam position. Do not try to force the glass wool ball into the cold finger or breakage may occur. *Note: Damage to dewars caused by breakage is not covered by the warranty.*

Installation

1. Insert the dewar holder (A) into the cuvette holder in the sample compartment.
2. The dewar (B) is shipped in a plastic net. Grasp the top edge of the dewar and pull it out of the net. Do not push on the cold finger as this may break the cold finger, or put finger prints in the optical path.

3. Very gently place the dewar into the holder (A). The cold finger extends into the cuvette holder. Fill the dewar with liquid nitrogen. **Caution:** do not try to fill the dewar all at once, or the liquid nitrogen will boil over due to the relative warmth of the dewar that is initially at room temperature.
4. Insert the sample tube (C) into the dewar. Place the foam lid (D) into the dewar to reduce the loss of liquid nitrogen. *Note:* the sample tube may fit entirely in the dewar below the foam cap. Figure 2 below with the sample tube poking above the cap is for illustration only.
5. Place the top hat lid over the open sample compartment and push down on it so that it fits snugly in the rim of the sample compartment. Figure 3 shows the top hat beside the dewar to show that the dewar has room above it under the top hat. Figure 4 shows the top hat pushed down to fit in the sample compartment rim.
6. Acquire the sample signal.

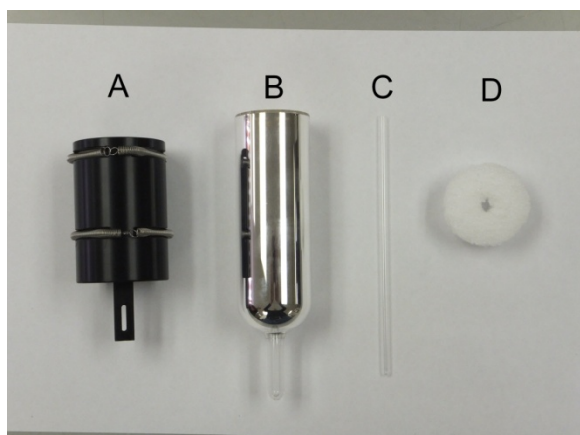


Figure 1: Components of the cold finger dewar



Figure 2: Cold finger dewar



Figure 3: Top hat beside dewar



Figure 4: Top hat in place

SWAPPING OPTIONAL SAMPLE HOLDERS IN THE EASYLIFE X

Tools required:

½ inch wrench

#1 Philips (cross-head) screwdriver

1. Close the EasyLife X program.
2. Turn off the power to the EasyLife console and remove the power, USB, and serial cables from the back of the EasyLife.
3. Remove any samples, cuvettes, filters, and polarizer holders from the EasyLife sample compartment.
4. Disconnect the BNC and 9-pin CPC cables from the back of the EasyLife X (at Pulsed Source Control).
5. On the back of the EasyLife X, lift up the LED from the mounting pins, and put it aside.
6. Facing the front of the EasyLife X, tip it over onto its left side (see figure 5).



Figure 5 - Placement of the EasyLife to access the sample holder bottom plate

7. Place a support surface 18 – 20 cm (7 – 8 inches) high against the bottom of the EasyLife to place the sample holder assembly on when removing or inserting it into the EasyLife, because it will be connected by the stirrer cable and coolant hoses during the process.

Removing the standard cuvette holder with stirrer and coolant hoses

8. Use a ½ inch wrench to loosen the nuts on the coolant hose fittings. Remove the nuts. Push the fittings inside the sample compartment. You may need to wiggle the fittings while pushing on them. You may also need to open the sample compartment lid and pull the coolant hoses and fittings inside the sample compartment.
9. Use a #1 Philips (cross-head) screwdriver to remove the four screws holding the sample holder bottom plate to the EasyLife X bottom plate (see figure 6). Hold the sample holder bottom plate when removing the last screw, as the sample holder as it may fall away from the EasyLife.

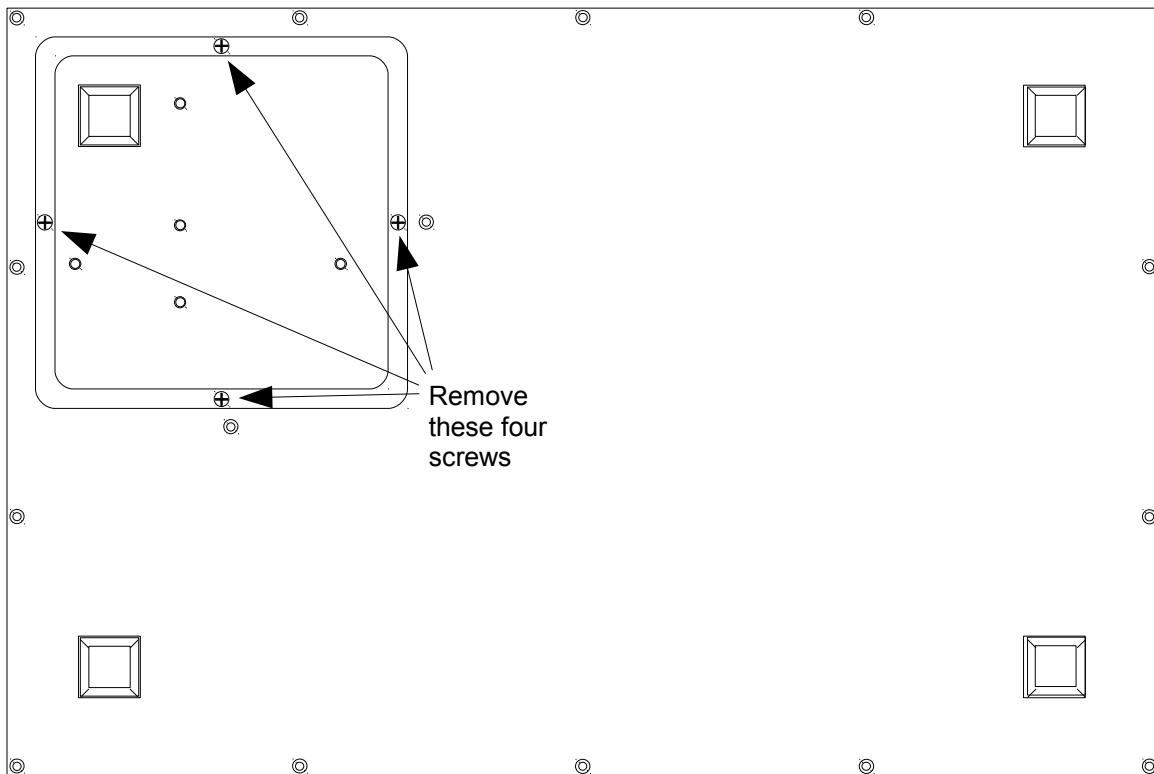


Figure 6 - Removal of the sample holder bottom plate

10. Check to see that one of the coolant hoses is not jammed beside one of the filter holder blocks.
11. As the sample holder bottom plate becomes loose from the EasyLife, check that the thin stirrer motor cable is not caught inside the EasyLife. Move it around a bit if it is caught.
12. On the connection between the cable from the stirrer motor and the cable from the EasyLife, grasp the connector connected to the stirrer in one hand and push down on the spring tab on the connector connected to the EasyLife with the other hand and pull the two connectors apart – you will have to wiggle the two connectors side-to-side while pulling them apart (see figure 7).

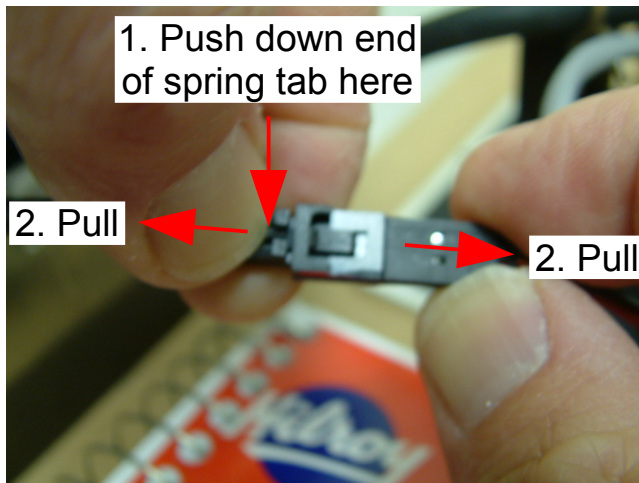


Figure 7 - Disconnecting the stirrer motor connection

13. Set the standard sample holder aside.
14. Push the wire connected to EasyLife back to the side of the sample compartment just behind the EasyLife bottom plate (see figure 8). Do **NOT** push the wire all the way back through its hole in the side of the sample compartment – then the EasyLife would have to be disassembled to retrieve it!

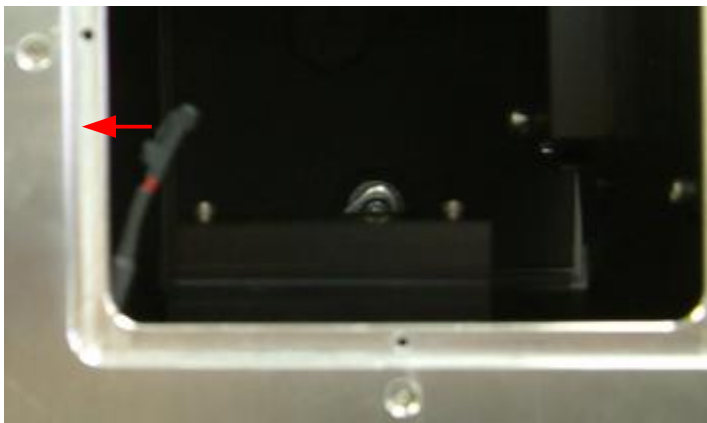


Figure 8 - Push the disconnected stirrer cable to the side of the sample compartment

Installing the solid sample holder

15. Open the lid of the sample compartment and put one hand inside to help guide the insertion of the sample holder.

16. Hold the solid sample holder bottom plate and slowly feed it through the hole in the EasyLife bottom plate so that the excitation and emission lens assemblies pass in front of their respective filter holder brackets. The rubber foot on the sample holder bottom plate should be close to the corner of the EasyLife bottom plate (see figure 6).
17. The sample holder bottom plate should fit into the recess in the EasyLife bottom plate. Screw in the four screws in the sample holder bottom plate.
18. Place and push in the rubber hole plugs into the Coolant 1 holes in the end plate.
19. Place the EasyLife upright on the table.
20. Hold the LED unit vertically (the peak wavelength label on top) and position it above the LED clips mount on the backside of the EasyLife, so the 3 pins on the LED unit are just above the clips. Press the LED unit down until it snaps into place in all three clips.
21. Connect one end of the 15" BNC cable to the LED unit and the other end to the 'PULSED SOURCE CONTROL' BNC connector on the backside of the EasyLife.
22. Connect one end of the 15" CPC cable to the LED unit and the other end to the 'PULSED SOURCE CONTROL' CPC connector on the backside of the EasyLife. Both ends have keyed connectors that you must rotate until you feel the connector move into the socket. Then, rotate the collar until it is in its locked position.
23. Reconnect the USB cable to the back of the EasyLife.
24. Connect the serial cable to the RS-232 port on the backside of the EasyLife.

Removing the solid sample holder

25. Follow steps 1 – 7 and 9 at the beginning of this section.
26. While holding the solid sample holder bottom plate, open the lid of the sample compartment, grasp the top edge of the emission lens mounting plate and slowly push the sample holder assembly out through the hole in the EasyLife bottom plate so that you can grasp the sample holder bottom plate securely.
27. Pull the sample holder assembly straight out from the EasyLife.
28. Remove the rubber hole plugs from the Coolant 1 holes by pushing out from the inside of the sample compartment.

Installing the standard cuvette holder with stirrer and coolant hoses

29. Place the standard cuvette holder assembly on the support surface with the cooling hoses facing you.

30. Gently pull the stirrer cable and connector from the corner of the sample compartment until it stops (see figure 8). Do **NOT** pull any more as this may damage electronics inside the EasyLife box.
31. Place the bottom of the spring connector on top of the bottom plate of the stirrer connector, so that the two connectors are horizontally aligned. Push the two connectors together slightly and make sure the bare pins in the stirrer connector fit inside the corresponding holes in the spring connector. On both connectors, grasp the connector ends and wires firmly and push the two connectors together until the stop on the spring connector slides under and is held by the bridge on the stirrer connector.
32. Put the left coolant hose and fitting through the hole in the EasyLife bottom plate and into the sample compartment.
33. Open the lid of the sample compartment and feed the coolant hose fitting into the upper Coolant 1 hole on the EasyLife end plate. You may have to wiggle the fitting while pushing it through the hole.
34. Similarly, feed the right coolant hose and fitting into the sample compartment and into the lower Coolant 1 hole. You may have to tilt the sample holder assembly toward the EasyLife for the fitting to reach and go into the hole (see figure 5).
35. Place the nuts over the fittings and turn the nuts until they are tight. You may have to hold the fitting ends, or hold the fittings and hoses inside the sample compartment while tightening the nuts.
36. Use a ½ inch wrench to tighten the nuts.
37. Hold the sample holder assembly bottom plate and slowly feed it through the hole in the EasyLife bottom plate so that the excitation and emission lens assemblies pass in front of their respective filter holder brackets. While doing so, push the stirrer cable and coolant hoses inside the sample compartment. The rubber foot on the sample holder bottom plate should be close to the corner of the EasyLife bottom plate (see figure 6).
38. The sample holder bottom plate should fit into the recess in the EasyLife bottom plate. Screw in the four screws in the sample holder bottom plate.
39. Place the EasyLife upright on the table.
40. Facing the back of the EasyLife, open the sample compartment lid and check that the coolant hoses are not kinked along any part of their length. Move the hoses around to relieve any kinks as necessary.
41. Follow steps 19 to 24 in the section 'Installing the solid sample holder'.

SOFTWARE INSTALLATION

If you have purchased a computer with the EasyLife X system from OBB, the software is already completely installed.

If you did not purchase a computer from OBB with your EasyLife X system, install it by the following:

1. Insert the EasyLife X CD in a CD drive. The AutoRun menu may take up to one minute to appear.
2. If your computer has an RS-232 port then do not install the USB-to-Serial Adapter driver and skip to step 3 (the PL-2303 USB-to-Serial driver consumes extra resources on the computer even if the cable adapter is not in use).

If your computer does not have an RS-232 port, then you will have to use the PL-2303 USB-to-Serial adapter cable and install the driver for this adapter cable. Do not connect the adapter cable to the computer at this time – wait until step 6 below (after all the software has been installed). Click on **USB-to-Serial Driver** and choose the defaults (**Next**, etc.). When this driver has finished installing, do not restart the computer, but wait until **Measurement Computing InstaCal** and **EasyLife X** have been installed.

3. In turn, click on each of the other menu items from top to bottom and choose all the defaults (**Next**, etc.).
 - a. During **Measurement Computing InstaCal** installation, you may get a message that the Windows Firewall has blocked the MccSkts Module and asks what you should do. Choose *Unblock this module*.
 - b. When **Measurement Computing InstaCal** has finished installing, do not restart the computer, but wait until **EasyLife X** has been installed, and then Windows Updates are checked for (step 4).
 - c. During installation of **EasyLife X**, you will be asked to agree to a License Agreement for the use of the software. You must agree to the License Agreement to install the software.
 - d. After **Measurement Computing InstaCal** and **EasyLife X** have been installed, click Exit on the AutoRun window.
4. Installation of EasyLife X also installs Microsoft .NET Framework 2.0 and Windows Installer 3.1 (KB893803) if these do not already exist on the computer. If the computer is connected to the Internet, click on the Windows desktop Start button and choose Windows Update to get any updates to these programs and others for your computer, if you do not already have them.
5. Remove the EasyLife X installation CD from the CD drive bay.
6. If your computer does not have an RS-232 port, then connect the PL-2303 USB-to-Serial adapter cable between an unused USB port on your computer and the RS-232 cable connected to the EasyLife.

7. Turn on the EasyLife.
8. Restart the computer.
9. You must run the InstaCal program once to initialize the interface:
 - a. On the desktop, click **Start, Programs, Measurement Computing, InstaCal**.
 - b. You should see the InstaCal program window, and then a **Plug and Play Detection** window saying a USB-1208FS device has been detected. Click OK. This board will be added to the PC Board list in the InstaCal window.
 - c. Close the InstaCal program. This board detection needs to be done only once at install and not again during EasyLife X operation.
10. Launch the EasyLife X program.
11. On the menu bar, click on **Configure, Hardware, Delay Gate Generator**.
 - a. From the Delay Gate Generator choice list, choose **Highland T550**.
 - b. Click on **Setup** for the Delay Gate Generator. From the COM port choice list select the COM port on your computer that is connected by RS-232 cable to the EasyLife.

If your computer does not have an RS-232 port and you are using the PL-2303 USB-to-Serial adapter cable, then Windows automatically configures the next available COM port for use by this Setup dialog. **Warning:** Do not disconnect the USB-to-Serial adapter cable while the computer is powered ON. This will cause Windows to increment the number of COM ports and change the one used for the EasyLife. You will then have to reconfigure the COM port used by the Delay gate Generator.
 - c. Leave the Command Delay at 0 ms.
 - d. Click **OK** to close the Delay Gate Setup dialog.
12. Configure, Hardware, Temperature Controller
 - a. If you do not want to control temperature, choose **none** in the choice list.
 - b. If you want to control the temperature of the sample, choose **QNW TLC50-EasyLife** in the choice list.
 - i. Click on the **Setup** button to show the **QNW TLC50-EasyLife Setup** dialog.
 - ii. From the COM port choice list select the COM port on your computer that is connected by RS-232 cable to the EasyLife.
 - iii. In the **Temperature Reached Detection** section, click on the **Use Internal Monitor** radio button and select the parameters for determining that a target temperature has been reached. When setting a temperature target, EasyLife X will wait until the temperature has reached the target temperature within the **Delta Temperature** for the **Stabilization Time** before starting or finishing acquiring data.

13. The Analog To Digital Converter (Interface) choice list should show MC USB-1208FS (the only board type allowed).
 - a. Click on **Setup** for the **Analog To Digital Converter (Interface)**.
 - b. The first text box should show that a board is connected and active.
 - c. Leave the **Channel** set to: **Diff. Channel 0 (CH0/CH1)**.
 - d. Click on the **Display** radio button beside **Min/Max** and leave the range as **-2048 to 2047**.
 - e. Click **OK** to close the ADC Setup dialog.
 - a. Click **OK** to close the Hardware Configuration dialog.

The EasyLife X installation is now complete!

GETTING STARTED

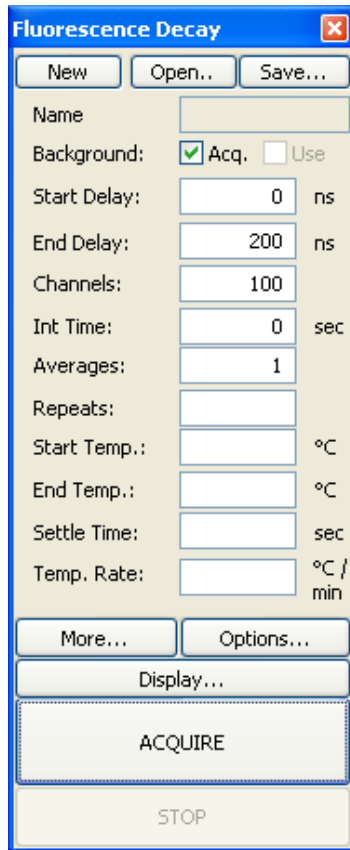
SAFETY PRECAUTIONS

1. The LED head emits visible or invisible radiation when the EasyLife X is turned ON and the LED head is connected to the main unit via BNC and CPC cables. Do not look directly inside the LED unit when it is powered!
2. Always switch OFF the EasyLife X before removing or installing the LED head.
3. The sample compartment has a mechanical safety shutter, which blocks the excitation light when the sample compartment lid is open. Always wear safety glasses appropriate for the wavelength range emitted by the LED if you are going to defeat the safety shutter.
4. Never attempt to use LED heads other than those supplied by OBB.
5. Do not disassemble the EasyLife X or the LED head – there is no maintenance required for either unit. Unauthorized attempts to access and interfere with internal components may cause electrical and radiation hazards and may void the warranty.

BASIC OPERATION

Warning: Do not disconnect the USB-to-Serial adapter cable while the computer is powered ON. This will cause Windows to increment the number of COM ports and change the one used for the EasyLife X. You will then have to reconfigure the COM port used by the Delay gate Generator.

- A. You will need the following items to carry out a fluorescence lifetime measurement:
1. Fluorescent sample, which can be one of the following:
 - Solution in a 10 mm x 10 mm cuvette
 - Solution in a microcuvette, which is adaptable to 10 mm x 10 mm cuvette holder
 - Solid sample (e.g. slide) mounted on an EL-1000 solid sample holder (optional accessory)
 - Sample placed in a K-158 liquid nitrogen dewar (optional accessory)
 2. Scatterer solution (to acquire the instrument response function, IRF), which can be one of the following:
 - Colloidal silica (available as Ludox from Aldrich) highly diluted in water
 - Glycogen in water
 - In an emergency situation, powdered non-dairy creamer in water can also be used
 3. Emission filter (typically a cut-off type) to cut off the excitation light
 4. K-136 set of neutral density filters (optional, but helpful accessory)
- B. To prepare the instrument for the measurement:
1. Turn the EasyLife X power switch ON. The red LED power indicator on the EasyLife X front panel should be lit.
 2. Turn the computer ON.
 3. Make sure the emission slit is partly open, e.g. start with a 3-mm opening (2 full turns = 1 mm).
 4. Place the sample in the sample holder
 5. Place the emission filter in the filter holder
- C. The next step is to set the acquisition parameters in EasyLife X. The following example is based on a 1 nM fluorescein solution in 0.1 N NaOH.
1. For a basic experiment, **Configure, Hardware**, choose **none** from the **Temperature Controller** choice list. **OK**.
 2. Click **Acquire** on the menu bar and select **Fluorescence Decay** on the drop down menu. This brings up the **Fluorescence Decay** dialog box. Set the required parameters:



- **Background:** check the Acq. box.
- **Start Delay** (ns). Enter a value, which is about 3 to 5 ns in front of the excitation pulse. If you don't know where the excitation pulse is, enter 0.
- **End Delay** (ns). Add about 5 to 8 times your sample lifetime to the **Start Delay** and enter here. If you have no idea what the lifetime of your sample is, enter a relatively large value, e.g. 200 ns.
- **Channels.** This is the number of data points defining the decay curve. Enter a desired value, e.g. 200 (use less channels to speed up the experiment and more channels to improve accuracy of the measurement).
- **Int Time** (s). This controls the time over which the output signal is averaged at each time delay step. Enter 0 s for a quick scan. Use a lower value to speed up the experiment or a larger value to improve signal to noise.
- **Averages.** Number of scans to be acquired and averaged. Enter 1 for a quick scan, use more averages to improve the signal to noise.
- Leave the other text boxes blank.

3. The **Fluorescence Decay** dialog box has three buttons **More...**, **Display...**, and **Options...** which lead to further dialog boxes. See **Common Acquisition Controls** and **Fluorescence Decay** for more information.

D. In order to acquire the fluorescence decay:

1. Click **ACQUIRE (PREP)** and then **START**, which acquires the background, and then continues to acquire the decay curve. **Note:** The green Trigger LED on the front panel of the EasyLife X should be lit now. The **Background Acq** checkbox will then dim, and the **Use** box will be checked.

After the scan is completed, you will likely see a decay trace, which will look like one of 3 traces depicted in figure 9.

First of all, it's obvious that the time window is much too large. We deliberately selected a broad time range in order to locate the signal. Now we can narrow the time window to a meaningful range. In this case we go back to **Acquire** menu and change **Start Delay** to 65 ns and **End Delay** to 95 ns. A rule of thumb is to use the total time range (i.e. range = **End Delay** - **Start Delay**) of approx. 5 to 8 times the lifetime value. The lifetime can be approximated as the full width of the decay trace at half maximum. The **Start Delay** should be a few nanoseconds in front of the excitation pulse, so the pre-pulse baseline could be determined.

If the intensity is too high, the decay will look like trace A, i.e. it will have a truncated top part. If this is the case, reduce the slit width and repeat the measurement (Step D) with the new **Start Delay** and **End Delay** settings. Alternatively, you may use a neutral density filter (optional) to reduce the intensity. Keep in mind that a filter with optical density (OD) of 0.3 will reduce the intensity approximately 2 times.

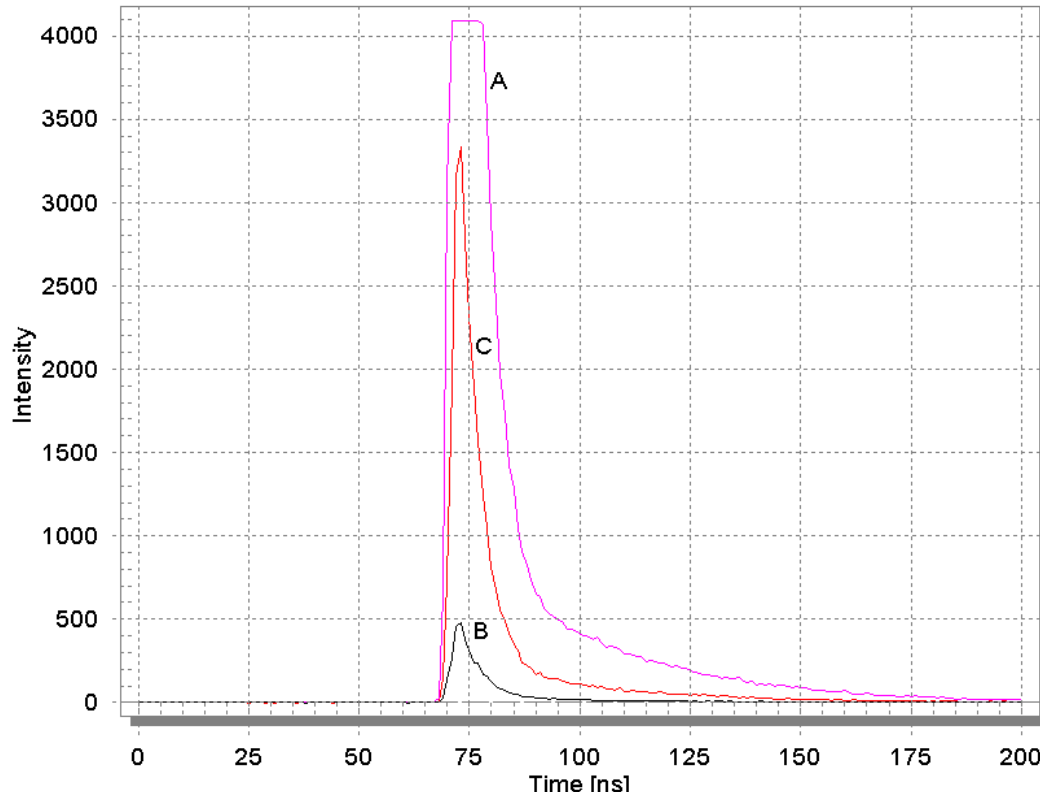


Figure 9. Three different outcomes of decay curve measurement: A – intensity too high; B – intensity too low; C – good level of intensity. The time range is too broad for all 3 traces.

If the intensity is too low (i.e. figure 9 trace B), you may want to increase the slit opening to admit more light to the detector. Open the slit wider and repeat the measurement (Step D) with the new **Start Delay** and **End Delay** settings. If the slit is fully open (6 mm) and the intensity is still low, you can decrease the gain setting in the Hardware Configuration, ADC Setup dialog to allow a small signal to fill the allowed range. The way to improve the signal to noise is to increase the integration time, use more averages, or both.

If your decay looks like trace C in figure 1, the intensity is adequate, so you can just repeat the measurement (Step D) with the new **Start Delay** and **End Delay** settings. The final decay should look like trace A in figure 10.

- E. The next step is to record the Instrument Response Function (IRF) using the scatterer solution. To record the IRF do the following:
1. Remove the sample and place the cuvette with scatterer in the sample holder.
 2. Remove the emission filter.
 3. **DO NOT CHANGE** such parameters as **Channels** or **Start Delay** and **End Delay**.

You may change **Integration Time** or **Averages** if required.

4. Click **ACQUIRE (PREP)** and then **START** to measure the IRF.

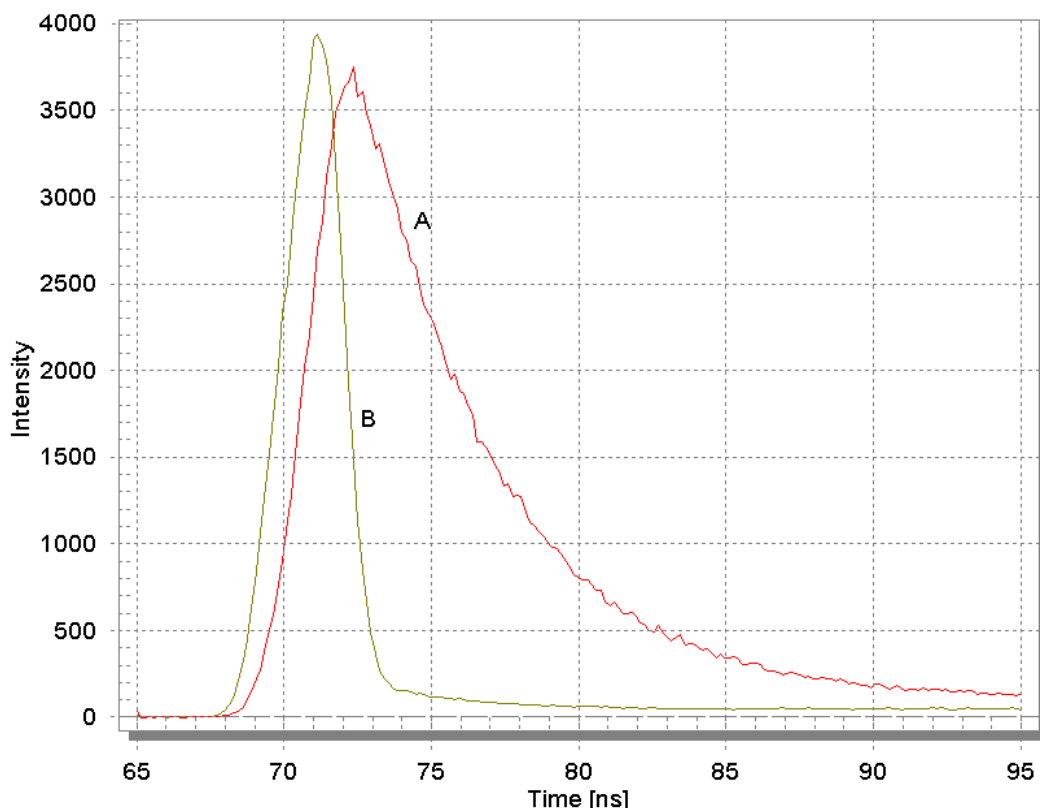
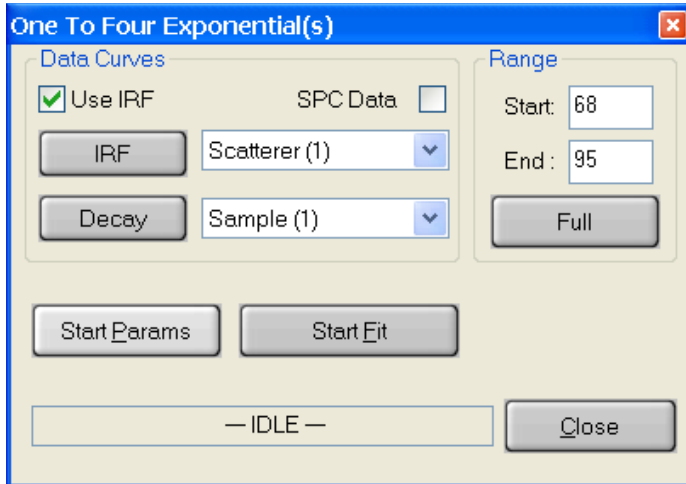


Figure 10. A typical outcome of correctly conducted fluorescence decay measurement. A – fluorescence decay; B – instrument response function (IRF).

If the intensity is too high (truncated top), lower the concentration of the scatterer or place a neutral density filter in the excitation or emission filter holder and repeat the measurement. Avoid changing the emission slit opening in order to adjust the intensity of the scatterer, as it may change the size of the illuminated spot on the PMT photocathode leading to potential undesirable artifacts.

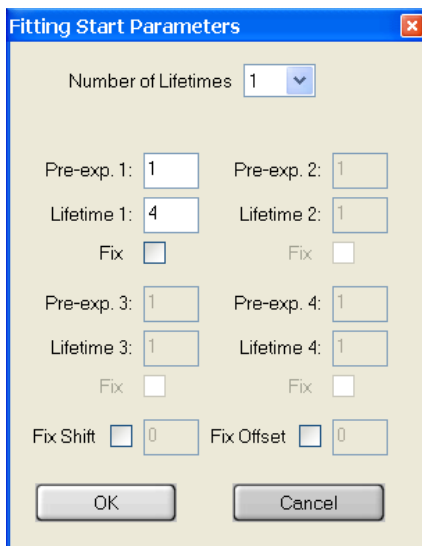
If the intensity is too low, increase the concentration of the scatterer and repeat the measurement. As stated above, avoid changing the emission slit opening to adjust the intensity of the scatterer.

- F. Once the fluorescence decay and the IRF have been measured (figure 10), the fluorescence lifetime can be determined with the use of analysis software.
 1. Click **Math** on the menu bar, then **Data Analysis** on the drop down menu and across to highlight **1 To 4 Exp. Lifetime** on the second drop down menu. Click on this to bring up the **One To Four Exponential(s)** dialog box shown below. Make the following selections:



- **Use IRF** – controls whether the instrument response function will be used in the analysis. In most cases the IRF is used except when the lifetime of the sample is very long compared to the temporal width of the LED pulse. Toggle this option on or off by checking/unchecking the box.

- **SPC Data** – Used only when single photon counting data has been imported for analysis. Toggle this option on or off by checking/un-checking the box. Leave it unchecked for GX-EL data.
- **IRF** - Select the scatterer decay curve by clicking on the name of the appropriate curve in the legend. Click on the IRF button. The name of the curve will appear in the window.
- **Decay** - Select the sample decay curve by clicking on the name of the appropriate curve in the legend. Click on the decay button. The name of the curve will appear in the window.
- Click **Start Parameters** to enter the fitting parameters.



- **Number of Lifetimes** - The number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4.
- **Pre-exp., Lifetime, Fix** - For each of the lifetimes to be used in the fit an initial guess for the lifetime and the pre-exponential factor (**Pre-exp**) must be given. Only the relative values of the pre-exponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are too far off. If this occurs, try changing the starting values.
- **Fix Shift** – The shift parameter accounts for some instrumental artifacts, such as the ‘color shift’ effect or potential instrumental instabilities. It corrects for a zero-time shift of the excitation pulse relative to the decay (see the Data Analysis chapter for details). The shift parameter may be fixed at the input

- value (in nanoseconds) or allowed to float in the fit. Toggle this option on or off by clicking on it. A value of 0.0 is usually a good initial guess.
- **Fix Offset** – Since fluorescence decays are obtained with an analog measurement, there will always be a small offset over imposed on the decay signal. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it.
 - Click **OK** to accept fitting parameters and exit the dialog.
 - **Start, End** – Position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be shown on the screen in a different color. Alternatively, the start delay and end delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard.
 - Click **Full** if you want to analyze the decay over the entire time range.
 - Click **Start fit** to initiate lifetime analysis.

The progress of the analysis is shown in the box marked -IDLE- in the above illustration. The fit may be stopped at any time by clicking the **STOP** button.

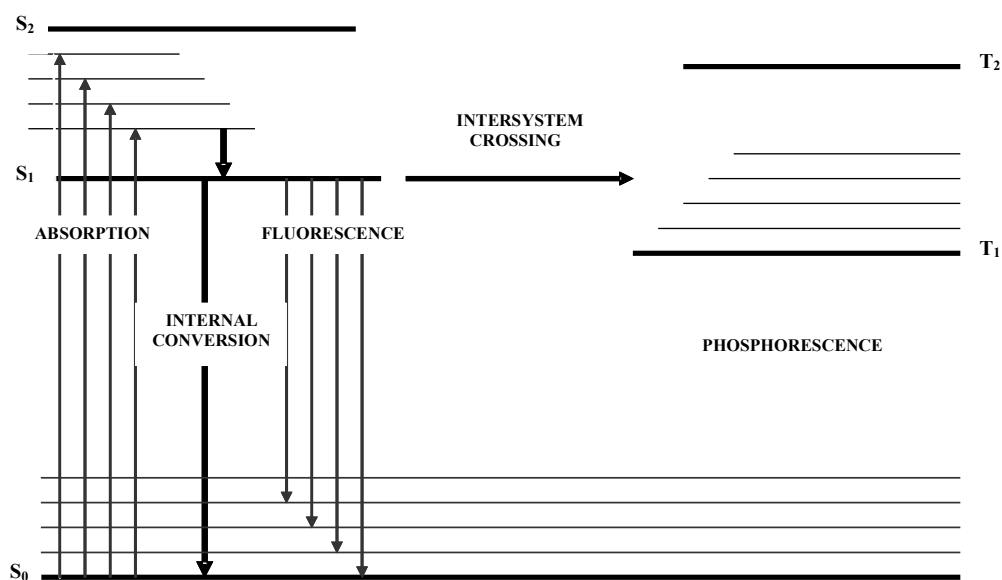
The results of the fit are shown in two different ways. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace. The values of the parameters found in the fit and some statistical information on the fit are entered into a text editor, which pops up in a window at the end of the fit.

Click **Close** to exit this analysis mode.

SIMPLE THEORY OF FLUORESCENCE DECAY

Fluorescence is the emission of light from a molecule resulting from a transition from one electronic state to a lower electronic state of the same multiplicity. The most commonly observed fluorescence from organic molecules is caused by a transition from an excited singlet state to the ground singlet state. A highly simplified *Jablonski diagram* illustrates the processes involved.

The various processes that occur in the excited state of a molecule include:



- Emission of a photon - this results in fluorescence if the transition is between states of the same multiplicity and in phosphorescence if the transition is between states of different multiplicities.
- Internal conversion (IC) - the non-radiative loss of excitation energy through conversion of an excited state to a highly excited vibrational level of a lower state of the same multiplicity. The excitation energy in this case dissipates as heat.
- Inter-system crossing (ISC) - the non-radiative transition from a singlet state to a triplet state (or vice versa).
- Quenching - the non-radiative loss of excitation energy via encounter with another molecule (quencher).

Each of these processes has a characteristic time in which it occurs. Emission and internal conversion typically occur on the picosecond through hundreds of nanoseconds time scale. Inter-system crossing also occurs in this time range when it is an effective mechanism for de-excitation of the excited state. Quenching is concentration-dependent, but commonly will compete with emission at millimolar and higher concentrations for good quenchers, as the quenching process is a diffusion-controlled process. Lastly, the molecule may undergo a photochemical conversion such as dissociation.

The Kinetic Scheme

It is possible to describe the de-excitation of a simple fluorescent molecule A by a first order kinetic scheme.

PROCESS	RATE CONSTANT	DESCRIPTION
${}^1A^* \rightarrow A$	k_r	radiative emission
${}^1A^* \rightarrow A$	k_{ic}	internal conversion
${}^1A^* \rightarrow {}^3A^*$	k_{isc}	inter-system crossing
${}^1A^* \rightarrow B + C$	k_d	dissociation
${}^1A^* + Q \rightarrow A$	k_q	quenching

Scheme 1

The rate of loss of the excited singlet state may thus be given by

$$\frac{-d[{}^1A^*]}{dt} = [{}^1A^*] \{ k_r + k_{ic} + k_{isc} + k_d + k_q[Q] \} \quad \text{Eq. 1}$$

This equation may be solved to yield an exponential decay law:

$$[{}^1A^*] = [{}^1A_0^*] \exp\left(\frac{-t}{\tau}\right) \quad \text{Eq. 2}$$

where $[{}^1A_0^*]$ is the initial excited state concentration and the lifetime τ is given by:

$$\tau = \frac{1}{\{ k_r + k_{ic} + k_{isc} + k_d + k_q[Q] \}} \quad \text{Eq. 3}$$

If other processes such as energy transfer or excimer formation occur, this kinetic scheme becomes more complex and the decay law may become non-exponential.

Convolution

A fluorescence decay $D(t)$ of a single fluorophore in homogeneous solution can be usually described as a single-exponential function in accordance with the 1st order kinetic equation 2:

$$D(t) = a \exp\left(-\frac{t}{\tau}\right) \quad \text{Eq. 4}$$

where τ is the lifetime defined in Eq. 3.

If the sample contains more than one fluorophore or the fluorophore is contained in a heterogeneous environment, the fluorescence decay can be better described as a sum of exponential functions:

$$D(t) = \sum_{i=1}^n a_i \exp\left(-\frac{t}{\tau_i}\right) \quad \text{Eq. 5}$$

where τ_i are fluorescence lifetimes of various fluorescent forms and a_i are corresponding pre-exponential factors. The interpretation of a_i depends on the nature of the sample: if the emission comes from a single fluorophore in different conformational forms or environments, the a_i factors are proportional to respective populations. If multiple lifetimes result from the presence of several fluorophores in the sample, the a_i pre-exponential factors will depend not only on their populations, but also on radiative probability constants and molar extinction coefficients of respective fluorophores.

The decay curves described by equations 4 and 5 will only be observed if the sample is excited by an infinitely narrow pulse. In most cases the temporal width of the excitation pulse cannot be neglected and the observed decay $D_{\text{obs}}(t)$ will be distorted by convolution with an instrument response function $L(t)$ in accordance with the following equation:

$$D_{\text{obs}}(t) = \int_0^t L(t-s)D(s)ds \quad \text{Eq. 6}$$

The instrument response function (IRF) $L(t)$ can be determined experimentally by using a scatterer solution instead of a sample. The IRF accounts for the shape of the excitation pulse and for the temporal response of the detection system. Figure 11 shows an example of the traces obtained in a typical fluorescence decay experiment.

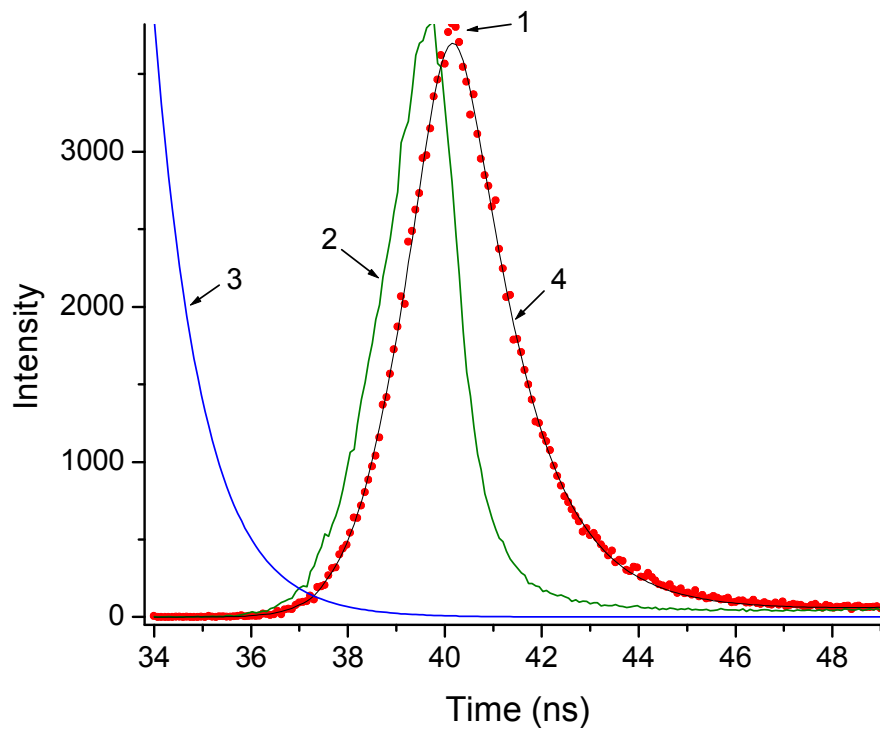


Figure 11. Illustration of the convolution effect as described in Eq. 6: 1 - experimental decay $D_{obs}(t)$; 2 - instrument response function $L(t)$; 3 – undistorted exponential decay $D(t)$; 4 – the best fit to experimental decay, i.e. convolution of curves 2 and 3.

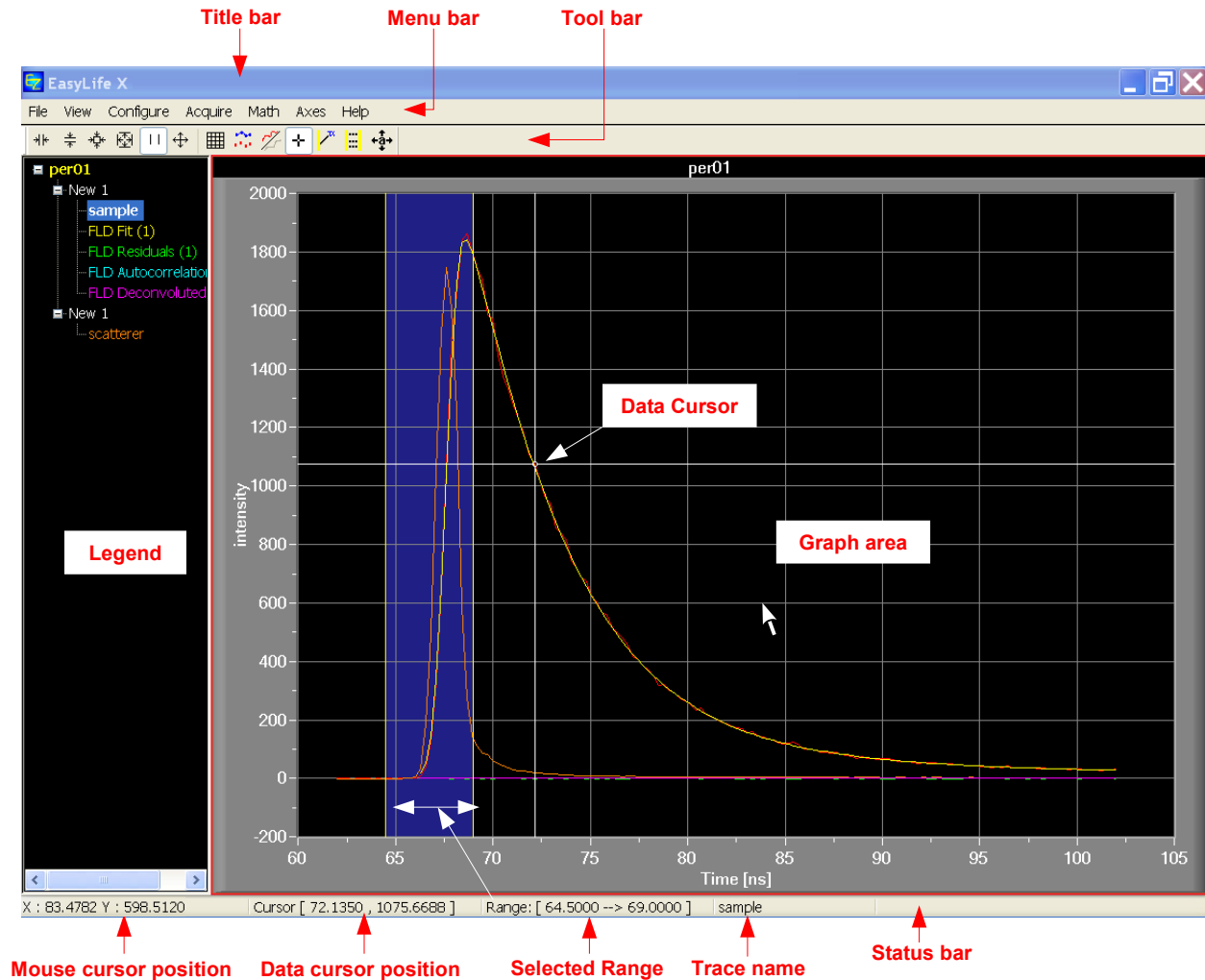
Once the $D_{obs}(t)$ and $L(t)$ have been measured, the analysis software performs iterative reconvolution according to Eq. 6 varying the fit parameters a_i and τ_i until the best fit to the experimental decay is obtained.





SOFTWARE

A QUICK TOUR OF EASYLIFE X

Note: in this manual the terms *curve* and *trace* are used interchangeably.

The principal parts of the EasyLife X screen are shown here and described below.



Across the top of the window runs the Title Bar. At the right end of the Title bar are three Windows buttons for Minimizing , Reducing , or Maximizing , and Closing  the window. These four commands are also available by right-clicking on the Title Bar. To move a window occupying less than the full monitor screen, click and drag on the title bar. To resize a window occupying less than the full monitor screen, click and drag on an edge or corner of the window.

Beneath the Title Bar is the Menu Bar. Each heading in the Menu Bar contains a group of related commands. Below the Menu Bar is the Toolbar. The icons on the Toolbar provide instant access to a number of the most frequently used commands.

Beneath the Menu Bar and Toolbar is the space where acquisitions are opened for the viewing and processing of traces. Multiple acquisitions can be opened within the Workspace although only one will be active at any given time.

The Legend along the left side lists the names of the records, groups and traces. A record is comprised of one or more groups, each containing one or more traces. The dividing bar between the legend and graph area can be moved by clicking and dragging it with the mouse cursor. Right-clicking on a acquisition, group or trace name in the Legend will show different command menus (see Legend submenus below). Double-clicking on a acquisition or group name in the Legend will contract or expand the list below it. You can also click on a + sign in front of an acquisition or group name to expand it, or click on a - sign in front of an acquisition or group name to minimize it.

The right side of this window has an area where traces are plotted in Graph Mode, or displayed in spreadsheet form in Grid Mode. Right-clicking on the graph or grid area will show a menu of relevant commands (see Graph submenus below).

Across the bottom of the Workspace is the Status Bar that gives information about cursor positions and trace names. From left to right are fields for the current mouse cursor position (or most recent position if the mouse cursor has left the graph area), the data cursor position, the marked range, and the name of the trace that the mouse cursor is pointing to (if the mouse cursor is not near a data point on a trace, this field will be blank).

TOOLBAR

Note: whether the graph is at Full Autoscale or displaying an expanded region, the arrow keys on the keyboard can shift the graph in any direction as long as the Data Cursor is not active. When the Data Cursor is active, then the arrow keys move the Data Cursor around and Shift+arrow keys move the graph around. When the Graph Window is first opened, a new graph must be rescaled in any way before it can be moved around.

Zoom X

Zoom X causes the selected region to fill the entire X-Axis in the window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse left or right in the graph area. Releasing the mouse button will expand the desired region.


The X-Axis is scaled in six modes. The other modes are:

Full Autoscale on the Axes menu and toolbar 

2x X-Zoom In on Axes menu

2x X-Zoom Out on Axes menu

Fixed X-Min & Max on Axes menu

Zoom Rect on the toolbar 

Zoom Y

Zoom Y causes a selected region to fill the entire Y-Axis in the window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse up or down in the graph area. Releasing the mouse button will expand the desired region.


The Y-Axis is scaled in one of six modes. A symbol will appear in the Axes menu next to Full Autoscale, Autoscale from 0, Fixed Y-Min & Max, and Logarithmic Y-scale depending on the mode in use. The other modes are:

Full Autoscale on the Axes menu and toolbar 

Autoscale from 0 on the Axes menu

Fixed Y-Min & Max on the Axes menu

Logarithmic Y-Scale on the Axes menu

Zoom Rect on the toolbar 


 **Zoom Rect**

Zoom Rect causes a selected rectangular region to fill the entire window. The toolbar shortcut is the only location to use this function. Select the icon, click and drag the mouse in the graph area. Releasing the mouse button will expand the desired region.

 **Full Autoscale**

Displays the full range of data. This mode is also available on the Axes menu.

 **Range Toggle**

Some math functions are performed on a selected region of a curve (a subset of the X values). To select this region, first choose the target trace by clicking on its name in the legend. Then select the **Range Toggle** icon () from the graph toolbar and use the mouse to click and drag within the graph display over the desired region of the curve. For more precise control, you can then enter *Low X* and *High X* values into the text boxes provided. The selected region will be highlighted, and the desired math value will be displayed. The math function dialog box can be left open while different regions are selected, and math values, when displayed, will change dynamically.

 **Pan Graph**

Select this icon then click and drag on the graph and the graph will move with the cursor.

 **Grid View**

Toggles the display between Grid mode and Graph mode. In grid mode curve(s) will be presented numerically on a grid in spreadsheet fashion.

 **Change Plot Mode**

This icon can be pressed 3 times to get different plot modes: dot, dot+line, line.

 **Toggle Trace Visibility**

Toggles the visibility OFF/ON of the selected traces or groups.

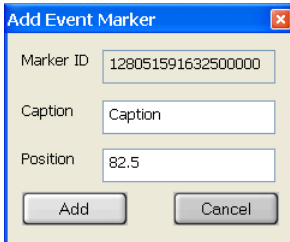
Data Cursor toggle

Toggles the Data Cursor ON/OFF. The Data Cursor highlights a data point with a small circle at the intersection of a horizontal and a vertical line. When the Data Cursor is enabled the trace in the legend that it is assigned to will become italic. Use the left or right keys to move the Data Cursor along a trace, and the up or down keys to switch between traces. The Data Cursor can also be enabled/disabled by double-clicking on a trace in the Legend. Each record can have its own Data Cursor.

Note: if you use the up or down keys to switch between traces, then you must use a left or right key to move the data cursor before the data cursor position will be updated on the status bar.

Note: if you click outside the graph area then the graph area is no longer the active window and the Data Cursor will not move in response to arrow keys. You must click on the graph area again to make it and the Data Cursor active.

Add Event Marker



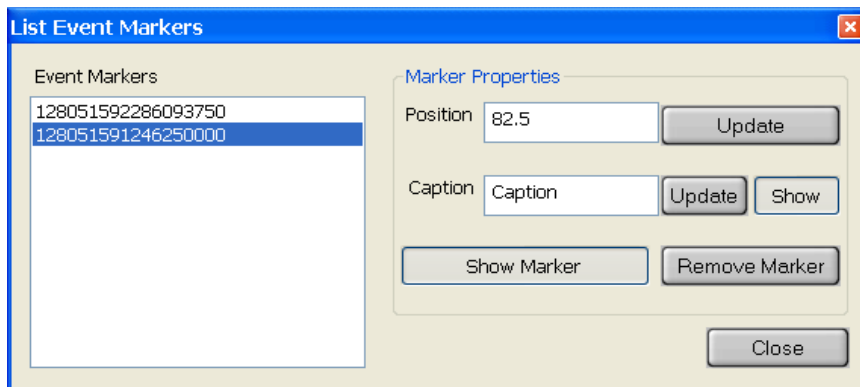
Opens the “Add Event Marker” dialog, where events can be manually created.

Caption: Enter the caption label.

Position: Enter the X-axis value for the placement of the event marker.

A vertical line will be placed at **Position** on the graph area and the **Caption** will be connected to this line by an arrow.

List Event Marker



Opens the “List Event Markers” dialog where events can be edited, deleted or hidden. Events are attached to their respective records.

Event Markers: Shows a list of the event markers. A unique numerical ID is created for each event. When the dialog opens, you must click on a specific event in the list to make all other buttons active.

Position: Position refers to time along the X-axis. If you edit the position, then you must click the position **Update** button to the right to accept this change.

Caption: You can edit the caption for that event marker. You must then click the caption **Update** button to accept this change. The caption **Show** button will toggle the visibility of the caption and arrow off/on.

Show Marker: This button will toggle the visibility of the selected event marker on/off.

Remove Marker: This button will delete the selected event marker.



Move Annotation Captions

Allows you to move the caption of a marker.

To reposition the arrow move the caption to a Y value where you would like the arrow and double-click the caption. This will create a horizontal arrow using the new position of the caption.

You can activate the **Move Annotation Captions** by double-clicking on a caption.

LEGEND MENUS

A record is comprised of one or more groups, each containing one or more traces.

Right-click on a record, group, or trace name to show the following menus, respectively.

Record:

Rename: renames the record

Close: closes the record

New Group: creates a new group

Import Group: imports a file as a new group. The group file format can either be EasyLife Group File (*.elg), or EasyLife Text Files (*.txt).

Export Record: exports all groups, traces and events in a record to a file using the formats shown at **File\Open Record**

Import Events: import events from a file

Export Events: export events to a file using the format shown at **File\Open Record** (active only if there are events)

Unmatch events: this will unmatch the events from a trace and removes the caption merging

Axis Properties: this makes adding/deleting X and Y axes and editing the axes titles possible. Note: the Z-axis is not available at this time.

Group

Toggle Visibility: toggles the visibility of traces inside the selected group(s)

Hide All/Show All: hide/show all the traces in the selected group(s)

Plot Mode: specifies the plot mode of the traces inside the group

Rename: renames the group

Delete: remove the selected group(s) and all traces inside

Export Group: exports the group to a file using either the EasyLife Group File (*.elg), or EasyLife Text Files (*.txt) format.

Axis Properties: this makes adding/deleting X and Y axes and editing the axes titles possible. Note: the Z-axis is not available at this time.

Trace:

Toggle Visibility: changes the visibility of the selected trace(s)

Color: change the color of the trace

Plot Mode: change the plot mode for the selected traces

Rename: rename the trace (same as clicking twice)

Delete: remove the selected trace(s)

Trace Math: traces can be arithmetically combined and data can be fitted, smoothed, averaged, integrated, normalized, differentiated, etc... See the description of the Trace Math Commands

Align Events: this will use the points from the trace to match them to the events that were imported or unmatched

Properties: shows some trace properties. Not fully implemented at this time.

NOTE: some Group/Trace commands are available when selecting multiple items

GRAPH MENUS

Right-click in the graph area to show the following menu.

File: Shows the same menu as the main File menu.

View: Shows the same menu as the main View menu.

Graph: here you can reach the following submenus:

Axes: Shows the same menu as the main Axes menu.


Math: Shows the same menu as the main Math menu.

Event Markers: **Add** and **List** show the same dialogs as seen by clicking the **Add Event Marker** and **Edit Event Markers** toolbar icons.

Help: : Shows the same menu as the main Help menu.

FILE MENU

Open Record

Use the **Open Record** command to open one or more data files stored on disk or on the network. The **Open** dialog box is a standard Windows Open dialog and shows the file name, and by clicking on the View Menu icon, , and choosing Details, you can also show the time and date the file was last saved, and the file size.

To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

Note: EasyLife X allows a maximum of 16 acquisitions to be open at one time.

File name

Type a file name in the text box, or select the file name from the list.

Files of type

You can choose to show EasyLife Record Files (*.elr), EasyLife Text Files (*.txt), or Aligned Text Files (*.atf). FelixGX Record Files (*.elr) are saved in a binary format and preserve groups and group names, events, and axes names. Felix Text Files (*.txt), and Aligned Text Files (*.atf) are tab delimited text (ASCII) and remove group name and axes name information – when reopened, all traces appear in one group named “*Group-1*” with unlabeled axes. EasyLife Text Files (*.txt), and Aligned Text Files (*.atf) must have the following format.

LINE 0: Aligned Text Files (*.atf) have “ALIGNED_FORMAT” inserted before the first line of the following.

LINE 1: number of curves, followed by carriage return/line feed

LINE 2: number of data pairs for first curve, two tab characters, number of data pairs for second curve... This line must end with one tab character and carriage return/line feed after the number of data points for the last curve.

LINE 3: first curve label, two tab characters, second curve label... This line must end with one tab character and carriage return/line feed after the curve label for the last curve.

LINE 4: first curve X-Axis label, tab character, first curve Y-Axis label, tab character, second curve X-Axis label, tab character, second curve Y-Axis label... ending with carriage return/line feed after the Y-axis label for the last curve.

LINES 5-N: first curve X value, tab character, first curve Y value, tab character, second curve X value, tab character, second curve Y value... ending with carriage return/line feed.

Note: All unused data fields must be padded with tab characters. For example, if the first curve has fewer data pairs than the second, tab pairs must be used prior to the remaining second curve values. However, if latter curves have fewer data points than preceding curves, do not pad these data fields.

Note: In *.txt format files all curves start data in line 5. In *.atf (*aligned text format*) files, the X-Y data pairs for each curve are placed so same X-data are placed on the same row starting with the smallest X-data in row 5.

Maximum number of characters per field is 32.

Maximum number of curves (or data pairs) is 30.

Maximum number of data pairs for any curve is unlimited.

For *.txt files the file name will be displayed as the acquisition name. The information on line 4 (axes labels) will not be displayed on the screen. To label the axes, select the group from the legend and use the Axes/Edit Axis Properties dialog.

Example of a *.txt format data file:

The following ASCII file contains data for three curves. The first is called TEST1 and has 3 data pairs representing Time versus Counts. The second is called TEST2 and has 5 data pairs representing Time versus Intensity. The third is called TEST3 and has 4 data pairs representing Wavelength versus Intensity.

```

3 \R \N
3 \T \T 5 \T \T 4 \T \R \N
TEST1 \T \T TEST2 \T \T TEST3 \T \R \N
Time \T Counts \T Time \T Intensity \T Wavelength \T Intensity \R \N
10 \T 100 \T 1 \T 160 \T 340 \T 1655 \R \N
20 \T 110 \T 2 \T 165 \T 345 \T 1650 \R \N
30 \T 120 \T 3 \T 170 \T 350 \T 1630 \R \N
\T \T 4 \T 175 \T 355 \T 1570 \R \N
\T \T 5 \T 180 \R \N

```

\R is a carriage return, ASCII code 10.

\N is a line feed, ASCII code 13.

\T is a tab character, ASCII code 9.

Note: Spaces are used in this example for clarity. No spaces would be used in the ASCII file. Tab characters are the only acceptable field delimiters.

If the file contents of the sample file above is viewed in a text editor (such as Windows Notepad), it would appear as follows:

3

3 5 4

TEST1 TEST2 TEST3

Time Counts Time Intensity Wavelength Intensity

10 100 1 160 340 1655


20 110 2 165 345 1650

30 120 3 170 350 1630

4 175 355 1570

5 180

Save Record As...

Use the **Save Record As** command to name and save the contents of the active acquisition. The **Save** dialog box is a standard Windows Save dialog and shows the file name, and by clicking on the View Menu icon, , and choosing Details, you can also show the time and date and file size of existing files. Select **Save**, or select **Cancel** whereby the dialog box will close and nothing will be saved.

To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

File name

Type a file name in the text box, or select the file name from the list. If you try to save as an existing filename, you will get a message that the file already exists. You must answer **Yes** to this question to save the data under an existing filename. Otherwise, choose **No** and enter a new filename.

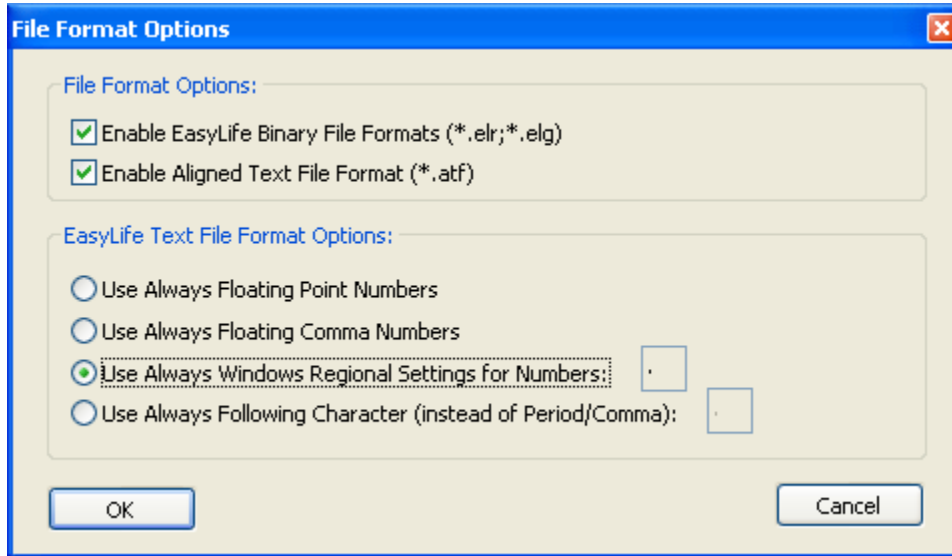
Save as type

You can choose to save as a EasyLife Record File (*.elr), EasyLife Text File (*.txt), or Aligned Text File (*.atf). FelixGX Record Files (*.elr) are saved in a binary format and preserve groups and group names, events, and axes names. EasyLife Text File (*.txt) and Aligned Text File (*.atf) are tab delimited text (ASCII) format and remove group name and axes name information – when reopened, all traces appear in one group with unlabeled axes.

Save: saves the file.

Cancel: the dialog box will close and nothing will be saved.

File Format Options



Sets the default file formats for saving/exporting and opening/importing data.

File Format Options

Enable EasyLife Binary File Formats (*.elr, *.elg): EasyLife Record Files (*.elr) and EasyLife Group Files (*.elg) are saved in a binary format and preserve groups and group names, events, and axes names.

Enable Aligned Text File Format (*.atf): See **File, Open Record** for a description of the *.atf format.

If both of the above options are disabled, then only the EasyLife Text format (*.txt) can be used for saving/exporting and opening/importing data. See **File, Open Record** for a description of the *.txt format.

EasyLife Text File Format Options

Set the type of decimal point to be used.

Always use Floating Point Numbers: Uses a period for the decimal point.

Always use Floating Comma Numbers: Uses a comma for the decimal point.

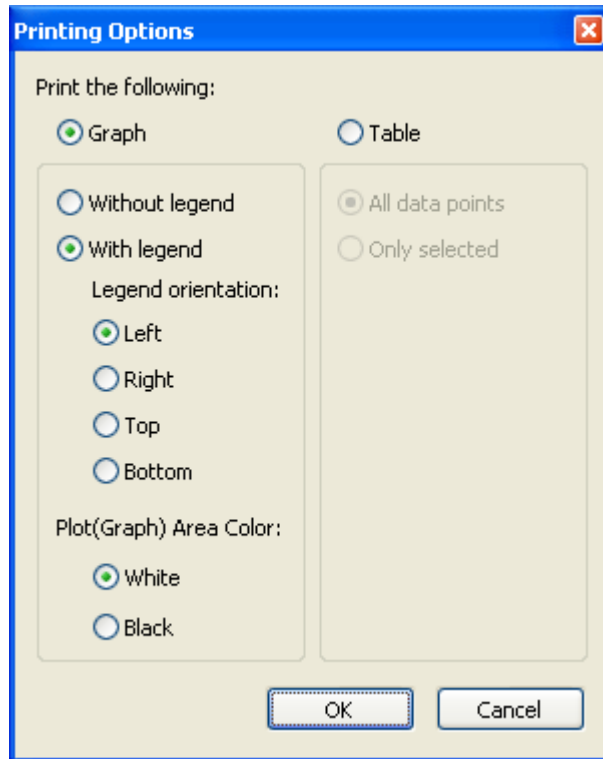
Use Always Windows Regional Settings for Numbers: Uses the decimal point format set by Windows Regional Settings.

Use Always Following Character (instead of Period/Comma): Uses the character entered into the text box for the decimal point.

Close All

Closes all open records. There is no warning if you have not (re)saved any data if you have altered or acquired any of the data.

Print Options...



This dialog sets the options for printing a graph or table. Only those active traces in the active record will be printed in a graph or a table. If the Plot Area Color is made white, then a white trace will print as a black line.

Print

Use this command to print the contents of the active workspace. This command opens a dialog box where you can specify the range of pages to be printed, the number of copies, the destination printer, and other printer options. Refer to Windows documentation and online help for details on using this dialog box.

Exit

Closes the EasyLife X program.

VIEW MENU

Math/FitOutput

When doing any kind of **Data Analysis** a text window named **Math/Fit Output** pops up containing identification information, fitted parameters and various statistics associated with the fit. Since this is a text window, the text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window.

This command reopens the **Math/Fit Output** text window.

File menu commands

Open: Opens a text file (for example, the result file from a previous performed analysis).

Save: Saves text in the **Math/Fit Output** window as a text file (default extension .txt).

Edit menu commands

Cut: Cuts selected text area out of the text.

Copy: Copies selected text area.

Paste: Pastes from clipboard (for example text area which was cut / copied).

Select All: Selects all the text in the text window.

Clear: Deletes all text in the text window.

Identification Information

Analysis Function: Type of analysis.

Curves: Names of the curves the analysis is based on.

Time Range: Characterized by Start Time and End Time.

Start Parameters: Fixed or floating start values of the used parameters.

Statistic Results

Fitted Curve: Name of the curve generated by the fitting procedure.

Residuals: Name of the curve displaying the weighted difference between the calculated fit and the real data.

Autocorrelation: Name of the Autocorrelation curve.

Deconvoluted: Name of the Deconvoluted curve.

Chi2: Chi Square Statistic for testing correlation.

Durbin Watson: Durbin-Watson parameter for testing correlation.

Z: Parameter expressing the result of a Runs Test.

Pre-exponential: Defined as a_i in the equation $I(t) = \sum [(a_i)\exp(-t/\tau_i)]$, where t is time and τ is the lifetime.

Lifetime: Defined as τ in the equation $I(t) = \sum [(a_i)\exp(-t/\tau_i)]$, where t is time and a_i is the pre-exponential factor.

F1: Relative integrated intensities defined as $F_i = a_i\tau_i / \sum a_i\tau_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

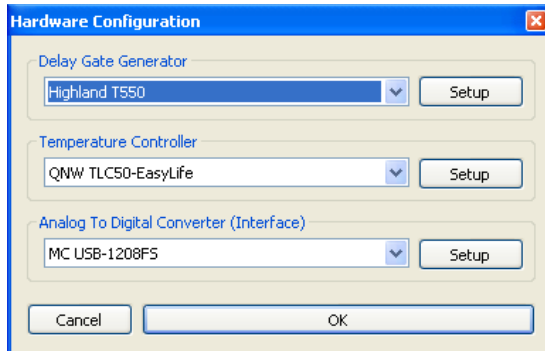
Tau-av1: Steady state average lifetime defined as $\text{Tau-av1} = \sum a_i\tau_i^2 / \sum a_i\tau_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

Tau-av2: Amplitude average lifetime defined as $\text{Tau-av2} = \sum a_i\tau_i / \sum a_i$, where a_i and τ_i are the pre-exponential factors and lifetimes, respectively.

Fitted Parameters: Values and Deviations of the curve parameters resulting from the fit.

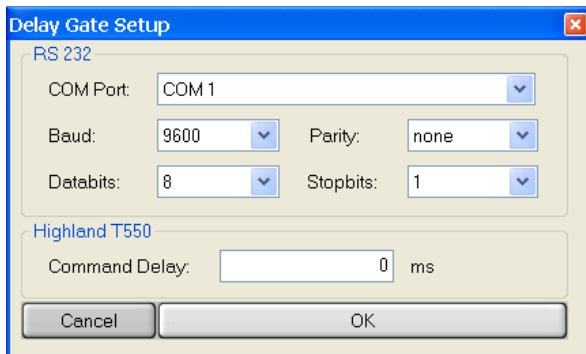
CONFIGURE MENU

Hardware...



Use these dialogs to configure the EasyLife X hardware settings. With Windows XP, you should close and reopen EasyLife X for any changes to the configuration to take affect. Otherwise attempting to acquire data after such changes may show the message “Initialization of Analog Data Capture Device failed!” With Windows 7, you do not need to close and reopen EasyLife X after making changes to the hardware settings.

Delay Gate Setup



On the **Hardware Configuration** dialog, click on the **Delay Gate Generator** choice list to choose **Highland T550**. Click on the Setup button to open the associated **Delay Gate Generator Setup** dialog. Select the COM port on the computer that the RS-232 cable from the EasyLife X is connected to. Enter the other parameters as shown above.

Warning: If your system uses the USB-to-Serial adapter cable, do not disconnect this cable while the computer is powered ON. This will cause Windows to increment the number of COM ports and change the one used for the EasyLife X. You will then have to reconfigure the COM port used by the Delay gate Generator in this dialog.

Temperature Controller

COM Port: Select the COM port on the computer that the USB cable from the EasyLife X is connected to.

Properties: Keep the **Use default Properties** box checked.

Temperature Reached Detection: Click on the **Use Internal Monitor** radio button and select the parameters for determining that a target temperature has been reached. When setting a temperature target, EasyLife X will wait until the temperature has reached the target temperature within the **Delta Temperature** for the **Stabilization Time** before starting or finishing acquiring data.

Use QNW Stable Temperature Notification: uses a Delta Temperature of 0.02 °C and a Stabilization Time of 60 s that is usually too precise and causes an extremely long wait before starting a scan.

ADC Setup

In the **Hardware Configuration** dialog, the ADC board **MC USB-1208FS** is the only option. Click on the Setup button to show the **ADC Setup** dialog.

The asterisk in Board Number, Channel and Gain text boxes indicates that these are the current settings (before the dialog box was opened).

The **Board Number** is fixed, but this box shows whether the EasyLife X is communicating with the computer.

The **Channel** and **Number** are fixed.

The **Gain** is selectable from the ranges listed in the choice list. The setting selected will give the maximum dynamic range. Thus, for a smaller signal you would want to choose a smaller voltage range.

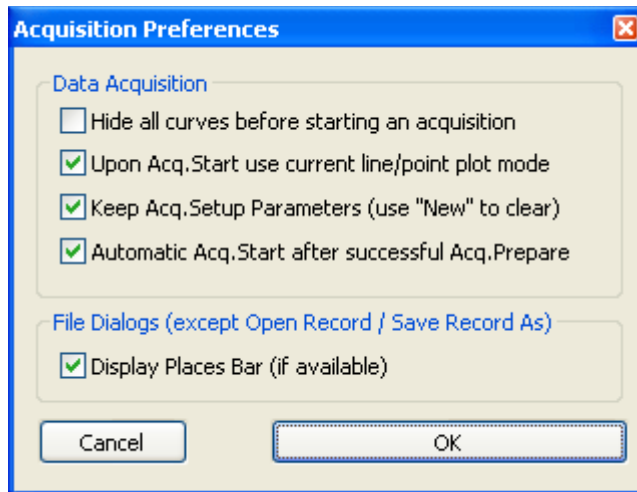
The **ADC Rate** is the signal sampling rate with an editable allowed range of 1 to 50,000 Hz. The choice list suggests commonly used values.

The **Screen Update Rate** is the screen refresh rate with an editable allowed range of 0.01 to 1 seconds. The choice list suggests commonly used values.

Display as: Volts: The maximum allowed data range is the **Gain** setting in the dialog.

Display as: Min/Max: The gain range is rescaled to editable **Min** and **Max** settings. The default range is -2048 to +2047 since the ADC is a 12-bit Analog to Digital Converter.

Preferences...



Hide all curves before starting an acquisition. When checked, all previous traces in the active acquisition will be hidden. If unchecked, previous traces that are visible will remain visible.

Upon Acq.Start use current line/point plot mode. When checked a new scan will show in the graph with the current line/point plot mode. This plot mode is also saved when saving an acquisition setup. If unchecked, new scans will be displayed in line plot mode.

Keep Acq.Setp Parameters (use “New” to clear). When checked, then if, while EasyLife X is open, you close an acquisition setup, then the next time you open that setup type, the last used parameters will be displayed.

Automatic Acq. Start after successful Acq. Prepare. When checked, acquisition will start immediately after the acquisition is prepared. If unchecked, then the Acquire button will change to a **Start** button after the acquisition is prepared.

Display Places Bar (if available). When checked the Places Bar will be shown in the **Open** and **Save** dialogs for the **File** menu and the acquisition setups. The **Import** and **Export** dialogs from the **File** menu and legend submenus always have the Places Bar showing.

ACQUIRE MENU

Experiments are invoked and data is acquired by selecting Acquire and the type of acquisition desired for the experiment from the drop down menu. Each acquisition command opens a data acquisition setup dialog box. The items in each box are organized according to the experiment type.

Decay: In decay mode the delay is scanned in time. Decay experiments are used to measure the lifetimes of samples.

Timebased: In timebased mode the delay remains fixed throughout the experiment and the intensity is measured as a function of time. Timebased experiments usually involve kinetic measurements but are also useful in maximizing intensity when adjustments are being made to the instrument.


Common Acquisition Controls

All acquisition dialog boxes contain buttons that reset, load and save setup files, start, stop, and pause the data acquisition process, and allow additional setup controls etc.

New:

Resets the acquisition parameters, collection mode and number of background points to defaults.

Open:

Use the **Open** command to open an acquisition setup file stored on disk or on the network. The **Open** dialog box shows the file name, and by clicking on the View Menu icon, , and choosing Details, you can also show the time and date the file was last saved.


To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

File name: Type a file name in the text box, or select the file name from the list.

Files of type: There is only one file type for each acquisition type.

The opened acquisition will replace the currently active acquisition window.

Save:

Use the **Save** command to name and save the contents of the active acquisition. The **Save** dialog box shows the file name, and by clicking on the View Menu icon, , and choosing Details, you can also show the time and date and file size of existing files. Select **Save**, or select **Cancel** whereby the dialog box will close and nothing will be saved.

To choose a different file location, click on the **Look in** text box and browse the file structure to find the location you want.

File Name: Type a file name in the text box, or select the file name from the list. If you try to save as an existing filename, you will get a message that the file already exists. You must answer **Yes** to this question to save the data under an existing filename. Otherwise, choose **No** and enter a new filename.

Name:

Shows the name of the acquisition setup if it has been saved to a file.

Background

Check the **Acq** box to acquire the background when the acquisition is started. On subsequent scans the **Acq** checkbox is automatically cleared, the **Use** box is automatically checked, and the just-acquired background values will be subtracted from the current measured values in real time. The background values will remain in effect for subsequent measurements until cleared in the Display dialog box, or by removing the check mark in the **Use** box, or by checking the **Acq** checkbox, which will clear the previous value and force a new background to be acquired.

Toggling **Use** keeps the background value in memory for future use. It is important to measure the background during the first scan, otherwise the signal may be distorted. This function only measures the electrical background on the signal integrator, i.e. it measures the pre-acquisition signal before the light source is used. It does not account for an optical background due to stray light, solvent, etc. It is important to re-measure the background every time the integration time or ADC gain setting are changed.

ACQUIRE / START

Clicking this button prepares the system for the acquisition of data. If a Peltier stage is included with the system and has been configured, then the **Temperature Control Panel** will appear the first time the **Acquire** button is clicked. The **Acquire** button will change to and remain as **Wait** until the **Start Temperature** has been reached. After this is done, the button label changes to **START**. If the Background **Acq**. box has been checked then clicking **START** will acquire a background value, and the button reverts to **ACQUIRE**; if the Background **Acq**. box has not been checked then this step will be skipped and the previous background value will be used for data acquisition. When **START** is clicked, data acquisition begins and the label changes to **PAUSE**.

PAUSE / CONTINUE

This button temporarily suspends data acquisition and the button label changes to **CONTINUE**. Acquisition resumes when **CONTINUE** is clicked.

ABORT / STOP

Click **ABORT** to cancel the current acquisition preparation and return the system to standby mode. The **ABORT** button changes to a **STOP** button once data acquisition via the **START** button has begun.

More...

Clicking this button brings up a dialog box where the number of data points to be used in calculating the average background is set.

Display...

Record, Group and Trace:

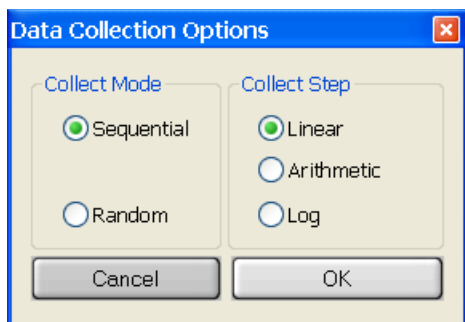
These are the labels given to the record, group and traces. You can type characters in the text boxes before the left parentheses so they will appear in the legend. The numbers in the respective brackets will be incremented each time you start a new record, group or trace according to the selected radio button. Starting a new acquisition type will by default create a new record. However, if that acquisition type has been used previously and the data still is in the workspace and legend, and Display Setup has “Next Run will create a New: Group or Trace”, then the data will be appended as a new group or trace accordingly.

Background:

The background value is shown here. It may be either acquired or from opening a saved acquisition setup.

Options...

This dialog box controls the several aspects of how decay data are collected.



Collect Mode

Radio buttons allow the selection of **Sequential** or **Random**, which controls the order in which data points are collected.

Sequential: Causes the data to be collected in “conventional” order, i.e. from the shortest delay to the longest delay.

Random: Causes the data to be collected in random order. This can be useful in situations where photochemical reactions are suspected of producing systematic effects on sample lifetimes.

Collect Step

Radio buttons allow the selection of **Linear**, **Arithmetic** or **Logarithmic**, which controls the spacing between consecutive time delays.

Linear: The conventional choice and divides the time between the start delay and end delay into equal time increments.

Arithmetic: Adds a constant time increment on to the previous time step to obtain the next time step. Thus the time between data points increases as the delay increases.

Log: Multiplies the previous time step by a constant factor to obtain the next time step. With this option, time between data points increases even faster than it does with the Arithmetic option.

The Arithmetic and Logarithmic options are particularly useful when the sample decays with several very different lifetimes. In such cases, it may be necessary to have good data at both short and long time delays. Good data at short time delays could be obtained by choosing Linear and a small time increment. However, this would require many channels for this small time increment to be extended to long delays. Choosing Arithmetic or Logarithmic concentrates the points in the short delay region but still gives coverage in the long delay region.

Temperature settings

Start Temp: The start temperature for a temperature ramp, or when using a static temperature.

Temperature: The static temperature at which to acquire data.

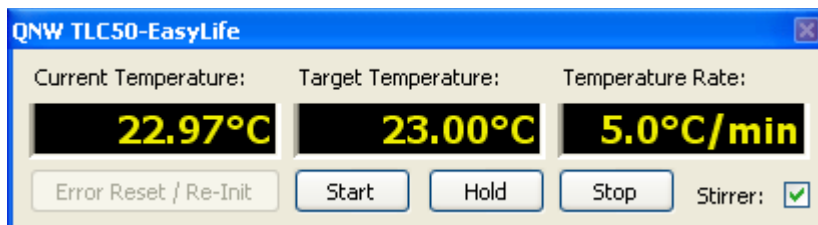
End Temp: The end temperature for a temperature ramp.

Settle Time: The time to remain at the start temperature before beginning data acquisition. This is to allow the sample to equilibrate at the start temperature.

Temp. Rate: The rate to change the temperature during a temperature ramp. If the rate text box is left blank while setting other temperature parameters, then the approach to the target temperature occurs by the fastest means possible, which is a non-linear approach.

Note: If a Peltier stage is included with the system and has been configured, but the temperature settings are left blank, then the acquisition dialog does not control the temperature. However, the Temperature Control Panel will still appear, allowing you full control of the temperature, including turning the stirrer ON/OFF.

Temperature Control Panel



Current Temperature: Displays the current temperature of the Peltier stage.

Target Temperature: Displays the target temperature as set by the acquisition dialog. Alternately, the user can click on or tab to this box, enter a target temperature, and then press the <Enter> key.

Temperature Rate: Displays the temperature rate as set by the acquisition dialog. Alternately, the user can click on or tab to this box, enter a temperature rate, and then press the <Enter> key. If this box is edited to be empty, then “no rate” appears in the box and the approach to the target temperature occurs by the fastest means possible, which is a non-linear approach.

Start: Using the values in the Temperature Control Panel, clicking this button starts the temperature change from the current to the target temperature at the temperature rate.

Hold: Sets the target temperature to be equal to the current temperature.

Stop: Stops the change in temperature and sets the target temperature to be equal to the current temperature.

Stirrer: Toggles the magnetic stirrer ON/OFF.

The Temperature Control Panel may show the values in different colors with the following meanings:

Yellow: The temperature is not being controlled (idle mode).

Purple: The user is editing the value in the box.

Pink: The edited value has been accepted and sent to the temperature controller and is waiting for an acknowledgement.

Red: The instrument reports an error. All three boxes showing “error” means there is no connection to the temperature controller. Otherwise the value just edited is not possible with the instrument.

Orange: The instrument is controlling the temperature - heating or cooling on the way to the target temperature.

Blue: The instrument is controlling the temperature - keeping the target temperature.

Grey: Unknown intermediate state (during startup, shutdown, or between operations).

Fluorescence Decay

The screenshot shows the 'Fluorescence Decay' dialog box. It has a title bar with a close button. The main area contains several input fields and checkboxes. The 'Background' section has two options: 'Acq.' (unchecked) and 'Use' (checked). The 'Start Delay' is set to 0 ns, 'End Delay' to 200 ns, 'Channels' to 100, and 'Int Time' to 0.1 sec. 'Averages' is set to 1. There are also fields for 'Repeats', 'Start Temp.', 'End Temp.', 'Settle Time', and 'Temp. Rate'. At the bottom, there are buttons for 'More...', 'Options...', 'Display...', 'ACQUIRE', and 'STOP'.

Start Delay (ns): Enter a value, which is about 3 to 5 ns in front of the excitation pulse. If you don't know where the excitation pulse is, enter 0.

End Delay (ns): Add about 5 to 8 times your sample lifetime to the **Start Delay** and enter here. If you have no idea what the lifetime of your sample is, enter a relatively large value, e.g. 200 ns.

Channels: This is the number of data points defining the decay curve. Enter a desired value, e.g. 200 (use less channels to speed up the experiment and more channels to improve accuracy of the measurement). Although a limit of 2000 channels is imposed by the software, the time taken to collect and analyze decays may become excessive for large numbers of points.

Int Time (s): This controls the time over which the output signal is averaged at each time delay step. Enter 0 s for a quick scan. Use a lower value to speed up the experiment or a larger value to improve signal to noise.

Averages: Number of scans to be acquired and averaged. Enter 1 for a quick scan, use more averages to improve the signal to noise.

Repeats: The number of times to repeat the decay acquisition after the first run.

Fluorescence Timebased

Delay (ns): Enter the delay at which the detection window will be opened.

Points/sec: Enter the number of points per second to record. The integration time for each point decreases inversely as the number of points increase. Time resolution can be increased by increasing the number of points per second at the expense of signal to noise. The choice depends on the time scale of the kinetics to be studied. For use in adjusting the instrument, values between 5 and 20 are found to give adequate response.

When enabled, **Single Point Screening** will enter the values measured into a spreadsheet grid, one at a time, so that you can evaluate the progress of an experiment in detail. A value is measured and entered in the grid when the **CAPTURE** button is pressed. **Note.** If **Enable Single Point Screening** has been selected the **PAUSE** button will be replaced with **CAPTURE**. An acquisition using this option can only be stopped not paused.

Duration (s): Data collection will continue until the time interval entered has elapsed. If the **Repeats** are set to a value greater than zero the duration is only the time of each repetition.

Repeats: Enter the number of times the experiment will be repeated. The time axis will continue to increment throughout the **Pauses** and subsequent **Repeats**, and all of the repeated experiments will be saved in the same file. By using **Repeat** and **Pause**, you can examine a dynamic sample over a long period of time. This is not available for wavelength scanning or temperature ramp experiments.

Pause (s): Enter the time to pause between repeated experiments. Pause only becomes available if the **Repeats** are greater than zero.

View Window (s): Enter the time segment that will be displayed on the X-axis. The **View Window** setting can be less than or equal to that of the **Duration**. If the **Repeats** are set to a value greater than zero, than the default View Window is equal to the sum of all repeated durations, plus **Pause** times. The data will be automatically shifted across the X-axis as each window is filled. Use a short view window for highest resolution of time.

Ultra Fast Lifetime – Basic

Upon opening this dialog a record labeled “*UFL*” with the “*Last used IRF*” trace will be added to the Graph window if it is not already there. It is hidden by default. To see it, select it, **Toggle Visibility ON**, and then click on **Full Autoscale**.

Initialize – A new IRF and sample decay will be acquired, and then a lifetime trace. There will be prompt messages to insert the scatterer and sample when required.

Use Previous Settings – Only a lifetime trace will be acquired. This will use the last acquired IRF and the T_1 and T_2 delay times based on the last acquired sample decay or on user defined delay times set in the Ultra Fast Lifetime Advanced dialog. If there is no previous IRF, then the Initialize procedure will automatically be used.

Duration: This is the length of the lifetime trace to be acquired. Data collection will continue until this time interval has elapsed or the **STOP** button is pressed.

View Window: Enter the time segment that will be displayed on the X-axis. If the View Window is smaller than the Duration the View Window will be shifted as each window is filled.

Lifetime Scan: Choose the integration time for the lifetime scan data points. Default values are Fast = 0.5 s, Medium = 1 s, Slow = 2 s.

Ultra Fast Lifetime – Advanced

Upon opening this dialog a record labeled “UFL” with the “*Last used IRF*” trace will be added to the Graph window if it is not already there. It is hidden by default. To see it, select it, **Toggle Visibility** ON, and then click on **Full Autoscale**.

Acquire new IRF: If this box is checked, then a new IRF trace will be acquired. If this box is not checked, then the IRF trace can be selected from the **IRF:** drop list.

IRF: Shows the most recently acquired IRF trace. You can also select a different IRF trace from this list to use if **Acquire new IRF** is unchecked.

Determine new Delays Ti: If this box is checked then a new TxDecay trace will be acquired.

Delay T1 and T2: If **Determine new Delays Ti** is unchecked then a new lifetime trace will be created using these Delay times.

Int Time (s): This controls the time over which the output signal is averaged at each time delay step. Enter 0 s for a quick scan. Use a small value to speed up the experiment or a larger value to improve signal to noise.

Duration: This is the length of the lifetime trace to be acquired. Data collection will continue until this time interval has elapsed or the **STOP** button is pressed.

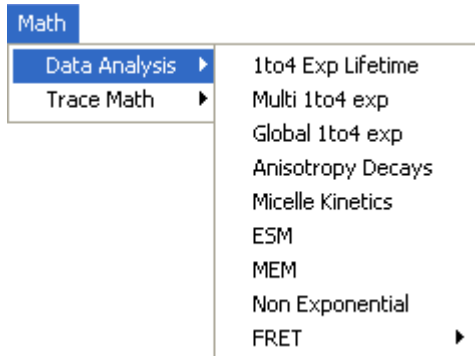
View Window: Enter the time segment that will be displayed on the X-axis. If the View Window is smaller than the Duration the View Window will be shifted as each window is filled.

Repeats: Enter the number of times the experiment will be repeated. The time axis will continue to increment throughout the **Pauses** and subsequent **Repeats**, and all of the repeated experiments will be saved in the same file. By using **Repeat** and **Pause**, you can examine a dynamic sample over a long period of time. This is not available for wavelength scanning or temperature ramp experiments.

MATH MENU

Acquired data can be processed mathematically through the commands in the Math submenus. The Trace Math menu is also available as Graph\Math\Trace Math by right-clicking on the graph area. **Data Analysis:** decay analysis functions which include but are not limited to 1-4 exponential fit, global analysis, MEM and ESM, and anisotropy decays. **Trace Math:** traces can be arithmetically combined and data can be fitted, smoothed, averaged, integrated, normalized, differentiated, etc.

Data Analysis



The various methods of data analysis are found under Math\Data Analysis. They are:

1. 1 To 4 Exp. Lifetime
2. Multi 1 To 4 Exp.
3. Global 1 To 4 Exp.
4. Anisotropy Decays
5. Micelle Kinetics
6. ESM
7. MEM
8. Non Exponential
9. FRET

These methods are covered in separate sections that are independent of each other. Thus, only the section of interest needs to be read. However, it is recommended that the **General Introduction** is read first as most of the concepts and topics used in the other sections are introduced there.

General Introduction

Fitting function

Every method of lifetime analysis depends on a model or fitting function for the decay of luminescence intensity. This may be as simple as a single exponential decay or as complicated as schemes for micelle kinetics including quenchers and distributions of micelle sizes etc. The various methods of analysis presented here differ mostly in the model they employ. The fitting function (explicitly dependent on time) is denoted in what follows as $D(t)$ and can be thought of as the time dependent luminescence excited by a delta function (infinitely short) excitation pulse.

Convolution

In any pulsed excitation fluorescence lifetime instrument the finite width of the excitation pulse will distort the free decay of fluorescence as described by $D(t)$. This distortion is known as convolution and the mathematical description is given by *Equation 1*:

$$I(t) = \int_0^t L(t-s)D(s)ds \quad \text{Eq. 1}$$

where $L(t)$ is the instrument response function (IRF) also known as the excitation pulse curve, and $I(t)$ is the experimentally determined decay intensity at time t . The meaning of Equation 1 is that the intensity of the decay at time t is determined by both the continuous re-pumping of the fluorescence excitation during the emission from the nanosecond flash lamp or laser and the decay of fluorescence emission that has occurred up to time t .

The convolution distortion cannot be removed experimentally. Instead, the IRF $L(t)$ is measured by using a scattering solution in a companion measurement prior to determining the fluorescence decay of the sample. This experimental $L(t)$ is then used to actually determine $I(t)$ from Equation 1 by the procedure known as iterative reconvolution.

Curve Fitting Procedure

The fitting procedure uses an iterative fitting procedure based on the Marquardt algorithm (Bevington, 1969) where the experimental data are compared to a model decay based on Equation 1. Deviations from the best fit are characterized by the reduced chi-square statistic, χ^2 , as shown in *Equation 2*:

$$\chi^2 = \frac{1}{N-n-1} \sum_1^N \frac{(I(i)_{\text{calc}} - I(i)_{\text{exp}})^2}{s(i)^2} \quad \text{Eq. 2}$$

where N is the number of data points (or channels), n is the number of fitting parameters, and s is the standard deviation (see below). The best fit is determined when chi-square is minimized. If the standard deviations are estimated correctly, a perfect fit to the data will produce a chi-square close to 1.0. Good results typically produce χ^2 's of 0.9 to 1.2.

It is necessary to incorporate an estimate of the data precision when using a statistical fitting procedure. For the case of the stroboscopic optical boxcar the standard deviation is determined from within the decay by actually measuring the “noise” at time t and applying a special procedure developed for this type of experimental data. (James *et al*, 1992)

Artifacts

There are several well-known artifacts due to the intrinsic nature of photomultiplier tubes, etc... which must be accounted for during the analysis procedure.

Color Shift Artifact Correction: Photomultiplier tubes do not respond identically at all wavelengths of incident light. This is due to the fact that the photoelectrons ejected from the photocathode will have excess kinetic energy when the incident photon is more energetic, i.e., bluer. This effect manifests itself primarily, although not exactly, as a zero-time shift in the excitation position relative to the decay. This time shift can be approximated by a single parameter δ as shown in *Equation 3*:

$$I(t) = a \exp\left(\frac{-(t + \delta)}{\tau}\right) \quad \text{Eq. 3}$$

This parameter may be either determined experimentally or incorporated in the fitting procedure as a variable parameter. Both options are available in this program. The fitting procedure for using the variable time shift parameter is based on that from *Time-Correlated Single Photon Counting* by O'Connor and Phillips.

Analog Baseline Offset: Since the stroboscopic technique is based on an analog measurement, there will always be a small difference between the measured baseline for the IRF and that for the fluorescence decay, typically on the parts per thousand level. This offset can cause inaccuracies to the determination of fluorescence lifetimes since it will be treated as due to a true convolution effect. The effects of this offset can be removed (James *et al*, 1992) by determining the offset during the data analysis by assigning a non-convolved constant, c , to the decay intensity as shown in *Equation 4*:

$$I(t)_{\text{corrected}} = I(t)_{\text{uncorrected}} + c \quad \text{Eq. 4}$$

Useful Statistical Parameters

A variety of statistical parameters have been developed to assist in determining the quality of the analysis. First among these is the reduced χ^2 parameter as discussed above. Others are:

Randomness of the Residual Pattern: The residual R_i is the difference between the calculated fit and the real data at time t_i . Weighted residuals r_i are the ratio of R_i to s_i , i.e., $r_i = R_i/s_i$ and should range from about -3.3 to 3.3.

A plot of these residuals should produce a flat pattern randomly distributed about zero with no features. Periodic oscillations or other deviations indicate a poor fit. This is a simple and reliable test for the goodness of the fit.

Autocorrelation Function of the Weighted Residuals: This function is calculated from Equation 5 (Grinvald and Steinberg, 1974):

$$Cr_j = \frac{\frac{1}{m} \sum_{i=n_1}^{n_1+m-1} r_i r_{i+j}}{\frac{1}{n_3} \sum_{i=n_1}^{n_2} [r_i]^2} \quad \text{Eq. 5}$$

where $n_3=n_2-n_1+1$, n_1 and n_2 are the first and last channels chosen to do the calculation over. An upper limit is set at $j = n_3/2$ to allow for maximal testing of a finite data set.

By definition $Cr(0)=1$. For the remaining points Cr_j should form a flat band of high frequency low amplitude noise about zero. Any pattern indicates a lack of fit. The autocorrelation function is very sensitive to any radio-frequency (RF) noise.

Durbin-Watson Parameter: This parameter was introduced by Durbin and Watson (Biometrika, 1950, 1951) to test for correlations. The parameter DW is defined in Equation 6 as:

$$DW = \frac{\sum_{i=n_1+1}^{n_2} [r_i - r_{i-1}]^2}{\sum_{i=n_1}^{n_2} [r_i]^2} \quad \text{Eq. 6}$$

where the other parameters are defined above.

This parameter may be interpreted as follows; the fit is likely satisfactory if the value of DW is greater than 1.7, 1.75 and 1.8 for single, double and triple exponential fits respectively (O'Connor and Phillips, 1984).

Runs Test: The runs test determines the number of positive and negative groups or runs of the residuals as defined in Equation 7:

$$z = \frac{zn}{\sqrt{(zd)}} \quad \text{Eq. 7}$$

where

$$zn = (nn + np) - \frac{(2 \times nvn \times nvp)}{(nvn + nvp)} + 1$$

$$zd = \frac{2 \times nvn \times nvp \times (2 \times nvn \times nvp - nvn - nvp)}{(nvn - nvp)^2 \times (nvn + nvp - 1)}$$

and np is the number of positive transitions, nn is the number of negative transitions, nvn is the number of negative residuals and nvp is the number of positive residuals.

A value of $-1.96 < Z$ indicates a satisfactory fit at the 95% confidence level (Hamburg, 1985).

The general structure of each analysis program is the same, so there is considerable similarity in running the programs. For each method the data to be analyzed and time range over which to analyze must be selected. Then the initial model parameters must be selected, perhaps holding some of them at pre-selected values. Finally, the fit is run and the results interpreted.

1 To 4 Exponential Lifetime

Theory

This is the simplest and arguably the most generally useful of the fitting procedures. It is suitable for the analysis of fluorescence decays consisting of up to 4 exponentials and associated pre-exponentials.

Fitting Function

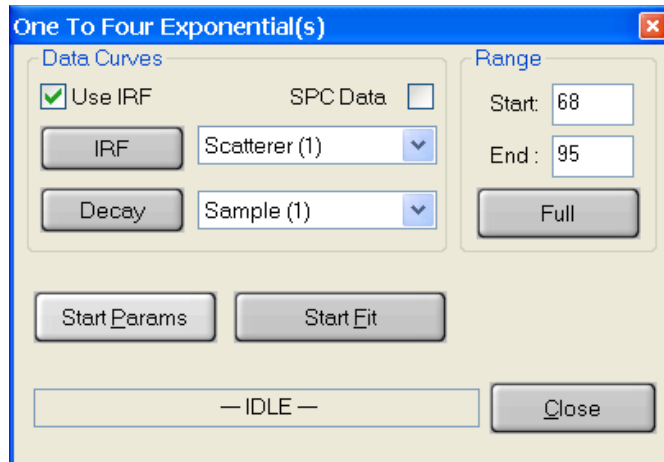
This analysis program can fit up to a 4 exponential decay which follows the fitting law:

$$D(t) = \sum a_i \exp \left\{ \frac{-t}{\tau_i} \right\} \quad \text{Eq. 1}$$

where $D(t)$ is the delta function generated decay at time t . This fitting function allows for negative a_i 's so that risetimes can also be determined with this program.

Using the Program

The initial **One To Four Exponential(s)** dialog box is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only

when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The **Start Parameters** dialog box for the **1 To 4 Exp. Lifetime** method is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Pre-exp., Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime and the pre-exponential factor (*Pre-exp*) must be given. Only the relative values of the pre-exponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit. Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the analysis.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

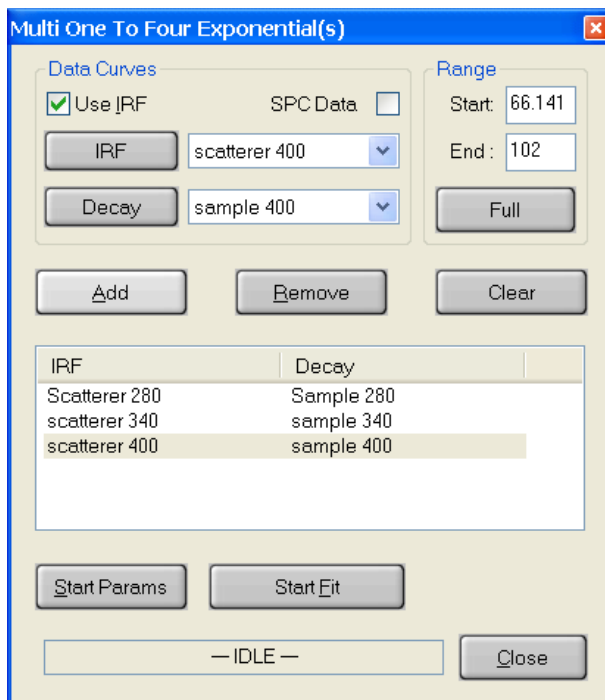
Multi 1 To 4 Exponential

The multiple file one to four exponential lifetime method, as its name implies, allows the analysis of multiple scatterer/sample pairs at the same time. Each pair will be separately analyzed over the same range with the same number of exponentials and the same options. The analysis results in a set of parameters (lifetimes and pre-exponential factors) for each data pair. The theory for this method is exactly the same as that for the **1 To 4 Exp. Lifetime** method.

This type of analysis is useful when a series of otherwise identical decay curves has been collected as a function of some parameter (temperature, composition or wavelength for example). Trends in the values of the lifetime parameters may then be recognized rather easily.

Using the Program

The initial dialog box for **Multi One To Four Exponential(s)** is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

Multiple scatterer/sample pairs are selected by first choosing a single pair.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Enter this data pair into the analysis by clicking the **Add** button at which point their names appear in the text window. Data pairs may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All sample pairs may be removed by clicking on the **Clear** button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The **Start Parameters** dialog box for the **Multi 1 To 4 Exp.** method is shown at left and is identical to the dialog for **1 to 4 Exp. Lifetime**.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curves. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Pre-exp., Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime and the pre-exponential factor (*Pre-exp*) must be given. Only the relative values of the pre-exponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Global 1 To 4 Exponential

Theory

This analysis program provides for the analysis of up to 4 exponential decays for a number of data files simultaneously. The global analysis assumes that the lifetimes are linked among the data files but that the associated pre-exponentials are free to vary.

Fitting Function

The analysis program can fit up to a 4 exponential decay, which follows the fitting law:

$$D(t) = \sum a_i \exp \left\{ \frac{-t}{\tau_i} \right\} \quad \text{Eq. 1}$$

where $D(t)$ is the delta function generated decay at time t . This fitting function allows for negative a_i 's so that risetimes can also be determined with this program.

Global Analysis

Global analysis is a procedure whereby several data sets, which have parameters in common may be analyzed simultaneously (Knutson, Beechem and Brand, 1983). This program assumes that the lifetimes are linked among the data files, i.e., the lifetimes are the same for all decays. This is accomplished by using a matrix mapping of the fitting parameters whereby the pre-exponentials are unique for each decay curve while the lifetimes are mapped to the same value for each decay.

For example, two linked lifetimes with 2 unique pre-exponentials each and 4 decay curves map as:

$$\begin{vmatrix} a(1,1) & a(1,2) & a(1,3) & a(1,4) \\ \tau(1,1) & \tau(1,1) & \tau(1,1) & \tau(1,1) \\ a(2,1) & a(2,2) & a(2,3) & a(2,4) \\ \tau(2,1) & \tau(2,1) & \tau(2,1) & \tau(2,1) \end{vmatrix} \quad \text{Eq. 2}$$

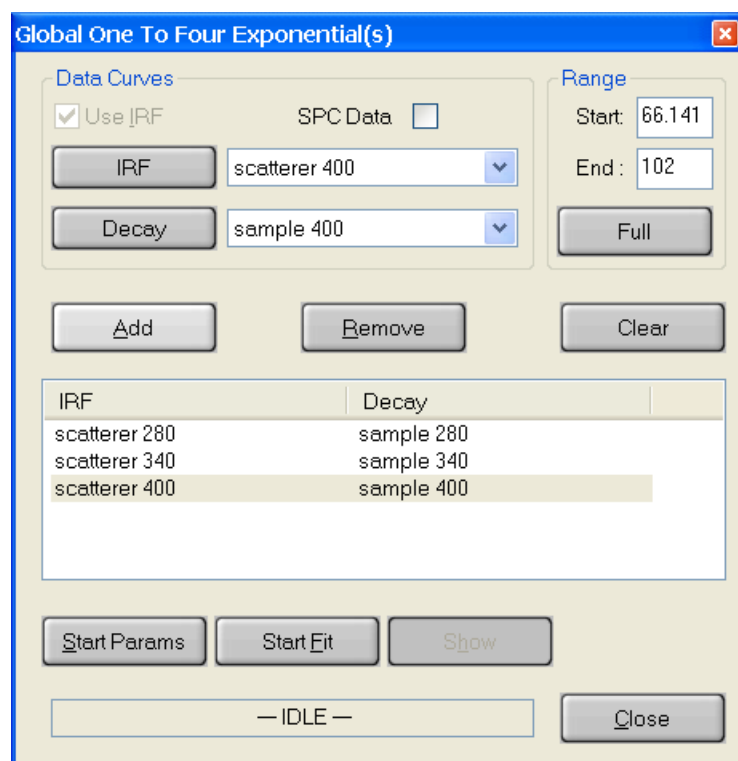
Least squares data analysis using the Marquardt algorithm is done on all data files simultaneously using the map to substitute parameters appropriately while minimizing the global χ^2_g :

$$\chi^2_g = \sum_j \chi^2_i \quad \text{Eq. 3}$$

where χ^2_i is given by Equation 2 of the **General Introduction**. Refer to the **General Introduction** for a discussion of fitting procedures and statistical parameters. This type of analysis is useful when a series of otherwise identical decay curves has been collected as a function of some parameter, which alters the relative amounts of two or more fluorophores without altering their lifetimes. For example, this form of analysis could be used for various mixtures of non-interacting fluorescent compounds.

Using The Program

The initial dialog box for **Global One To Four Exponential(s)** is shown below.



Data Curves

Note: Global 1-4 Exponential analysis requires that all the data curves to have the same time data (X-axis data).

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

Multiple scatterer/sample pairs are selected by first choosing a single pair.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

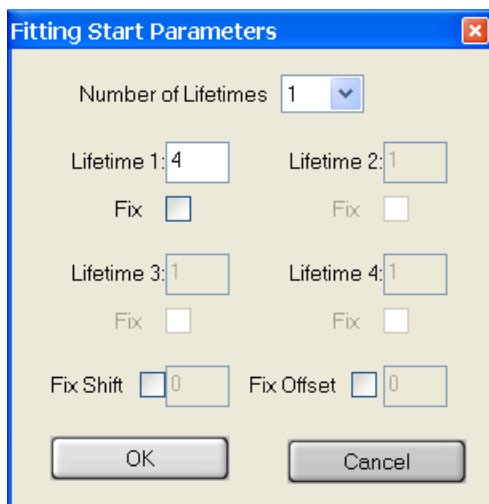
Enter this data pair into the analysis by clicking the **Add** button at which point their names appear in the text window. Data pairs may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All sample pairs may be removed by clicking on the **Clear** button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

Show: In order to avoid screen congestion, only selected analysis curves will be displayed. Data pairs are selected by clicking on the appropriate line in the text window to highlight the line. Clicking on the **Show** button will then display the fitted curve, the residuals, autocorrelation, and deconvoluted curves associated with this data pair.



The **Start Parameters** dialog box for the **Global 1 To 4 Exp.** method is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curves. Select a number between 1 and 4. Normally, for the first fit of a new sample, the number one is chosen.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analysis are displayed in two forms.

1. The results may be displayed in graphical form. However, in order to avoid screen congestion, only selected analysis curves will be displayed. Data pairs are selected by clicking on the appropriate line in the text window of the **Global One To Four Exponential(s)** dialog box to highlight the line. Clicking on the **Show** button will then display the fitted curve, the weighted residuals, autocorrelation and deconvoluted curves associated with this data pair.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Anisotropy Decays

Theory

This program allows for the calculation of up to four rotational correlation times plus a residual anisotropy term. The program first allows the user to calculate the fluorescence lifetime(s) from the parallel and perpendicularly polarized emission intensities. The user can then calculate the rotational correlation time(s).

Fitting Function for Fluorescence Lifetimes from Polarized Emissions

The analysis program can fit up to a 4 exponential decay, which follows the decay law:

$$D(t) = \sum a_i \exp\left\{\frac{-t}{\tau_i}\right\} \quad \text{Eq. 1}$$

where $D(t)$ is the delta pulse excited decay function at time t . This fitting function allows for negative a_i 's so that risetimes can also be determined with this program.

For polarized light, $D(t)$ may be calculated from the raw data:

$$D(t) = I(t)_{\text{par}} + 2 \times G \times I(t)_{\text{per}} \quad \text{Eq. 2}$$

where $I(t)_{\text{par}}$ is the intensity of light detected with a vertical excitation polarizer and a vertical emission polarizer (*i.e.*, the polarizers are parallel to each other), $I(t)_{\text{per}}$ is the intensity of light detected with a vertical excitation polarizer and a horizontal emission polarizer (*i.e.*, the polarizers are perpendicular to each other), and G is the correction term for the relative throughput of each polarization through the emission optics (see below).

Convolution

Refer to the **General Introduction** for information on convolution.

Decay of Anisotropy

Anisotropy, $r(t)$, is defined as:

$$r(t) = \frac{I(\text{par}) - G \times I(\text{per})}{I(\text{par}) + 2 \times G \times I(\text{per})} \quad \text{Eq. 3}$$

where $I(\text{par})$ and $I(\text{per})$ are as defined above and the time dependence is assumed. This function is known to decay with a multi-exponential decay law (Phillips *et al*, 1985):

$$r(t) = \sum_{i=1}^5 b_i \exp\left(\frac{-t}{\phi_i}\right) + b_{\infty} \quad \text{Eq. 4}$$

Although the sum can run to 5 terms for completely anisotropic rotational motion, at lower precision levels and with relatively symmetric rotors, equation 4 will only yield in practice 1 or 2 terms. The b_{∞} term refers to residual anisotropy remaining after all the transient terms have decayed and is commonly interpreted to imply restricted motion of the rotor.

G-Factor

The instrumental G-Factor is measured in a separate experiment that can employ the same sample for which the polarization is being measured. The experimental procedure is to set the excitation polarizer to the horizontal orientation and then measure the intensity of emission for both the vertical and horizontal orientations of the emission polarizer. The G-Factor is then defined as:

$$G = \frac{I_{HV}}{I_{HH}} \quad \text{Eq. 5}$$

Since the excitation polarization is horizontal, and thus perpendicular to both the vertical and horizontal orientations of emission, any movement of the emitting molecules will make equal contributions to both orientations of emission. Thus the intensity of both vertically and horizontally polarized light reaching the emission monochromator will be equal and will have identical time evolution. Any differences in the observed intensity must then be due to polarization artifacts of the detection channel (diffraction gratings are well known to have different efficiencies for light polarized parallel and perpendicular to the rulings). Equation 5 determines the ratio of such artifacts and provides a correction factor.

For luminescence decays the G factor may be determined in either of two ways.

The decay may be recorded with both orientations of the analysing polarizer and the G factor determined from the ratio of the integrated intensities.

$$G = \frac{\int I(t)_{HV} dt}{\int I(t)_{HH} dt} \quad \text{Eq. 6}$$

Since the time evolution of both decays is identical, the G factor may be determined from the ratio of signals recorded with both orientations of the analysing polarizer at *any* time. It is convenient to determine this ratio using Fluorescence Timebased measurements with the delay set just after the peak of the luminescence decay. This method is likely to be faster than the first method.

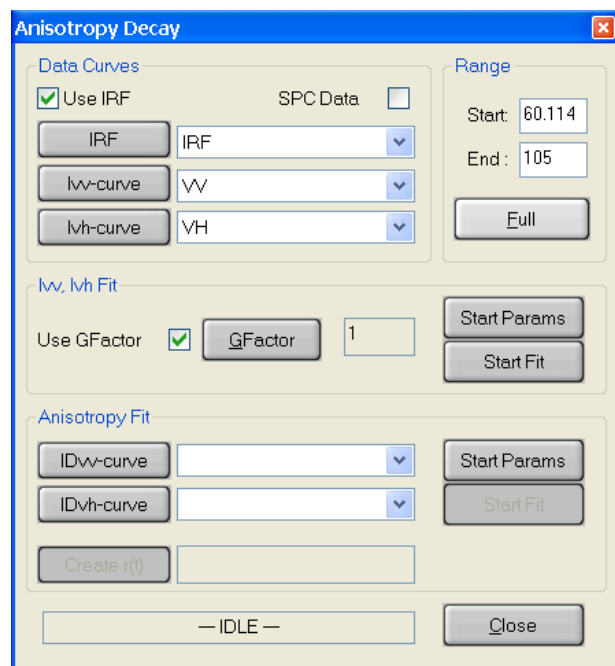
Curve Fitting Procedure

Refer to the General Introduction for information on fitting procedures and fitting statistics. Numerical analysis of anisotropy decay data is non-trivial. For this software, we chose to perform fits on the raw data files, I_{vv} and I_{vh} , without manipulating the curves prior to analysis. In the first step, the I_{vv} and I_{vh} curves are analyzed simultaneously by the global multi-exponential program. The fitted deconvoluted curves, ID_{vv} and ID_{vh} are then used to create the anisotropy function $r(t)$ according to Equation 3. The so-constructed $r(t)$ is free of any convolution effects and can be directly fitted to Equation 4. It must be remembered that $r(t)$, being constructed from fitted curves, contains no experimental noise and therefore typical fit criteria like the χ^2 value, randomness of weighted residuals, D-W parameters etc... do not apply. Instead, the guiding criterion should be the minimum value of the sum of the least squares obtained with different kinetic models, *i.e.*, different numbers of parameters.

The interpretation of the b_j 's is that b_j at $t=0$ is the initial polarization of the molecule (for single exponential decays, often known as $r(0)$) and b_∞ is the residual polarization, often known as r_∞ .

Using the Program

The initial **Anisotropy Decay** dialog box is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will

appear in the box beside the button.

The **Ivv-curve** and **Ivh-curve** buttons select the two curves to be analyzed. Select a curve by clicking on its name in the legend then click on the **Ivv-curve** or **Ivh-curve** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Ivv, Ivh Fit

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered (see below).

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The user has the option of using a G factor in the analysis or not. Toggle this option on or off by clicking on **Use GFactor**. Clicking on the **GFactor** button opens a dialog box that allows the G factor to be entered in several different ways (see below).

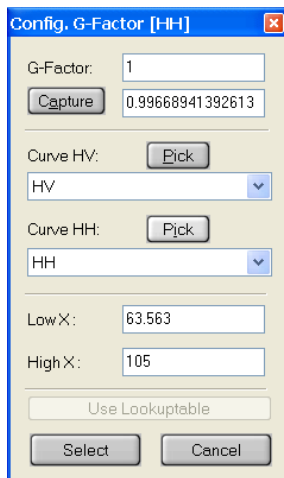
Anisotropy Fit

The **IDvv-curve** and **IDvh-curve** buttons select the two deconvoluted curves to be analyzed. Select a curve by clicking on its name in the legend then click on the **IDvv-curve** or **IDvh-curve** button. The name of the selected curve will appear in the box beside the button.

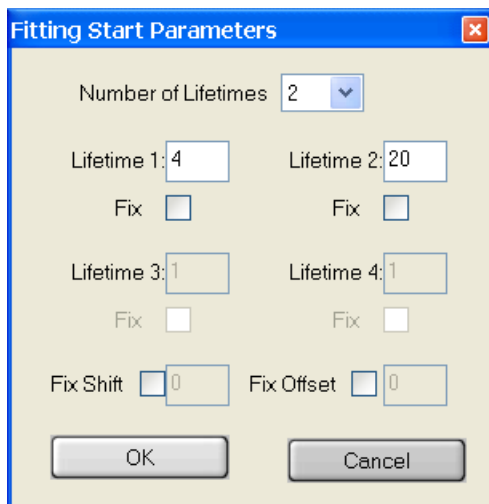
The **Create r(t)** button calculates $r(t)$ from the ID_{vv} and ID_{vh} curves.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box, which allows these values to be entered (see below).

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Configure G-Factor** dialog box is shown at left. The G-factor may be entered directly into the **G-factor** text box or captured from HV and HH decays. To capture the G-factor select the HV curve in the left legend and click on the **Curve HV Pick** button. Select the HH curve in the left legend and click on the **Curve HH Pick** button. Select the region of the curves to be used in calculating the G-factor in the normal manner (usually this is the whole decay curve). The ratio of the integrals under the HV and HH curves is displayed in the **Capture** text box. Click on **Capture** to accept this value for the G-factor, then click on the **Select** button to copy this value to the Anisotropy Decay dialog and close the Configure G-Factor dialog. It will be displayed in the **G-Factor** text box. **Use Lookuptable** is not available at this time.



The **Start Parameters** dialog box for the **Ivv, Ivh Fit** is shown at left.

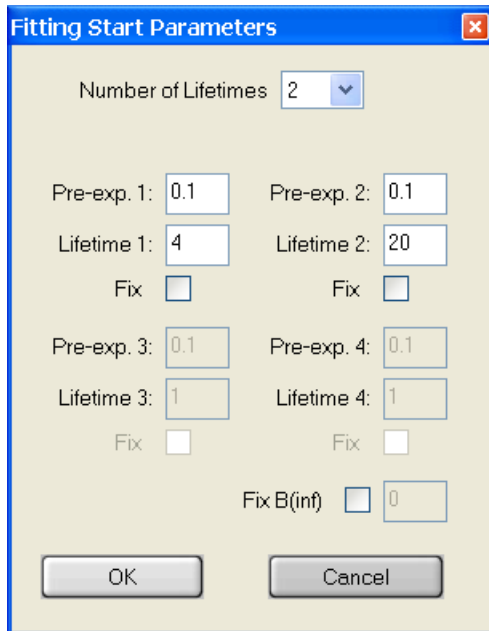
The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Study of the portion of the sample and scatterer curves before the laser pulse may indicate whether an offset is required. However, it is often better to adjust the sample and scatterer curves to average zero before the pulse (using the math functions provided in the graphics module) than to trust the fit. Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.



The **Anisotropy Fit** start parameters dialog box is shown at left.

The **Number of Lifetimes** text box selects the number of different lifetimes used to analyze the $r(t)$ curve. Select a number between 1 and 4.

Lifetime, Fix: For each of the lifetimes to be used in the fit an initial guess for the lifetime must be given. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix B(inf): B_{∞} is the long time residual polarization and may be included as a parameter in the analysis. The

parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis of $r(t)$.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function, the deconvoluted decay curves (i.e. $D(t)$ or ID_{vv} for example), the anisotropy, etc... appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Micelle Kinetics

Theory

This program allows for the analysis of quenching processes in micelles.

Fitting Function

The analysis program uses the “stretched exponential” fitting function (Rogers *et al.*, 1978). This function can be used to describe the quenching in micelles when quencher molecules are Poisson distributed among the micelles. The fitting function is:

$$D(t) = a_1 \exp\{-a_2 t - a_3 [1 - \exp(-a_4 t)]\} \quad \text{Eq. 1}$$

For the case of quenching in micelles these parameters can be interpreted as:

a_1 = scale factor for the fitting function

$a_2 = 1/\tau$, the reciprocal of the unquenched fluorophore lifetime

a_3 = aggregation number

$a_4 = 1/k_q$, the reciprocal of the quenching rate constant

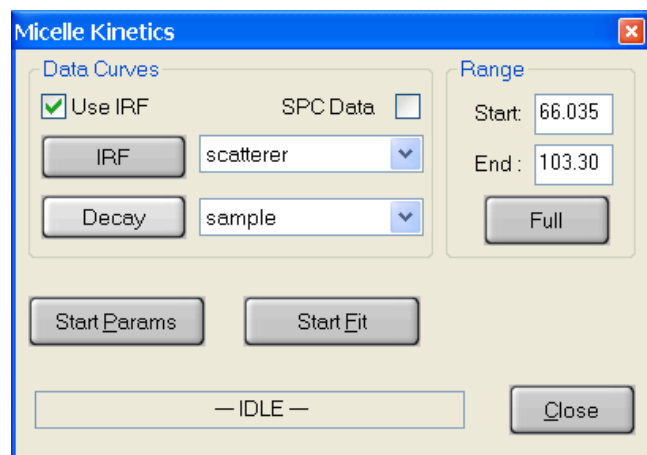
providing that the quenching process is fast relative to exchange of species.

Equation 1 implies that the fluorescence decay can be represented by a set of exponential decays with Poisson distributed amplitudes and discretely spaced lifetimes, which means that this type of data can also be analyzed with the OBB Maximum Entropy Method program (Siemiarczuk and Ware, 1990).

Please see the **General Introduction** for a discussion of the fitting procedures and statistics.

Using The Program

The initial dialog box for **Micelle Kinetics** is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The **Parameters** dialog box for the **Micelle Kinetics** method is shown at left.

The fitting function is shown in a text box as a reminder of what the various parameters are.

A1-A4: For each of the parameters to be used in the fit an initial guess must be given. Each of the parameters chosen for the analysis may be fixed at the input value (except **A1**) or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Time Domain: This method can be used to analyze fluorescence or phosphorescence data and imported data. The units used on the time axis may be different for each of these cases. For OBB instruments the units are nanoseconds for fluorescence and microseconds for phosphorescence.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

Non-Exponential Decay

Theory

This program allows for the analysis of data by a general fitting function consisting of two exponentials multiplied together each with variable exponents of time. The exponents can be either varied or fixed which provides a powerful general function for models such as Förster energy transfer and time-dependent quenching.

Fitting Function

The fitting function is:

$$D(t) = a_1 \exp(-a_2 t^n) \exp(-a_3 t^m) \quad \text{Eq. 1}$$

The parameters are:

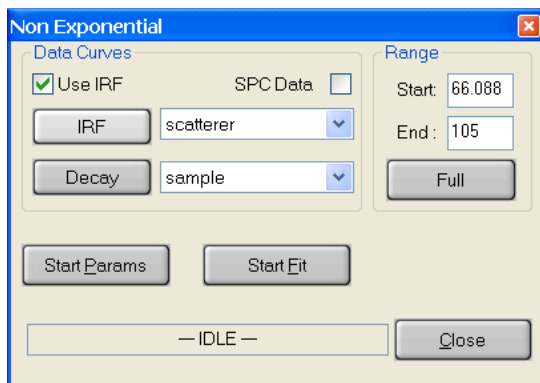
- a_1 = scale factor for the fitting function
- $a_2 = 1/\tau$, the reciprocal of the “slow” decay component
- $a_3 = 1/\tau$, the reciprocal of the “fast” decay component
- n = exponent of the “fast” component
- m = exponent of the “slow” component if $n > m$.

The exponents can be held constant or found as parameters of the fit. For example, by setting $n=1$ and $m=0.5$, this fitting function is suitable for Förster energy transfer kinetics (Förster, 1949, Birks, 1948, Steinberg *et al.*, 1983) or time-dependent quenching (Ware and Andre, 1983). Any other decay law, which can be modeled by two exponentials multiplied together, can be analyzed by this program.

Refer to the **General Introduction** for a discussion of the fitting procedures and statistical parameters.

Using the Program

The initial dialog box for **Non-exponential Decay** is shown below.



Data Curves

The **Use IRF** check box selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** check box is used only when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

Non Exponential Parameter

$$D(t) = A_1 e^{-A_2 t^n} e^{-A_3 t^m}$$

A1	A2 [1/s]	A3 [1/s]	n	m
1	100000000	100000000	1	1
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Fix Shift 0 Fix Offset 0

Time Domain

ps ns μs ms s

OK Cancel

The **Parameters** dialog box for the **Non-exponential Decay** method is shown at left.

The fitting function is shown in a text box as a reminder of what the various parameters are.

A1-A3, m, n: For each of the parameters to be used in the fit an initial guess must be given. Each of the parameters chosen for the analysis may be fixed at the input value (except **A1**) or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are very poor. If this occurs, try changing the starting values.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Fix Offset: Because of difficulties in establishing a noise free baseline there may be a small intensity offset for a decay curve. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the offset is allowed to float, a value of 0.0 is used as the initial guess.

Time Domain: This method can be used to analyze fluorescence or phosphorescence data and imported data. The units used on the time axis may be different for each of these cases. For OBB instruments the units are nanoseconds for fluorescence and microseconds for phosphorescence.

Results

The results of the analysis are displayed in two forms.

1. The fitted curve, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.

ESM – Exponential Series Method

Theory

Fluorescence lifetime measurements often result in complex decays requiring a more sophisticated approach than a single- or double-exponential fitting function (James and Ware, 1986, Siemiarczuk *et al.*, 1990). This applies especially to the emission originating in such intrinsically complex systems as:

- bichromophoric molecules exhibiting distributions of conformers in the excited state
- fluorophores adsorbed on surfaces
- fluorophores attached to polymers
- fluorescent probes in micelles and liposomes
- fluorescent probes in biomembranes and other biological systems
- fluorophores in monolayers
- intrinsic fluorescence from proteins
- systems undergoing Förster-type energy transfer
- and many others...

Even intuitive considerations would lead one to expect distributions of lifetimes in these systems. Quite often, however, especially for low precision data, a good fit can be obtained with a double- or triple-exponential function for a system, which in fact represents a continuous distribution of lifetimes. In general, however, the parameters recovered from such a fit have no physical meaning.

The Exponential Series Method (ESM) is designed to recover lifetime distributions without any *a priori* assumptions about their shapes. This method uses a series of exponentials (up to 200 terms) as a probe function with fixed, logarithmically-spaced lifetimes and variable pre-exponentials. This allows covering a lifetime range of several orders of magnitude. In many situations the ESM is capable of differentiating between continuous distributions and discrete, multi-exponentials decays.

Fitting Procedure

The fluorescence decay is approximated by the exponential series:

$$F(t) = \sum_{i=1}^N a_i \exp\left(\frac{-t}{\tau_i}\right) \quad \text{Eq. 1}$$

where a_i are the variable amplitudes, τ_i are the lifetimes which are fixed and logarithmically-spaced, and N is the number of terms. Initially all a_i are set equal. In order to recover amplitudes a_i the ESM uses an iterative procedure to minimize the chi-square function that is defined as follows:

$$C = \left(\frac{1}{n}\right) \sum_{k=1}^n \frac{\left(Y_k - \sum_{i=1}^N D_{ki} a_i\right)^2}{\sigma_k^2} \approx 1.0 \quad \text{Eq. 2}$$

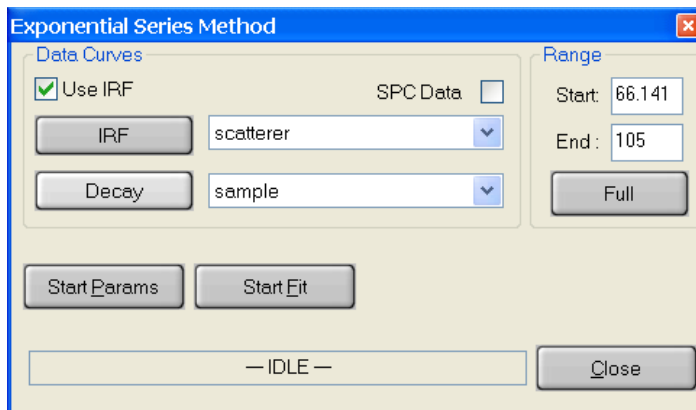
where Y_k represents the fluorescence intensity (e.g. number of photons) in the k th channel, σ_k is the standard deviation in the k^{th} channel, n is the number of data points (or channels), D_{ki} is the convolution matrix:

$$D_{ki} = \int_0^{t_k} L(t_k - t) \exp\left(\frac{-t}{\tau_i}\right) dt \quad \text{Eq. 3}$$

where $L(t)$ comprises the excitation pulse profile and the instrument response function.

Using the Program

The initial **Exponential Series Method** dialog box is shown below.



Data Curves

The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.

The **Fitting Parameters** dialog box for the **Exponential Series** method is shown at left.

The **# Lifetimes** check box and text box select the number of different lifetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

The **# Risetimes** check box and text box select the number of different risetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

Additional Lifetimes

Additional fixed lifetimes may be entered one at a time in the text box. Clicking on the **Add** button enters this value on the lower text window. Lifetimes may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All lifetimes may be removed by clicking on the **Clear** button. This option is useful when there are some lifetimes lying far outside the range of the distribution. Extending the range of the lifetime distribution to include these would be very wasteful since most of the lifetimes would lie in regions with zero amplitude.

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analyses are displayed in several forms.

1. The fitted curve, lifetime distribution, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace. Initially, only the fitted curve and the lifetime distribution are displayed the others being hidden to avoid clutter. The lifetime distribution curve contains most of the information from this analysis. Commonly, all other files must be hidden to see this curve since the Y scale is much smaller than most data curves. The numerical values associated with the distribution are not included in the Math/Fit Output text window since they are, typically, very numerous. The numerical values can be viewed by hiding all other curves except the distribution curve and using the **Grid View** button to display a spreadsheet of the results.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.
3. While the fit is executing a **Fit Status** window displays the current lifetime distribution and residuals on a logarithmic time scale. Once this window is closed it cannot be recovered. Should the user wish to capture this window, this can be done by making **Fit Status** the active window (click on title line), saving the active window to the clipboard (**Alt+ Print Scrn**), opening a graphics program e.g. Paint, and pasting the clipboard into the program (**Ctrl+V**).

MEM – Maximum Entropy Method

Theory

Fluorescence lifetime measurements often result in complex decays requiring a more sophisticated approach than a single- or double-exponential fitting function (James and Ware, 1986, Siemiarczuk *et al*, 1990). This applies especially to the emission originating in such intrinsically complex systems as:

- bichromophoric molecules exhibiting distributions of conformers in the excited state
- fluorophores adsorbed on surfaces
- fluorophores attached to polymers
- fluorescent probes in micelles and liposomes
- fluorescent probes in biomembranes and other biological systems
- fluorophores in monolayers
- intrinsic fluorescence from proteins
- systems undergoing Förster-type energy transfer
- and many others...

Even intuitive considerations would lead one to expect distributions of lifetimes in these systems. Quite often, however, especially for low precision data, a good fit can be obtained with a double- or triple-exponential function for a system, which in fact represents a continuous distribution of lifetimes. In general, however, the parameters recovered from such a fit have no physical meaning.

The Maximum Entropy Method (MEM) is designed to recover lifetime distributions without any *a priori* assumptions about their shapes (Skilling and Bryan 1989, Smith and Grady, 1985). This method uses a series of exponentials (up to 200 terms) as a probe function with fixed, logarithmically-spaced lifetimes and variable pre-exponentials. This allows covering a lifetime range of several orders of magnitude. In many situations the MEM is capable of differentiating between continuous distributions and discrete, multi-exponentials decays.

Fitting Procedure

The fluorescence decay is approximated by the exponential series:

$$F(t) = \sum_{i=1}^N a_i \exp\left(\frac{-t}{\tau_i}\right) \quad \text{Eq. 1}$$

where a_i are the variable amplitudes, τ_i are the lifetimes which are fixed and logarithmically-spaced, and N is the number of terms. Initially all a_i are set equal. The MEM theory utilizes the Shannon-Jaynes entropy function:

$$S = -\sum_{i=1}^N a_i \log \left(\frac{a_i}{\sum_{i=1}^N a_i} \right) \quad \text{Eq. 2}$$

which has to be maximized in order to recover the least biased set of amplitudes $\{a_i\}$ out of all feasible solutions. On the other hand, to ensure that the recovered solution is in agreement with the experimental decay, the following constraint based on the chi-square statistics is implemented:

$$C = \left(\frac{1}{n} \right) \sum_{k=1}^n \frac{\left(Y_k - \sum_{i=1}^N D_{ki} a_i \right)^2}{\sigma_k^2} \approx 1.0 \quad \text{Eq. 3}$$

where Y_k represents the fluorescence intensity (e.g. number of photons) in the k th channel, σ_k is the standard deviation in the k th channel, n is the number of channels, D_{ki} is the convolution matrix:

$$D_{ki} = \int_0^{t_k} L(t_k - t) \exp\left(\frac{-t}{\tau_i}\right) dt \quad \text{Eq. 4}$$

where $L(t)$ comprises the excitation pulse profile and the instrument response function. Conditions (2) and (3) can be combined in one function:

$$Q = \alpha S - C \quad \text{Eq. 5}$$

where α is a Lagrange multiplier. Q is then maximized by an iterative procedure thus ensuring simultaneous maximization of S and minimization of C until constraint (3) is satisfied. After a target value of chi-square is reached, the program keeps maximizing S with C kept constant until the entropy test parameter:

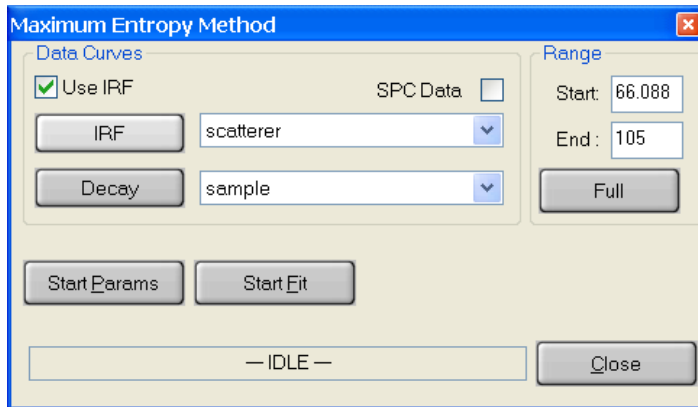
$$0.5 \left[\left(\frac{\text{grad}C}{|\text{grad}C|} \right) - \left(\frac{\text{grad}S}{|\text{grad}S|} \right) \right] < 0.1 \quad \text{Eq. 6}$$

This condition ensures that the global maximum of Q has been reached.

Using the Program

The initial **Maximum Entry** method dialog box is shown below.

Data Curves



The **Use IRF** checkbox selects whether an instrument response function (scatterer) will be used in the analysis or not. Normally, an IRF is used. However, if the lifetime of the sample is long compared to the width of the excitation pulse or the range of data to be analyzed starts at a delay long compared to the width of the excitation pulse an IRF is not required.

The **SPC Data** checkbox is used only when single photon counting data has been imported.

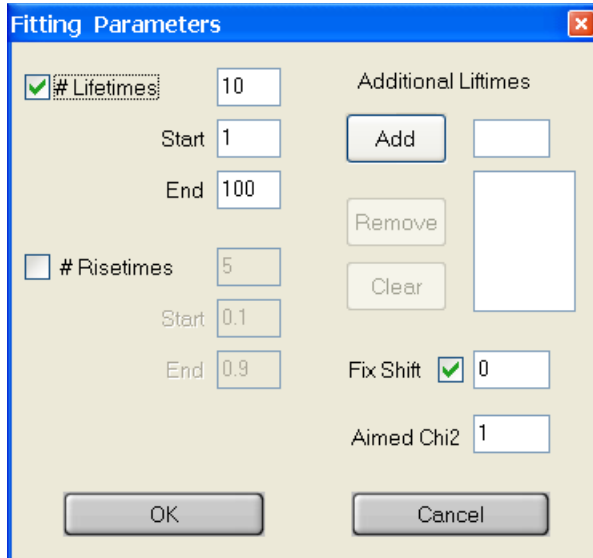
The **IRF** button selects the curve to be used as scatterer. Select a curve by clicking on its name in the legend then click on the **IRF** button. The name of the selected curve will appear in the box beside the button.

The **Decay** button selects the curve to be analyzed. Select a curve by clicking on its name in the legend then click on the **Decay** button. The name of the selected curve will appear in the box beside the button.

Range: The **Start** delay and **End** delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard. Alternatively, select the **Range Toggle** icon from the graph toolbar and position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be highlighted on the screen. If the entire range is to be used in the analysis, click the **Full** button.

Start Params: The non-linear-least-squares fit used in the data analysis requires estimated starting values for the various parameters. Clicking on the Start Params button opens a dialog box that allows these values to be entered.

Start Fit: Clicking the **Start Fit** button starts the analysis program. The box showing *IDLE* in the above picture changes to show the progress of the fit. The **Close** button in the above picture changes to a **Stop Fit** button. Clicking the **Stop Fit** button immediately aborts the analysis. Upon completion of a fit analysis the resulting fit parameters and statistics are automatically appended to the Math/Fit Output text window.



The **Fitting Parameters** dialog box for the **Maximum Entropy Method** is shown at left.

The **# Lifetimes** check box and text box select the number of different lifetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

The **# Risetimes** check box and text box select the number of different risetimes used in the analysis of the decay curve. These are distributed in a logarithmic manner between the **Start** lifetime and the **End** lifetime.

Additional Lifetimes

Additional fixed lifetimes may be entered one at a time in the text box. Clicking on the **Add** button enters this value on the lower text window. Lifetimes may be deleted by clicking on the appropriate line in the text window to highlight the line then clicking on the **Remove** button. All lifetimes may be removed by clicking on the **Clear** button. This option is useful when there are some lifetimes lying far outside the range of the distribution. Extending the range of the lifetime distribution to include these would be very wasteful since most of the lifetimes would lie in regions with zero amplitude.

Aimed Chi2: Enter the target value of χ^2 .

Fix Shift: There may be a small time shift between the sample and the scatterer decay curves (see the **General Introduction** for details). This shift may be included as a parameter in the analysis. The shift parameter may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. If the shift is allowed to float, a value of 0.0 is used as the initial guess.

Once all parameters have been set, click the **OK** button to return to the previous dialog box and then **Start Fit** to start the Analysis.

Results

The results of the analyses are displayed in several forms.

1. The fitted curve, lifetime distribution, the weighted residuals, the autocorrelation function and the deconvoluted decay (i.e. $D(t)$) appear in the workspace. Initially, only the fitted curve and the lifetime distribution are displayed the others being hidden to avoid clutter. The lifetime distribution curve contains most of the information from this analysis. Commonly, all other files must be hidden to see this curve since the Y scale is much smaller than most data curves. The numerical values associated with the distribution are not included in the Math/Fit Output text window since they are, typically, very numerous. The numerical values can be viewed by hiding all other curves except the distribution curve and using the **Grid View** button to display a spreadsheet of the results.
2. A text window named **Math/Fit Output** pops up containing identification information, the lifetimes and pre-exponential factors and various statistics associated with the fit. The text may be edited, saved or printed as the user desires. The results are not deleted from this window when another analysis is run. This feature allows the results of several analyses to be combined. However, this feature may also lead to very long files if many trial analyses are run without clearing the window. To clear the window select **Edit** in the **Math/Fit Output** window and **Clear** from the drop-down menu. If this window is closed it can be reopened by the **View\Math/Fit Output** command.
3. While the fit is executing a **Fit Status** window displays the current lifetime distribution and residuals on a logarithmic time scale. Once this window is closed it cannot be recovered. Should the user wish to capture this window, this can be done by making **Fit Status** the active window (click on title line), saving the active window to the clipboard (**Alt+ Print Scrn**), opening a graphics program, e.g., Paint, and pasting the clipboard into the program (**Ctrl+V**).

Fluorescence Resonance Energy Transfer (FRET)

Theory

The Fluorescence Resonance Energy Transfer (FRET) takes place between an excited donor molecule (D) and the ground-state acceptor molecule (A) over a range of distances, typically 10-100 Å. FRET is a non-radiative process (i.e. there is no photon emitted or absorbed during the energy exchange). The efficiency of FRET is strongly dependent on the D-A distance and is characterized by the Förster critical radius R_0 , a unique parameter for each D-A pair. When the D-A distance is R_0 , the efficiency of energy transfer is 50%. Once R_0 is known, the D-A pair can be used as a molecular ruler to determine the distance between sites labeled by D and A.

There are two basic methods to determine the efficiency of FRET: a) by measuring a decrease of fluorescence intensity of D in the presence of A, and b) by measuring the fluorescence lifetime of D, which becomes shortened as a result of FRET. In some cases, one can also monitor an enhancement of the acceptor fluorescence or the acceptor lifetime, if it is much shorter than the donor lifetime. OBB provides specialized systems for both steady state and time-resolved FRET techniques, which can be used for a variety of FRET applications.

If any quantitative information is expected from a FRET experiment, it is imperative that the R_0 value is known. The R_0 value can be calculated as follows:

$$R_0 = 0.2108 (\kappa^2 \Phi_D n^{-4} J_{DA})^{1/6} \quad \text{Eq. 1}$$

where κ^2 is the orientation factor, Φ_D is the quantum yield of D in the absence of A, n is the refraction index of the medium, and J_{DA} is the spectral overlap integral between the excitation spectrum of A and the emission spectrum of D. The overlap integral J_{DA} can be calculated if the absorption (excitation) spectrum of A and the fluorescence emission spectrum of D are known, i.e.

$$J_{DA} = C \int_0^{\infty} I_D(\lambda) E_A(\lambda) \lambda^4 d\lambda \quad \text{Eq. 2}$$

where I_D is the emission spectrum of D, E_A is the absorption (excitation) spectrum of A and C is the normalization factor defined as:

$$C = \frac{\varepsilon(\lambda_{\max})}{E_A(\lambda_{\max}) \int_0^{\infty} I_D(\lambda) d\lambda} \quad \text{Eq. 3}$$

where $\varepsilon(\lambda_{\max})$ is the molar extinction coefficient of A at the absorption (excitation) maximum.

The value of κ^2 depends on a relative orientation of D and A transition moments. If the transition moments have fixed orientations, κ^2 will vary from 0 (transition moments perpendicular) to 4 (transition moments collinear). For parallel transition moments $\kappa^2 = 1$. When A molecules are randomly distributed about D in a rigid medium, $\kappa^2 = 0.476$. If D and A undergo a rotational motion, which is faster than the decay time of D, $\kappa^2 = 2/3$.

The energy transfer efficiency E can be calculated from either fluorescence intensity or lifetime measurements for D alone and D in the presence of A.

$$E = 1 - \frac{I_{DA}}{I_D} \quad \text{Eq. 4}$$

where I_D and I_{DA} are fluorescence intensities of D in the absence and presence of A, respectively, or

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \quad \text{Eq. 5}$$

where τ_D and τ_{DA} are fluorescence lifetimes of D in the absence and presence of A, respectively.

Once R_o and E are known, the distance r between D and A can be calculated:

$$r = \left(\frac{1}{E} - 1 \right)^{1/6} R_o \quad \text{Eq. 6}$$

If the lifetime of D is known, the FRET rate constant can also be calculated:

$$k_{ET} = \frac{1}{\tau_D} \left[\frac{R_o}{r} \right]^6 \quad \text{Eq. 7}$$

Using the FRET Calculator

Clicking on **Math, Data Analysis**, and then on **FRET** can access the FRET Calculator. The FRET drop down menu gives three choices: **Determine R_o** , **Calculate FRET Parameters (steady-state)** and **Calculate FRET Parameters (lifetimes)**.

Determine R_0

FRET - Determine R0

$$R_0 = 0.2108 \sqrt{\frac{\kappa^2 \Phi_D n^{-4} \epsilon(\lambda_{\max}) \int_0^{\infty} I_D(\lambda) E_A(\lambda) \lambda^4 d\lambda}{E_A(\lambda_{\max}) \int_0^{\infty} I_D(\lambda) d\lambda}}$$

Data Curves

Donor Emission: Id(lambda)

Acceptor Absorption: Ea

λ_{\max} = 553

Parameters

κ^2 = 0.6666666666 n = 1.333333373 Set To Default

Φ_D = 1 $\epsilon(\lambda_{\max})$ = 20000

Förster distance (Å)

R₀ = 36.004 Calculate R₀ Close

Donor Emission: This button selects the curve to be used as donor emission spectrum. Select a curve by clicking on its name in the legend and then click on the **Donor Emission** button. The name of the selected curve will appear on the box beside the button.

Acceptor Absorption: This button selects the curve to be used as acceptor absorption (excitation) spectrum. Select a curve by clicking on its name in the legend and then click on the **Acceptor Absorption** button. The name of the selected curve will appear on the box beside the button.

Once the curves have been defined, the acceptor absorption (excitation) maximum wavelength will be displayed in the λ_{\max} box.

κ^2 : Enter the value for the orientation factor in the box or leave the default value of 2/3 for the fast rotation limit.

Φ_D : Enter the value for the donor emission quantum yield in the box (the default value is 1).

n: Enter the value for the index of refraction in the box (the default value is 1.33333 for water).

$\epsilon(\lambda_{\max})$: Enter the value for the molar extinction coefficient for the acceptor at the absorption (excitation) maximum in the box (the default value is 20000).

Calculate R_0 : Click on this button to show the Förster distance R_0 in the **R_0** box.

Set To Default: Click on this button to reset all the parameters to default values.

Calculate FRET Parameters (steady-state)

Calculate FRET Parameters (steady-state)

Data Curves

Range

Parameters

$R_0 = 36.004$ (Å)

$\tau_D = 5e-009$ s

Intensity Input Mode

Enter values manually

Define using data cursor

Calculate by integration

Calculate by average

Intensity Values

Donor 536800

D/A 327640

Calculate

E = 0.39

$r_{DA} = 38.8$ (Å)

$K_{ET} = 1.2768e+008$ (1/s)

Close

R_0 : In the **Parameters** box, either enter the value of the Förster distance R_0 or retain the value calculated in the **Determine R_0** option.

τ_D : In the **Parameters** box, enter the donor lifetime if you want the FRET rate constant to be calculated.

- To enter intensity values manually, click on the **Enter values manually** radio button in the **Intensity Input Mode** box. Then type in intensity values for donor alone in the **Donor** box and for donor in the presence of acceptor in the **D/A** box. Click on **Calculate** and the FRET efficiency **E**, donor-acceptor distance (**r_{DA}**) and FRET rate constant **k_{ET}** will be displayed. The **k_{ET}** value will only have any meaning if the correct **τ_D** has been entered, otherwise it should be ignored.

- To enter donor intensities using the data cursor, click on the **Define using data cursor** radio button. The **Data Curves** box becomes available.

Calculate FRET Parameters (steady-state)

Data Curves

Donor alone

Donor + Acceptor

Parameters

R₀ = 36.004 (Å)

τ_D = 5e-009 s

Intensity Input Mode

Enter values manually

Define using data cursor

Calculate by integration

Calculate by average

Intensity Values

Donor 533805

D/A 364589

E = 0.317

r_{DA} = 40.918 (Å)

K_{ET} = 9.2826e+007 (1/s)

The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

Select Donor values

X 500

Y 229094

In the **Intensity Values** box click on the **SET** button next to the **Donor** box, which opens the **Select Donor values** box. The data cursor initially is at the left edge of the selected trace. Using the mouse or the left and right arrow keys move the cursor to the desired position for the intensity readout. Click **Auto Find** to automatically position the cursor at the maximum of the donor curve. Click on **Select** to capture the intensity of the Donor and close the dialog.

Similarly, in the **Intensity Values** box click on the **SET** button next to the **D/A** box, which opens the **Select D/A values** box. The data cursor initially is at the left edge of the selected trace. Using the mouse or the left and right arrow keys move the cursor to the desired position for the intensity readout. Click **Auto Find** to automatically position the cursor at the maximum of the D/A curve. Click on **Select** to capture the intensity of the Donor in the presence of the acceptor and close the dialog.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance (**r_{DA}**) and FRET rate constant **k_{ET}** will be displayed. The **k_{ET}** value will only have any meaning if the correct **τ_D** has been entered, otherwise it should be ignored.

- To calculate donor and D/A intensities by integration, click on the **Calculate by integration** radio button. The **Data Curves** box becomes available and the **Selected Range** function is activated.

The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

Range: To select the integration range, use the mouse to drag the left and right edges of the **Selected Range**. Alternatively, type in the start and end values for the range and click on the **UPDATE** button. The integrated intensity values will be displayed in the **Intensity Values** box. If the integration is to be carried out over the entire range, just click on the **FULL** button and the intensities will be captured and displayed.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance (r_{DA}) and FRET rate constant k_{ET} will be displayed. The k_{ET} value will only have any meaning if the correct τ_D has been entered, otherwise it should be ignored.

- To calculate donor and D/A intensities by average, click on the **Calculate by average** box. The **Data Curves** box becomes available and the **Selected Range** function is activated.

Calculate FRET Parameters (steady-state)

Data Curves

Donor alone

Donor + Acceptor

Range

D 500 700

A 500 700

Parameters

ρ_0 = 36.004 (Å)

τ_D = 5e-009 s

Intensity Input Mode

Enter values manually

Define using data cursor

Calculate by integration

Calculate by average

Intensity Values

Donor 134649.091044776

D/A 91965.3312935325

E = 0.317

r_{DA} = 40.918 (Å)

K_{ET} = 9.2826e+007 (1/s)

The **D only emission** button selects the donor emission curve. Select a curve by clicking on its name in the legend, then click on the **D only emission** button.

The **D/A emission** button selects the donor emission curve measured in the presence of acceptor. Select a curve by clicking on its name in the legend, then click on the **D/A emission** button.

Range for D: To select the averaging range for the donor alone, click on the **D** radio button in the **Range** box, and use the mouse to drag the left and right edges of the **Selected Range**, or type in the start and end values for the range and click on the **UPDATE** button. The average intensity value for **D** will be displayed in the **Intensity Values** box. If the averaging is to be carried out over the entire range, just click on the **FULL** button and the intensity will be captured and displayed.

Range for D/A: To select the averaging range for the donor in the presence of acceptor, click on the **A** radio button in the **Range** box, and use the mouse to drag the left and right edges of the **Selected Range**, or type in the start and end values for the range and click on the **UPDATE** button. The average intensity value for **D/A** will be displayed in the **Intensity Values** box. If the averaging is to be carried out over the entire range, just click on the **FULL** button and the intensity will be captured and displayed.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance (r_{DA}) and FRET rate constant k_{ET} will be displayed. The k_{ET} value will only have any meaning if the correct τ_D has been entered, otherwise it should be ignored.

Calculate FRET Parameters (lifetimes)

Calculate FRET parameters from lifetimes

Input

Lifetimes

τ_{DA} = 2.36e-009 s

τ_D = 5.74e-009 s

R_0 = 36.004

Output

E = 0.588850174216028

r_{DA} = 33.9117324965 Å

K_{ET} = 2.4951e+008 1/s

Calculate Close

In the **Input** box, enter the lifetime value of donor in the presence of acceptor (τ_{DA}) and the donor alone (τ_D). At the bottom of the **Input** box, either enter the value of R_0 or retain the value calculated in the **Determine R_0** option.

Click on the **Calculate** button and the FRET efficiency **E**, donor-acceptor distance (r_{DA}) and FRET rate constant k_{ET} will be displayed.

Trace Math Commands

The commands in the Trace Math menu allow specific mathematical functions to be carried out on single curves or selected regions of a curve. Many of the math dialog boxes can be left open so that multiple operations can be performed.

Settings and controls that are common to all dialog boxes are presented under the heading Common Math Controls. The descriptions for the configuration dialog boxes that follow provide details on the specific math function as well as settings and controls that are unique to them.

Note. Some math functions are performed on a selected region of a curve (a subset of the X values). To select this region, first choose the target curve by clicking on its name in the legend. Then select the **Range Toggle** icon (II) from the graphing toolbar and use the mouse to click and drag within the graph display over the desired region of the curve. For more precise control, you can then enter **Low X** and **High X** values into the text boxes provided. The selected region will be highlighted, and the desired math value will be displayed. The math function dialog box can be left open while different regions are selected, and math values, when displayed, will change dynamically.

Common Math Controls

The trace name operand is shown in the dialog title bar after the trace math command name.

Create New Data

If checked, a new curve will be created. The original (source) data will be preserved.

Replace Old Data

If checked, the original curve will be permanently lost, as it will be replaced by the new data.

Label

Type the name of the new curve in the text box. If no label is specified, the new curve will be listed in the legend with a name comprised of a generic math function descriptor (e.g., Smooth, or Logarithm) added to the source curve's original name.

Execute

Carries out the operation. If you type in new values to select an X-axis region, **Execute** is required to perform the new calculation.

Lock to trace

The values in the dialog are locked to that curve, even if you then select another curve.

Close

Closes the math function dialog box.

Antilog

Calculates the antilogarithm of the selected curve.

Average

Calculates the average value of the Y-axis parameter on a selected region of a curve. The average value is the sum of the values divided by the number of points.

The standard deviation is also determined using the equation:

$$\sigma = \sqrt{\frac{\sum_i y_i^2 - \frac{1}{n}(\sum_i y_i)^2}{n-1}}$$

Where y_i is the Y-value of a data point and n is the total number of data points in the portion of the data trace being averaged.

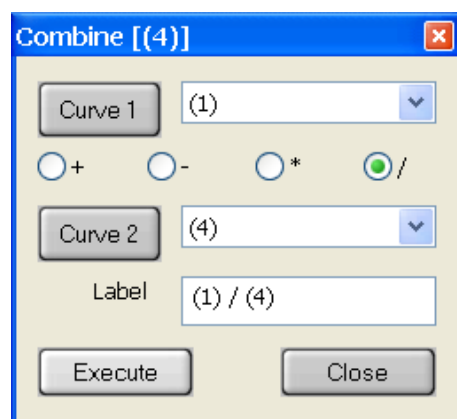
Distribution Average

Calculates the **Integrated Amplitudes** and **Distribution Average** of the selected region of a trace. The integrated amplitudes is the sum of the Y-values. The distribution average is determined using the equation:

$$\frac{\sum_i x_i y_i}{\sum_i y_i}$$

Combine

The combine command allows you to add one curve to another, subtract a curve from another, multiply a curve by another, or divide a curve by another. The math is performed in a point-by-point fashion. Only the portions of the curves that overlap are combined.



Curve 1, Curve 2

Select the curves for the operation by clicking on their names in the drop-down list boxes. Alternatively, select a curve from the legend and click on the Curve 1 or Curve 2 button.

Operation

Check an operator to add (+), subtract (-), multiply (x), or divide (/) Curve 1 by Curve 2.

XY Combine

This feature allows the user to construct a new data trace, using the X values of one trace, and the Y values of another trace. In this way, complex data, such as time-dependent temperature ramps and correlated data, can be converted into new traces that have compatible X axes to simplify the display and treatment of the data.

Source trace with X data

Use the drop-down menu to choose the trace from which to create the X data. Alternatively, select a curve from the legend and click on the **Pick** icon beside the **Source trace with X data** header.

Source trace with Y data

Use the drop-down menu to choose the trace from which to create the Y data. Alternatively, select a curve from the legend and click on the **Pick** icon beside the **Source trace with Y data** header.

Note: If the X-data of the two operands are different, then the resulting Y-values will be linearly interpolated or extrapolated from the two nearest Y-values of the second operand.

Differentiate

Differentiate takes the derivative of the selected curve. Subsequent application of the differentiate command results in the second derivative, etc. Differentiation is done using the 5 point Savitzky-Golay algorithm, which provides a smoothed derivative.

Integrate

This function integrates within the range of the selected region of a curve. The Total Area is the integral of the data above the absolute X-axis. The Peak Area is used to integrate a peak within a curve.

Total Area

Displays the total integrated area within the selected range. If there is negative data, then the total integrated area may also be negative.

Peak Area

Displays the integral of the peak above the background. The graph module projects an imaginary line between the points where the boundaries of the range intersect the curve. Peak Area is the integrated area above that line. If most of the curve data lies below this line, then the Peak Area will be a negative number.

Lock to trace

The values in the Integrate dialog are locked to that curve, even if you then select another curve.

Linear Fit

Calculates and overlays a linear fit to the selected region of a curve. The slope, intercept, and correlation coefficient are displayed.

Linear Scale

The Linear Scale is used to shift a curve or a selected region of a curve on either the X- or the Y-axis. The curve can be shifted on the Y-axis by a multiplier, divisor, or an addend. The curve can be shifted on the X-axis by an addend only.

Y and X Value

Multiplier: Multiplies all Y values in the curve by the specified multiplier.

Divisor: Divides all Y values in the curve by the specified divisor.

Offset: Adds the specified value to the X or Y value for each point in the curve.

Select Range

Applies the transformation only within the region selected by the user. The remainder of the trace is also copied but without transformation. The range is selected dragging the edges of the **Selected Range**.

Logarithm

Calculates the logarithm of the selected curve.

Normalize

Normalizes a curve to a set value. The normalization function reference may be either a peak or a specified point.

Reference

Select **Peak** or **Specified Point**. Enter the X value of the specified point in the text boxes.

Normalize to:

Enter the value to which the curve will be normalized.

Reciprocal

Calculates the reciprocal ($1/Y$) of the Y-axis data in the selected curve.

Smooth

This function performs a Savitzky-Golay smoothing of the selected curve.

Buffer Size

Select a 7, 15, 21, or 33-point buffer. A higher buffer results in greater smoothing.

Truncate

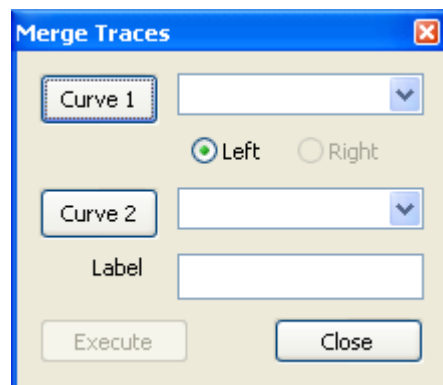
Truncate is used to reduce the X-axis range on the selected curve. The selected region of the curve is preserved and all X values above and below this region are permanently deleted. The region may be selected by entering the endpoints of the region, or by clicking and dragging the **Selected Range** edges.

Baseline

Baseline suppression causes a **Selected Range** of a curve to be set to a constant Y value (commonly zero). The range is selected as described in the introduction to this chapter. The chosen Y-value is entered into the text box and the function is performed by pressing the **Execute** button. This function is useful when noise in the baseline of the scatterer affects the lifetime of the sample. This happens because the IRF (scatterer) is convoluted with the sample lifetimes to give the observed decay. Thus noise in the scatterer is also convoluted and becomes a major problem for long-lived samples when observations are recorded out to many sample lifetimes. The convoluted noise has the same effect as small light pulses long after the real light pulse has ended. The derived lifetimes therefore appear to be smaller than they really are. See the **Analysis** chapter for further details.

Merge Traces

The Merge Traces command allows you to merge two traces together.



Curve 1, Curve 2

Select the curves for the operation by clicking on their names in the drop-down list boxes. Alternatively, select a curve from the legend and click on the Curve 1 or Curve 2 button.

Curve 1 must extend toward the right end of the X-axis.

Curve 2 must extend toward the left end of the X-axis.

Peak Finder

This function uses two algorithms. **Smoothed** fits a quadratic polynomial to sequential groups of data points to find peaks within the range of the **Selected Range** of a curve. Because of the polynomial fitting, the apparent X and Y **Peak values** and may be shifted slightly. **Analog** finds the global peak as the highest Y-value and local peaks as being higher than immediate left and right neighboring points.

X-range limits: Displays the low and high limits set by the **Selected Range**. These limits may be moved by using the cursor to grab and move the vertical edge lines.

Mark peak on graph: Shows a crosshair at the peak position on the graph.

Smoothed: Uses polynomial fitting to find the peaks.

T= Displays the threshold value. Only peaks above this threshold will be found.

W = Specifies the number of consecutive data points to use in the quadratic least squares fit. This value should not exceed approximately half of the half-width of the peak. It can be much smaller for noise-free data. The best choice for well-defined peaks is 3.

Analog: Does not smooth the data before finding peaks.

Search for:

Global peak: The peak within the selected range with the highest Y-axis value.

Local peak to right/left: Click on **Execute** to find the next peak to the right or left.

Peak values:

X: the X value of the specified peak.

Y: the Y value of the specified peak.


Peak #: Currently shown peak / Total peaks found.

AXES MENU

The commands in the axes menu allow expansion or contraction of the axes for viewing and analyzing specific regions of a trace. The labels applied to the axes may also be altered. Some of these commands will appear as buttons on the Toolbar and as the submenu Graph\Axes when right-clicking on the graph area.

Full Autoscale

Scales the X and Y-axis to provide maximum space for the displayed curves in the graph window. A check mark will appear next to this command when this scaling mode is in effect.

Shortcut:  Use the toolbar icon to re-scale the axes after zoom features are used.

Autoscale From 0

Scales the Y-axis to provide maximum space, starting at 0, for the displayed curves in the graph area. A check mark will appear next to this command when this scaling mode is in effect.

Fixed Y-Min. & Max...

Assigns a minimum and a maximum value to the Y-axis. The Y-scale will remain fixed within this range even when the X-axis is zoomed in or out. A check mark will appear next to this command in the menu when this scaling mode is in effect.

Logarithmic Y-Scale

Makes the Y-axis logarithmic. The default log scale is automatic decade selection. If there are zero or negative values in the displayed trace(s) the automatic log scale may not be optimized. You can change the number of decades to display the data over using the **Visible log Decades** command in the **Axes** menu. A check mark will appear next to this command in the menu when this scaling mode is in effect.

Visible log Decades

Use this menu to define the number of log decades to be displayed on the screen. The default will automatically scale the Y-axis using an appropriate number of decades. If there are zero or negative values in the displayed trace(s), automatic selection of log decades may not be optimal for the display. The user can select from two to eight log decades to display the data over. To view the displayed curves in log scale select **Logarithmic Y-Scale** from the **Axes** menu.

2x X-Zoom In

Expands the X-axis by a factor of 2, beginning at the center of the display.

2x X-Zoom Out

Contracts the X-axis by a factor of 2, beginning at the center of the display.

Fixed X-Min. & Max...

Assigns a minimum and a maximum value to the X-axis. The X-scale will remain fixed within this range even when the Y-axis is zoomed in or out. A check mark will appear next to this command in the menu when this scaling mode is in effect.

Edit Axis Properties

Here you can modify the Axis settings for the record or the groups (depending on which you select at the bottom radio button). If you click on an Enabled button (so that it is pushed down) it will add a new axis with the properties specified. If you unclick (doesn't stay down) it will remove the axis. You can add/remove group axes and edit the axes titles. Record axes titles can only be edited.

If you click on a Fixed button you can set the minimum and maximum values to that axis.

Selecting a unit from the Unit choice list will put that title in the Caption box where it can then be edited. If there are units in the parentheses these will show on the axis title. Note: changing a unit (e.g., from ms to s) changes the axis label only, it does not change the data point values.

NOTE: The graph module supports multiple X axes (each group can have one), although the graph only allows one to be displayed at once, so you have to click on the traces in the groups to make that axis become visible.

HELP MENU

About EasyLife X

This command gives version and copyright information about EasyLife X.

TROUBLESHOOTING

MESSAGES

Initialization of the DGG failed!

- Check if the EasyLife X is turned on.
- Check if the RS-232 cable is disconnected from the EasyLife X or the computer.

MC/UL Error 002: Digital device is not responding. Is base address correct?

- Check if the USB cable is disconnected from the EasyLife X or the computer.

BEHAVIOUR

Near zero signal only

- Check if the grey or BNC cables are disconnected from the LED housing or the EasyLife X.
- Check if there is a sample in the chamber.
- Check if there are filters or something else blocking the optical path.

SERVICE CALLS TO OBB

Before calling for service, please review the *Troubleshooting* section. To aid our Service Department in discussing your questions, as well as to aid in the timely solution of any problems, please assemble as much as possible of the following information before calling OBB:

- Your instrument serial number, or as many other component serial numbers as possible
- Your instrument type and hardware configuration (and software version, if applicable)
- The date on which your instrument was received
- As much detail as possible on the particular chain of events or circumstances that led to the problem. This information should include the complete instrument status and data gathering protocol.

Contact OBB Service at

Phone: 1-877-784-4349

E-mail: ptiservice@pti-nj.com

REFERENCES

1. Bevington, P.R., (1969) *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York.
2. Birks, J.B., (1948) *J. Phys. B. Ser 2*, **1**, 946.
3. Durbin, J. and Watson, G.S., (1950) *Biometrika*, **37**, 409-428.
4. Durbin, J. and Watson, G.S., (1951) *Biometrika*, **38**, 159-178.
5. Förster, T., (1949) *Z. Naturforsch.* **49**, 321.
6. Grinvald, A. and Steinberg, I.Z., (1974) *Anal. Biochem.*, **59**, 583-598.
7. Grynkiewicz, G., Poenie, M., Tsien, R.Y., (1985) *J. Biol. Chem.* **260**, 3440-3450.
8. Hamburg, M., (1985) *Basic Statistics*, Brace Harcourt Jovanovich, New York.
9. James, D.R., Siemiarzuck, A., Ware, W.R., (1992) *Review of Scientific Instruments*, **63** (2), 1710-1716.
10. James, D.R. and Ware, W.R., (1986) *Chem. Phys. Letters*, **126**, 7.
11. Knutson, J.R., Beechem, J.M. and Brand, L., (1983) *Chem. Phys. Letters*, **102**, 501-507.
12. O'Connor, D. and Phillips, D., (1984) *Time-Correlated Single Photon Counting*, Academic Press, London.
13. Phillips, D., Drake, R.C., O'Connor, D.V., and Christensen, R.L., (1985) *Analytical Instrumentation*, **14**, 267-292.
14. Rodgers, M.A.J., da Silva, M.E. and Wheeler, E., (1978) *Chem. Phys. Letters*, **53**, 165.
15. Siemiarzuck, A., Wagner, B.D. and Ware, W.R., J., (1990) *Phys. Chem. Letters*, **94**, 1661.
16. Siemiarzuck, A. and Ware, W.R. (1990) *Chem. Phys. Letters*, **160**, 285-290.
17. Skilling, J. and Bryan, R.K., (1984) *Mon. Not. R. Astron. Soc.*, **211**, 111.
18. Smith, C.R. and Grady, W.T., Jr., Eds., (1985) *Maximum Entropy and Bayesian Methods in Inverse Problems*, Reidel, Boston.
19. Steinberg, I.Z., Haas, E. and Katchalski-Katzir, E., (1983) *Time-Resolved Fluorescence in Spectroscopy and Biochemistry*, Cundall and Dale, Ed., Plenum, Pp. 411-450.
20. Valeur, B. (2002) *Molecular Fluorescence. Principles and Applications*, Wiley-VCH, Weinheim
21. Ware, W.R. and Andre, J.C., (1983) *Time-Resolved Fluorescence in Spectroscopy and Biochemistry*, Cundall and Dale, Ed., Plenum, Pp. 363-392.

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