PTI Fluorescence Master Systems

FelixGX 4.1.2 Software User's Manual

Photon Technology International

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FelixGX 4.1.2 Software User's Manual revision A4

PTI Standard Instrument Warranty

Warranty Period and Extent
Photon Technology International (PTI) warrants that its instruments will be delivered in a functional state and free from defect, and will meet stated specifications for a period of one (1) year. The warranty period will start on the date of shipment by PTI. In case of systems that include installation by PTI, the warranty will start from the date of installation or thirty (30) days after the shipping date, whichever is earlier.

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, nor will PTI provide training on its use free of charge. PTI shall not be responsible for any liability, loss or damages, caused or alleged to be caused, by the system, as a result of use or operation including, without limitation, consequential damages and loss of profit.

Specific Exclusions and Limitations
1) It is recognized that the performance of consumable items will diminish as a function of use, and that it may be necessary to replace such items to restore the stated specifications. Consumable items (arc lamps, filters, cuvettes, lenses, etc.) are not covered by the warranty.
2) The original manufacturer's warranty will be maintained for major system components not manufactured by PTI (e.g. computers, printers, microscopes, cameras and components thereof).
3) Fiber optic bundles are not covered by the warranty.
4) The use of arc lamps not supplied by PTI (or approved in writing by PTI) will void PTI's warranty on all illuminator subsystem components.
5) If there is any evidence of physical contact with coated optics (e.g. fingerprints), the warranty on that item will be voided.
6) If the optical components are realigned by the customer without specific permission from PTI, the warranty will be voided. Please note that the customer is responsible for changing lamps and aligning the lamp after installation. Aligning the lamp will not void the warranty unless other exclusions are applicable (nos. 4 and 5).
7) Instrument systems that are not authorized to be installed by anyone other than PTI service personnel will not be warranted.
8) In case of systems that include installation as part of the original purchase, unpacking the instrument by anyone other than PTI personnel will void the warranty.
9) Moving systems to another site within a facility or to another location, without specific permission from PTI, will void the warranty.
10) Damage or loss caused by shipping is not covered by the warranty.
11) Damage caused by improper operation of the instrument will void the warranty.
12) Damage caused by equipment not purchased from PTI that is attached to the instrument is not covered by the warranty.
13) Warranty is valid only in the state, province or country of the original purchase.
14) Warranty is valid only on systems having a computer supplied by PTI.
15) Software upgrades performed on the PTI computer workstation (e.g., adding word processors, image editors, etc.) not authorized by PTI will void the warranty.
16) Hardware upgrades performed on the PTI computer workstation (e.g., adding network boards, sound cards, etc.) not authorized by PTI will void the warranty.

Warranty Returns
A Return Material Authorization (RMA) Number must be obtained from the PTI Service Department before any items can be shipped to the factory. Returned goods will not be accepted without an RMA Number. Customer will bear all shipping charges for warranty repairs. All goods returned to the factory for warranty repair should be properly packed to avoid damage and clearly marked with the RMA Number.

Warranty Repairs
Warranty repairs will be done either at the customer's site or at the PTI plant, at our option. All service rendered by PTI will be performed in a professional manner by qualified personnel.

Software
PTI makes no warranties regarding either the satisfactory performance of the software or the fitness of the software for any specific purpose. PTI shall not be responsible for any liability, loss or damages caused or alleged to be caused by our software as a result of its use, including, without limitation, consequential damages and loss of profit, nor will PTI provide training on its use free of charge.

Contents

Chapter 1

Introduction

Welcome to the Family

Congratulations on your purchase of a fluorescence system from PTI. 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 User's Guide

What it does

This guide provides the following information:

1. Basic system startup procedure.
2. Installing and uninstalling the FelixGX program software.
3. Data acquisition for typical fluorescence techniques.
4. Data analysis functions for fluorescence data.
5. Saving and retrieving data.
6. Transferring data to and from other applications.
7. Basic hardware operation and maintenance.

What it does not do

1. Describe using Windows.
2. Explain the techniques of fluorescence spectroscopy.

For detailed information on fluorescence spectroscopy and time resolved measurements, please see the following references:

a) Guilbault, G., Practical Fluorescence, Marcel Dekker, New York (1990).

b) Lakowicz, J., Principles of Fluorescence Spectroscopy, Third Edition, Plenum Press, New York (2006).

c) Rendell, D., Fluorescence and Phosphorescence Spectroscopy, Analytical Chemistry by Open Learning, John Wiley & Sons, New York (1987).

d) Valeur, B., Molecular Fluorescence: Principles and Applications, Weinheim Wiley, New York (2002).

Guide Organization

The guide applies to PTI's FelixGX™ for Windows. FelixGX is the computer program used to control your instrument and to gather, analyze and store data. Since the operation of your instrument depends entirely on FelixGX, information on system startup and operation is included in the software section.

Note: Some systems may include options and accessories that are not covered in the hardware section of this manual. For information on those products, please refer to the materials provided with them. Conversely, there will be information on components that do not exist in your particular instrument configuration.

PTI Fluorescence Instrument lines Overview

All of PTI's fluorescence systems utilize an "open architecture" design that permits systems to be configured to best suit your needs. FelixGX features specialized and powerful data collection and analysis tools for all these systems. PTI has three open-architecture fluorescence instrument lines:

QuantaMaster™: Spectrofluorometers are used to measure steady state fluorescence and phosphorescence, as well phosphorescence lifetimes, luminescence, bioluminescence, and chemiluminescence.

TimeMaster™: Lifetime fluorometers measure fluorescence and phosphorescence lifetimes, time-resolved spectra, and timebased kinetics using PTI's patented strobe technique and gated detection.

RatioMaster™: Microscope based ratio spectrofluorometers measure dynamic ratio fluorescence on a millisecond timescale.

Chapter 2

Getting Started

Although we strongly recommend reading this manual before attempting to run your instrument, most users are anxious to get started. It is possible to learn as you go, but there are some basics to understand first. It should be noted the terms *trace* and *curve* are used synonymously throughout the manual.

Important: The system startup procedure has some critical steps that must become second nature to any system user. Otherwise, it is possible to damage the instrument as well as peripheral equipment attached to it.

What is FelixGX?

FelixGX software is used for fluorescence data collection and analysis. The program runs on computers using the Windows XP or 32-bit Windows 7 operating systems. Since FelixGX conforms to Windows conventions, the user interface is very similar to that of other Windows programs such as Word or Excel.

FelixGX controls the instrument, collects the fluorescence data, and provides a number of tools needed for data analysis and presentation. The acquired data can be displayed in a variety of ways during and after acquisition. The versatility of Windows makes it easy to customize the display format. In time, you will develop your own preferences depending upon how you organize your experiments.

After the data is acquired, it can be analyzed in a variety of ways. The traces can be zoomed to look at a small region and/or mathematically manipulated and combined. Calibration traces can also be constructed.

Within FelixGX, data can be exported to other Windows programs for additional analysis or other processing. You can either use the Windows clipboard to transfer data from one open Windows application to another, or export your data in standard text file format for subsequent importation.

Windows handles printing and plotting from FelixGX, so you have a wide choice of output devices. Older or obsolete printers and plotters may not have Windows drivers. Contact the printer manufacturer to see if the correct Windows operating system drivers for your printer are available.

Getting the most out of FelixGX

Are you familiar with Windows?

If you are familiar with Windows, you will be able to use FelixGX immediately. If you are not familiar with Windows read through the Windows documentation. There are also online Windows tutorials. Once you acquire basic Windows skills you will quickly find what you need to get started and then pick up more detailed information as you go.

Use the Help utility

FelixGX has an online Help utility that works similar to the Windows Help utility.

 **Hint**. You can get a good feel for how FelixGX works by reviewing some of the Help topics before using the program. You can do this without even running FelixGX by double clicking on the FelixGX Help icon that was added to the Windows Start Menu during installation. Explore the FelixGX menus and commands and learn what they do. Get to know the primary features of FelixGX and some of the terminology used throughout the program.

In addition to the Help utility, "Tool Tips" will pop up next to the cursor when it is positioned over most toolbar buttons. They will appear without having to press the mouse button.

Keep Help handy

You can resize and reposition the Help window to keep it out of your way.

To keep the screen less cluttered, you can toggle between the FelixGX and Help windows. After opening Help from within FelixGX and selecting the topic you want, press **Alt+Tab** simultaneously on the keyboard. Each time you press Alt+Tab, you will toggle between FelixGX and Help (this only works if Help and FelixGX are the only two windows opened, otherwise this will cycle through all opened windows). You can also make a hard copy of a Help topic by selecting the **Print** icon on the Help toolbar.

Basic System Startup Procedure

The following procedure can be used only after FelixGX has been installed on the computer. See **Installing FelixGX** in Chapter 3 System Setup in the FelixGX Software User's Manual.

Warning! The ignition of an arc lamp requires a very high voltage pulse. A high voltage transient may be injected into the electrical system of the instrument. This transient can cause a read/write error or even damage your computer or other system components. PTI recommends that the Lamp Power Supply be connected to a separate electrical line and that all other components (computer, motor driver, etc…) be connected to a surge suppressor on an isolated line. Contact PTI if you have any questions.

Warning! When starting your instrument, if your system has an Arc Lamp, ***always ignite the lamp before turning on anything else!*** Otherwise, damage to the computer, the detector, or other sensitive subsystems could result. This point cannot be overemphasized.

1. **For systems with an LPS-220 or LPS-220B Lamp Power Supply**
   On the Lamp Power Supply, set the LCD display control to Watts. Press the power button (it is illuminated when powered). Turn the current knob to vertical. Wait 10 seconds, then press and hold the Ignite button. You will hear an audible click as the lamp ignites. When the LCD display shows the wattage reading, the lamp has ignited. Release the Ignite button and allow the lamp to warm up for 15 minutes. Finally, adjust the Current control to display 75 watts. If the lamp does not ignite, see the Troubleshooting section at the end of this manual. It should also be noted that the Lamp Power Supply has the ability to auto-ignite. By putting the Ignite switch on the back of the power supply to the Auto the lamp will automatically ignite when the power button is pressed. All other steps must still be followed to maintain proper lamp operating conditions.

   For systems with an LPS-100 Integrated Lamp Power Supply and Igniter
   (75 watt Xenon lamp only)
   Press the ON/OFF rocker switch to ON position "-". The lamp will automatically ignite and adjust to 75 W operation.

2. If you have light sources other than the arc lamp, please follow the appropriate procedures outlined elsewhere.

3. In no particular order, power up (as applicable) the ASOC-10 box, the Motor Drive Box(es), Temperature Control, along with any other components your system may contain.

4. Turn on the computer and launch FelixGX.
   If the computer has been disconnected from the ASOC-10 and or the MD-4000 Motor Driver boxes, then you may have to follow the procedure in Chapter 14 Troubleshooting: Loss of communication with the ASOC-10 or MD-4000.

5. Click on a hardware configuration name on the Acquisition Bar to initialize the hardware. If you have just installed or upgraded FelixGX and it was not configured

for your hardware, you must create a hardware configuration that comprises the components in your system. Click on **Configure, Hardware, Configuration, New** to create a new hardware configuration (see Hardware in Chapter 10). To modify an existing hardware configuration, click on that hardware configuration on the Acquisition Bar, then **Configure, Hardware**, or **Configure, Hardware, Configuration, Open** and choose the desired hardware configuration.

6. To start collecting data, click on an acquisition name on the Acquisition Bar to open a previously saved acquisition or click on **Setup** on the Acquisition Control Panel to create a new acquisition (see Chapter 12 - Acquisition Setup). If selecting a new acquisition, a dialog box will open allowing you to select the type of acquisition you would like to perform (Emission Scan, Excitation Ratio, Timebased, etc…). Selecting one of these options will open an acquisition window where you can set the experimental parameters.
7. If your system has Photomultiplier Detector System, 610, 710, 810, or 814 housing(s) with ON/OFF switches turn the detector ON. They are powered from the ASOC-10 box or optional dedicated power supplies. The VOLT/SIGNAL switch should be switched to the VOLT position (down) and the High Voltage should be set to ~ 1100 volts, by turning the Voltage Adjust knob.

   If your system has 914 housing(s) using digital mode the voltage applied to the PMT is set by a potentiometer inside the 914 housing and in most cases should not need to be adjusted.

   For a 914 housing using analog mode the High Voltage should be set to ~ 1100 volts using the 914 Control Panel.
8. You can now acquire data.

Note. You can analyze previously collected data ("off-line analysis") without starting the instrument. Remember to turn the computer off should you want to subsequently start the instrument and ignite the arc lamp. Data analysis can be performed on a different computer loaded with FelixGX.

Now that your system is up and running, it is time to learn FelixGX. As a bare minimum, we recommend reading all of Chapter 4: A Quick Tour of FelixGX.

Chapter 3

System Setup

Computer Requirements

For proper operation, the Photon Technology FelixGX Software package requires:

1. PC compatible computer
2. 2.7 GHz Dual Core processor
3. 2 GB system RAM
4. 100 GB free hard drive space
5. 6 USB 2.0 ports
6. Internet connection (required only to register Windows and to get updates to Microsoft .NET Framework during FelixGX installation).
7. Operating system: FelixGX version 1.0 or 2.0 requires Windows XP Service Pack 3. FelixGX build 3.1.17.51 or later works with either Windows XP Service Pack 3 or 32-bit Windows 7. At this time FelixGX will not work with 64-bit Windows 7.

Note. Some anti-virus software packages may cause faults in the way FelixGX communicates with the hardware. For more information, please contact your PTI Customer Service Representative.

Contact PTI for up-to-date computer specifications.

Installing FelixGX and other software

Uninstalling FelixGX and other programs

If an older version of FelixGX is already installed on your computer, it must be uninstalled before a new version of FelixGX can be installed. FelixGX installation does not automatically update the program files. Use **Start, Control Panel, Add/Remove Programs, FelixGX, Remove** to uninstall FelixGX, and then use Windows Explorer to delete any remaining *.rep and *.txt files in the C:\Program Files\PTI\FelixGX\ folder.

Similarly, the current version of PTIGraphicX is 1.3.0.129, and the current version of InstaCal is 6.01. Use **Add/Remove Programs** to select these programs (**PTIGraphixcX Component** and **InstaCal for Windows**), then click on "**Click here for support information**" to check the version of these programs. Click **Remove** if they are older versions.

If your system has any devices that use an RS-232 connector, you may need to reinstall the driver for the RS-232 to USB converter cable. Make sure you have the CD or mini-CD with this driver.

Installing FelixGX on a new system

If your computer was supplied by PTI then FelixGX and InstaCal will already have been installed. Skip to step 8.

Use the installation CD to install FelixGX and InstaCal on your computer before installing any cards (e.g., the Becker & Hickl card for a TCSPC system) in the computer, or connecting any cables from the system hardware to the computer. Otherwise, the computer will detect the existence of the USB devices and be looking for non-existent drivers for them

To install FelixGX on a computer for off-line analysis only, follow only steps 1 - 3 below. You will not need to install InstaCal for Windows, and you will not need to restart the computer.

1 Turn on the computer. If you are starting with the computer already on, close all other program windows. Some of the installation windows may not be 'on top' and so be hidden by other program windows. Also, at the end of the InstaCal installation procedure, you will need to restart the computer.

2 Insert the FelixGX Install CD into a CD drive. The **Autorun** menu may take up to 30 seconds to appear.

3 Click on the **FelixGX** build #.#.#.# button. If FelixGX is already installed on your computer, a FelixGX Setup error message will appear. Clicking on Details states "Another version of this product is already installed. Installation of this version cannot continue. To configure or remove the existing version of this product, use Add/Remove Programs on the Control Panel…." Follow the **Uninstalling FelixGX** procedure above.

   a The FelixGX Setup for .NET Framework 2.0 SP2 (x86) window will appear. Click **Accept**. An install progress window for .NET Framework 2.0 SP2 (x86)

will appear. Installing .NET Framework 2.0 SP2 (x86) may take 10 to 20 minutes.

b The 'Welcome to the FelixGX Setup Wizard' window will appear. Click **Next**.

c The FelixGX License Agreement window will appear. Click on **I Agree** and then on **Next**.

d The 'Select Installation Folder' window will appear. Use the default installation folder C:\Program Files\PTI\FelixGX\. Click **Next**.

e The 'Confirm Installation' window will appear. Click **Next**. The files will be installed on the computer.

f The Welcome to the FTDIStepperboard USB Drivers Setup Wizard window will appear. Click **Next**.

g The Setup - FTDIStepperboard USB Drivers - Information window will appear. Click **Next**.

h The Setup - FTDIStepperboard USB Drivers – Ready to Install window will appear. Click **Install**.

i The Welcome to the Stepperboard Device Driver Installer window will appear. Click **Next**.

j The Congratulations! You are finished installing the drivers for the Stepperboard device window will appear. Click **Finish**.

k The Completing the FTDIStepperboard USB Drivers Setup Wizard window will appear. Click **Finish**.

l Two CleanUpOldRepFiles may appear in succession (if you are updating FelixGX from an earlier version). Click **OK**.

m The FelixGX Installation Complete window will appear. Click **Close**.

n Wait 5 to 30 seconds. The PTIGraphicX Component for .NET Framework 2.0 SP2 (x86) License Agreement window will appear. Click **Accept**.

o The Welcome to the PTIGraphicX Component Setup Wizard window will appear. Click **Next**.

p The PTIGraphicX Component License Agreement window will appear. Click **I Agree** and **Next**.

q The PTIGraphicX Component Confirm Installation window will appear. Click **Next**.

r The PTIGraphicX Component Installation Complete window will appear. Click **Close**.

s The PTIGraphicX Component Installation Complete window will disappear and the AutoRun menu will appear.

4 Click on the **Measurement Computing InstaCal** button. Note that InstaCal installation will automatically update/overwrite an existing version of InstaCal.

a A 'WinZip Self-Extractor' window will appear. Click **OK**.

b A second 'WinZip Self-Extractor' window will appear. Click **Setup**.

c The Preparing to Install window will appear. Wait for the next window.

d The 'Welcome to the InstallShield Wizard for InstaCal for Windows' window will appear. Click **Next**.

e The 'Destination Folder' window will appear. Click **Next** to accept the default destination folder.

f The 'Ready to Install the Program' window will appear. Click **Install**.

g The 'Installing the program' window will appear. Wait for the next window.

h The 'InstallShield Wizard Completed' window will appear. Click **Finish**.

5 The 'InstaCal for Windows Installer Information' window will appear stating: "You must restart your system for the configuration changes made to InstaCal for Windows to take effect. Click Yes to restart now or No if you plan to restart later." Click **No**.

6 Remove the FelixGX Install Cd from the CD drive.

7 If you have a TCSPC system, insert the B&H CD into the CD drive and install the software.

a Use Windows Explorer to create the folder C:\temp.

b Copy the file C:\Program Files\BH\SPCM\spcm.ini to C:\Temp.

c Rename the file C:\Temp\ spcm.ini to tcspc.ini.

d Shut down and restart the computer.

e **Start, Programs**, **BH**, **SPCM**, spcm.

f **Close** the SPC-130 dialog.

g Shut down the computer.

h Turn off the power to the computer and all devices connected to the computer. Turn off the power bar connected to the computer.

i Ground yourself by touching a screw or any metallic part on the back panel of the computer.

j Remove the side panel on the computer that allows access to the PCI slots.

k To avoid damage due to electrostatic discharge touch a metallic part of the computer with one hand and then open only the end of anti-static bag holding the SPC-130 board. While still touching a metallic part of the computer with one hand, grasp the SPC-130 board at the metallic back shield with the other hand. This will drain any potentially dangerous charge from you and the module. . Remove the Becker & Hickl SPC-130 board from its anti-static bag and install it in a free PCI slot in the computer.

l Place the side panel back on the computer.

m Leave the computer power off. The SPC-130 board in the computer supplies power to the PMH-100 detector (if you have one) whenever the computer is on. Therefore, connect cables only when the computer power is off.

8 Shut down the computer.

9 Assemble the system hardware and connect all cables according to the table in the section: **Connecting the System.**

10 Turn on the ASOC-10 and MD-4000 and any other control boxes (QNW TC controller, XenoFlash Control, EL-1000, etc.)

11 Turn on the computer.

12 The computer will detect the USB devices.

a The 'Welcome to the Found New Hardware Wizard' window will appear. Click "**No, not this time**" to the question "Can Windows connect to Windows Update to search for software?" Click **Next**.

b Depending on the hardware options included in your system and whether you had a previously installed copy of FelixGX, you will see Found New Hardware Wizards for some or all of the following:
- MCC USB2 Loader Device
- USB-2527
- USB Stepperboard Product
- USB HS Serial Converter
- USB Serial Port

c The 'Found New Hardware Wizard This wizard helps you install software for MCC USB2 Loader Device' window will appear. Choose "**Install the software automatically (Recommended)**". Click **Next**.

d The wizard searches, finds and installs the driver for the named device. The computer should find the driver in C:\Program Files\PTI\FelixGX\QNWDRV.

i If the Found New Hardware Wizard cannot find the software driver, then click the **Back** button.

ii Click on the option "**Install from a list or specific location (Advanced)**". Click **Next**.

iii In the 'Found New Hardware Wizard Please choose your search and installation options' window,
click on "**Search for the best driver in these locations**",
check "**Include this location in the search:**",
and enter or **Browse** to select
"C:\PROGRAM FILES\PTI\FELIXGX\QNWDRV".
Click **Next**.

e The 'Completing the Found New Hardware Wizard' window will appear. Click **Finish**.

f Similarly, Found New Hardware Wizards will appear to install other drivers.

g When all drivers have been installed, you will see a message callout in the lower right corner of the desktop stating "Found New Hardware Your new hardware is installed and ready to use." This callout will disappear after a few seconds.

13 Run **InstaCal** (**Start, Programs, Measurement Computing, InstaCal**. It may be useful to copy a shortcut to InstaCal onto the desktop. Right-click on the InstaCal icon, drag and drop it to the desktop, and select 'Copy here'.). InstaCal should automatically detect the 2527 board in the ASOC-10 and display its board and serial numbers. Close InstaCal.

14 Launch **FelixGX** (there should be a FelixGX icon on the desktop, or use **Start, Programs, PTI, FelixGX, FelixGX**).

Connecting the System

Electrical cables (and water hoses) should be connected according to the following table. The computer and all control boxes should remain off until any cards (e.g., the Becker & Hickl card for a TCSPC system) are installed in the computer and all cables are connected.

The table shows the device and connector for each end of the possible cable connections for systems using FelixGX software. The first two columns show a device and labeled connector on the device for one end of a cable, and the second two columns show the device and labeled connector for the other end of the cable. The fifth column shows the function of the cable connection. The last two columns show the type of cable and labels on the cable ends if they are labeled. Note that most circular cable ends are keyed – i.e., the cable end will attach to the connector in only one orientation and the collar can then be rotated to anchor it in place. In the cable type column, a '/' indicates that that end of the cable is split into 2 or more cables.

NOTE: Many different cables use the same connectors. Make sure that the cables connect only those devices that should be connected.

NOTE: The ASOC-10 has changed style. Connections and labels are shown as, e.g., ASOC-10 (side panel or back panel), where the first choice refers to the older 7.5 cm (3 inch) high box with connectors on the side and back panels, and the second choice to the new 11 cm (4.5 inch) high box with connectors only on the back panel.

Electronic and water connections: QuantaMaster, RatioMaster, or TimeMaster systems (with options)

FROM		TO		FUNCTION	CABLE	
DEVICE	**LABEL**	**DEVICE**	**LABEL**		**TYPE**	**LABEL**
Lamp Power Supply[1]	IGNITER	Igniter		Ignition and power	CPC 4 pin	
Water circulator[2]		Arc Lamp Housing	IN / OUT	Water cooling	Plastic hoses	
Power pack	Line (mains) outlet	ASOC-10	Power IN	Power	DIN 5 pin 180°	
ASOC-10	USB	Computer		Communication	USB	
ASOC-10 (side panel or back panel)	+/- 12 V or DC Out-#	Detector Housing	POWER	Power	DIN 3 pin to DB-9	
814 Detector Housing	SIGNAL OUT	ASOC-10 (side panel or back panel)	DIGITAL PMT-# (BNC) or Digital B-#	Digital signal (Photon Counting)	BNC	

814 Detector Housing	SIGNAL OUT	ASOC-10 (side panel or back panel)	Analog In #	Analog signal	BNC	
914 Detector Housing	A	ASOC-10 (side panel or back panel)	Analog In #	Analog signal	BNC	
914 Detector Housing	D	ASOC-10 (back panel)	D1 or D2 or Digital A #	Digital signal (Photon Counting)	BNC	
914 Detector Housing	Power	ASOC-10 (side panel or back panel)	Digital PMT-# or Control-#	914 Power and Control	DIN 5 pin 240° or CPC 9 pin to CPC 9 pin	
ASOC-10 back panel	+/-12 V or DC Out	ASOC-10	Ex Cor Signal	Signal	DIN 3 pin / BNC	S
		ASOC-10	Ex Cor Gain	Gain control	DIN 3 pin / BNC	G
		MP-1		Ex. Cor. Power and I/O	DIN 3 pin / right angle DIN 5 pin 240°	
ASOC-10	Ex. Cor. Gain[3]	DeltaRAM	POSITION	Wavelength Control	BNC	
ASOC-10	TTL OUT 1	DeltaRAM	SHUTTER	Shutter Control	BNC	
ASOC-10	TTL IN or OUT	Other devices		TTL I/O	BNC	
ASOC-10	FEATURE	Not used at this time			DB-9	
ASOC-10	STROBE	Not used at this time			DB-15	
MD-4000	USB	Computer	USB	Motor control	USB	
MD-4000[4]	EXTENSION OUT	MD-4000	EXTENSION IN	Slave Control	CPC 7 pin	
MD-4000	Motor #	Monochromator		Monochromator control	CPC 9 pin to Hex/Row	
MD-4000	Motor 1	Monochromator		Monochromator and Shutter[5] control	CPC 9 pin to Hex/Row	
MD-4000	Motor #	Motorized Slit		Slit Control	CPC 9 pin to DB-9	
MD-4000	Motor #	MP-1		Motorized polarizer control[6]	CPC 9 pin to Hex	
MD-4000	Stirrer	MP-1		Stirrer control	DIN	

InGaAs detector option						
Peltier Power Supply		InGaAs detector		Peltier power	CPC 9 pin to 4 pin Row connector	
InGaAs detector	SMA	Pre-Amplifier	Input	Signal	SMA to BNC	I
24 V Gelpac		Pre-Amplifier	12 V	Power	Hardwired to CPC 2 pin	
Pre-Amplifier	Output	SCITEC Lock-in Amplifier	Input			
SCITEC Lock-in Amplifier	Output	ASOC-10 (side panel or back panel)	ANALOG IN #			
OC-4000	Motor	Chopper wheel		Chopper control	RJ-12 (6 wire phone cable)	
OC-4000	DIRECT (back panel)	SCITEC Lock-in Amplifier	Reference	Chopper reference	BNC	
QNW Temperature Control option						
ASOC-10 or computer	USB-#	QNW TC-125	USB	Peltier control	USB	
QNW TC-125[7]	Sample holder	MP-1		Peltier control and power	M/F DB-15 (3 row)	
QNW TC-425[7]	Sample holder and turret	MP-1		Peltier and turret control and power	DB-15/DB-9 to DB-25	
Water circulator[7]		MP-1		Water cooling	Plastic hoses	
Phosphorescence Lifetime option						
XenoFlash Power Supply	X	XenoFlash Flash Lamp Housing	x	Power	BNC	
XenoFlash Power Supply	T	XenoFlash Flash Lamp Housing	t	Trigger Pulse	BNC	
ASOC-10	TTL OUT 1	XenoFlash Power Supply	IT	Timing	BNC	
or						
ASOC-10	TTL OUT 1	Nitrogen Laser[8]	TRIGGER IN	Trigger In to nitrogen laser	BNC	TI

VCI detector option						
ASOC-10	Feature / Strobe	ASOC-10	Feature / Strobe	Detector signal	BNC	A / A1
ASOC-10	Feature / Strobe	VCI	Feature / Strobe	Detector signal	BNC	A / A1
Electrometer	A	ASOC-10	A1	Detector signal	BNC	A / A1
LaserStrobe Lifetime option						
ASOC-10	Strobe / Feature connectors (unlabelled)	Electrometer	F	Integrator power	DB15/DB9 to 9 pin Hex	F
ASOC-10	Strobe connector (unlabelled)	Nitrogen Laser	TRIGGER IN	Trigger Into nitrogen laser	BNC	TI
Electrometer	A	ASOC-10	A1	Detector signal	BNC	A / A1
TCM-1000	STROBE PULSE K	Electrometer	K	Strobe pulse	BNC	K
TCM-1000	PHOTODIODE POWER	Dye laser	PD (inside dye laser cabinet)	Photodiode power	BNC	PD
Dye Laser	TO (inside dye laser cabinet)	TCM-1000	EXTERNAL TRIGGER ET	Trigger Out from dye laser	BNC	TO
TCM-1000	RS-232 DGG INTERFACE	Computer	USB	Delay timing	RS-232 to USB	
LED Lifetime option						
ASOC-10	+/- 12 V	Nano-electrometer	F	Avalanche Power	DIN 3 pin to Hex	F
Nano-electrometer	a	ASOC-10	A#	Detector Signal	BNC	
EL-1000	K	Nano-electrometer	k	Delay Gate Generator Control	BNC	K
EL-1000	TO LED	LED		LED Timing	BNC	
EL-1000	TO LED	LED		LED Power	CPC 9 pin	
EL-1000	RS-232	Computer	USB-#	Delay timing	RS-232 to USB	

TCSPC option (turn the computer off when connecting the TCSPC cables)[9]						
TCSPC light sources: PTI LED with EL-1000 or B&H Diode Laser						
EL-1000	TO LED	LED		LED Timing	BNC	
EL-1000	TO LED	LED		LED Power	CPC 9 pin	
EL-1000	RS-232	Computer		Delay timing	RS-232 to USB	
EL-1000	SYNC	SPC-130	SYNC	SYNC pulses	SMA to BNC with inverter[10]	
or						
Power pack		B&H Laser Switch Box		Power	DB9	
B&H Laser Switch Box	B&H Laser	B&H Diode Laser	Power & Control	Power & Control	DB9	
B&H Diode Laser	TRIG OUT	SPC-130	SYNC	SYNC pulses	SMA with inverter[10]	Delay time[11]
TCSPC detectors[9]: PTI 914 with pre-amplifier or PMH-100						
SPC-130	center plug	ACA-2-21-N pre-amplifier	+12 V	Pre-amplifier power[9]	RS-232 to radio plug	SPC-130
914	direct	ACA-2-21-N pre-amplifier	IN	Detector signal	SMA	
SPC-130	CFD	ACA-2-21-N pre-amplifier	OUT	CFD Signal	SMA	
or						
SPC-130	center plug	PMH-100	+12 V	Detector Power[9]	RS-232 to radio plug	
SPC-130	CFD	PMH-100	OUT	CFD Signal	SMA	

Notes

is an integer and depends on what other options the system has. The choice here must match what is set in the FelixGX hardware configuration.

USB cables may be plugged into any USB port on the computer. However, if a USB cable is moved to a different USB port on the computer, Windows may reassign the USB port assigned to that device and try to find the drivers for that device again.

1 The LPS-100 Integrated Lamp Power Supply and Igniter only requires a power cord. It has no electrical connection to the rest of the instrument.

2 The Arc Lamp Housing requires water cooling only for lamps with greater than 100 W power consumption.

3 Connect DeltaRAM Position to Ex. Cor. Gain connector on the back of the ASOC-10. This connection must be used for high speed operation of the DeltaRAM. For the older ASOC-10 boxes, the TTL I/O jumpers need to have been reconfigured to allow high-speed DeltaRAM operation with FelixGX version 4.0.2 or higher. Contact PTI Service to check if this has been done. The newer ASOC-10 boxes have the proper TTL I/O configuration for high-speed DeltaRAM operation with FelixGX versions 4.0.2 to 4.1.0. When connected this way, make sure the **Configure, Preference: ASOC with swapped XDAC0 / XDAC2** is enabled. With this Preference, the Excitation Correction "G" cable should be connected to the DeltaRAM Position connector on the back of the ASOC-10.

   If there is no DeltaRAM in the system, then the Excitation Correction "G" cable may be connected to the Ex. Cor. Gain connector on the back of the ASOC-10. In this case the **Configure, Preference: ASOC with swapped XDAC0 / XDAC2** should be disabled.

4 An MD-8000 is essentially two MD-4000's internally connected. The leftmost four motor connections on the back panel behave as a master MD-4000 and the rightmost four motor connections on the back panel behave as a slave MD-4000 (or as consecutive slave MD-4000's).

5 A shutter installed in a standard monochromator is only controlled using motor channel 1 of a master or slave MD-4000 or MD-8000.

6 To connect a motorized polarizer cable to one of the cables inside the sample compartment, align the white dots on the cable ends (also, a nib in one connector will fit into a key slot in the other connector) and push the cable ends together.

   To disconnect a motorized polarizer, squeeze the top (white dot) and bottom of the circular loop around the connection of the two internal cables and pull the cable ends apart. To remove a manual or motorized polarizer assembly from its mounting plate, pull up on the motor or polarizer wheel until the mounting plate is free of the three clips, then lift it free from the mounting plate.

7 The water circulator must be operating whenever the QNW TC-125 or TC-425 is turned ON to control the temperature of the Peltier sample holder and prevent overheating of the cuvette holder.

8 When using a laser on a QM (Phosphorescence) or QM(VCI) system, the TCM-1000 is not used.

9 The SPC-130 board in the computer supplies power to the PMH-100 detector whenever the computer is on. Therefore, connect and disconnect cables to the PMH-100 detector only when the computer is off, to prevent electrical shocks to the PMH-100 detector.

10 In-line Becker&Hickl A-PPI-D Pulse Inverter with SMA connectors on both ends.

11 Various cable lengths are supplied to allow changing the SYNC and CFD delays.

Changing the Screen Resolution

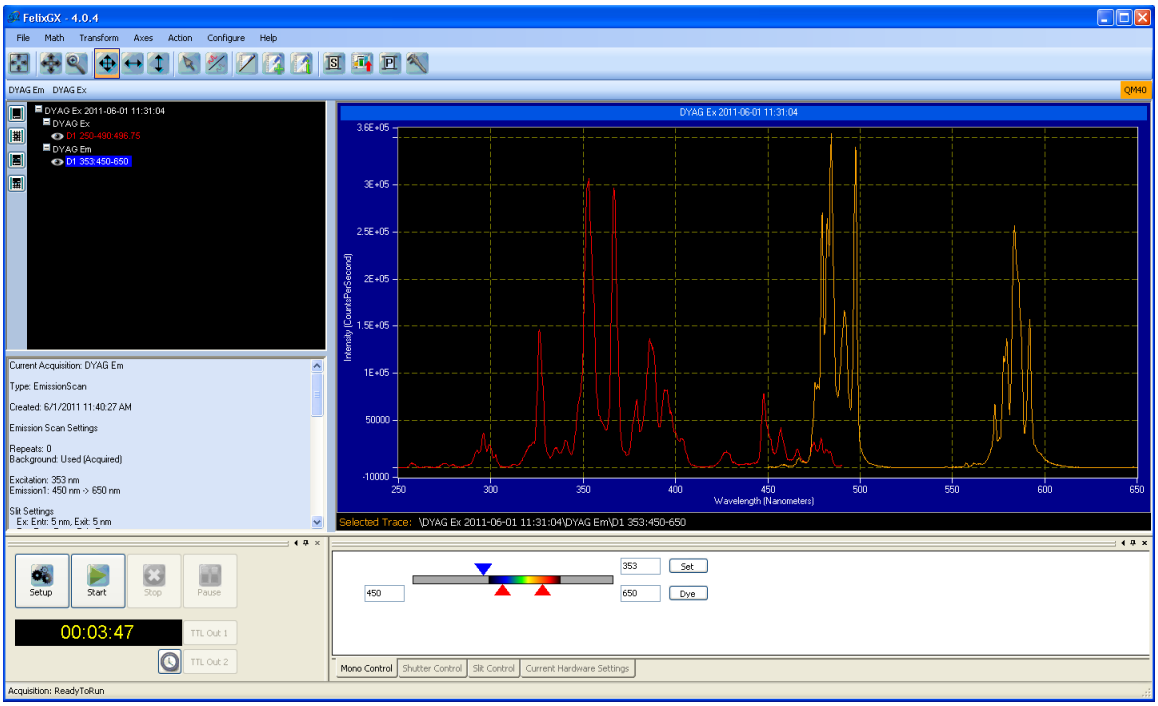
FelixGX is designed to work on most Windows-based computers at most screen resolutions. However, for the best operation and appearance, we recommend running your computer monitor at 1024 by 768 (or more, recommended. The minimum is 800 x 600) resolution.

To change the screen resolution, right-click anywhere on the desktop (except on a desktop icon) and choose **Properties**. Choose the **Settings** tab. Use the slider to change the screen resolution and the choice list to select the color quality. You may want to change the text font size – click on the **Advanced** button and choose a **DPI** setting on the **General** tab. **OK**, **OK** to close these dialog windows. Depending on the Compatibility settings on the General tab you may need to restart the computer.

Chapter 4

A Quick Tour of FelixGX

Workspace



When FelixGX is launched, the Workspace will be opened. Across the top, beneath the Title Bar, is the Menu Bar. Each heading in the Menu Bar represents a group of related commands. Below the Menu Bar is the Toolbar. The buttons on the Toolbar provide instant access to a number of the most frequently used commands for control of data display.

Beneath the Menu Bar is the Acquisition Bar that shows the names of user selected acquisition setups and hardware configurations. If the Acquisition Bar is full, then a choice list icon is shown at the right end of the Acquisition Bar. Click on this icon to show additional acquisition setups. To change the visibility of acquisition setups on the Acquisition Bar click on Recall Sessions on the Toolbar. To change the visibility of hardware configurations on the Acquisition Bar click on **Configure, Hardware, Repository** button.

Beneath the Acquisition Bar is the space where data is displayed.

The Legend along the left side lists the name(s) of the sessions, groups and traces. Right-clicking on a session, group or trace name in the Legend will show different command menus. Click on a + sign in front of a session or group name to expand the list below it, or click on a - sign in front of an session or group name to minimize the list. Note that clicking on the + or - buttons in front of a session or group name to expand or contract the session or group does not change what is seen in the graph area.

Below the legend is a box showing a description of the current acquisition setup.

The right side of this window has an area where traces are plotted in Graph Mode or displayed as a spreadsheet in Grid Mode. The screen shot above shows 'One-Graph' Mode. Clicking on the 'Two-Graph' Mode button to the left of the legend splits the graph area into two graphs, one below the other. The upper graph shows the same traces as in 'One-Graph' Mode. Clicking on either graph shows in the legend which traces are visible for that graph. You can change the visibility of traces independently for each graph. Clicking on the 'Graph-Grid' Mode button changes the lower graph to a spreadsheet display that shows the values of data visible in the upper graph. Clicking on the 'One-Graph' Mode or 'Two-Graph' Mode button changes the spreadsheet display back to a graph.

Below the acquisition description box and the graph area are the Control Panels. Depending on the current Hardware Configuration, this area shows various panels to control data acquisition and hardware operation.

The dividing bars between the legend, graph, acquisition description, and the control panels area, and between control panels can be moved by clicking and dragging them with the mouse cursor.

Menus

When you click on one of the menu items detailed below, a list of available commands will appear.

All of the commands are detailed in the following chapters.

File The File menu is used to open saved data files, save data, and import and export ASCII files and FelixGX data. Printing is also accomplished from this menu.

Math Acquired data can be processed mathematically through the commands in the Math menu. Two traces can be arithmetically combined, and data can be fitted, smoothed, averaged, integrated, normalized, differentiated, etc…

Transform Used to do post-acquisition transformation of data to concentration or pH. The transformation equations or Lookup Tables can be setup here.

Axes 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.

Action Used to perform calibrations against standards or automation jobs.

Configure The Configure menu is used to explicitly define the hardware components that are being used with FelixGX. It is critical that the hardware configuration be correct. The Configure menu is also used to save Control Panel Configurations and program preferences.

Help The Help menu provides access to information on using Help and the Search function.

Data Acquisition

A hardware configuration must first be set up that corresponds to the components in your system. Click on **Configure, Hardware** to create a new hardware configuration or to modify an existing one. Fluorescence experiments can then be set up using the Acquisition Setup dialog. Afterward, many settings can be modified using the Control Panels. The acquisition items represent basic experimental techniques; for instance, excitation, emission or synchronous wavelength scans, timebased scans, excitation or emission ratios, etc…

Each of the acquisition menu items is introduced and its experimental applications and procedures are presented in the *Acquisition Control* chapter. Hardware control functions unique to these procedures are also detailed.

Toolbar

Quick Full Scale X and Y Axes

Click on this button to display the full X- and Y-ranges of data. This mode is also available on the Axes menu. During acquisition, the X-axis is set to be at least as wide as the View Window. This command is also available on the Axes menu.

Panning is default graph interaction mode - no <ctrl> key press required

When checked, Panning (see below) is the default mode on the selected graph. Default means that you don't have to press the Ctrl key to turn on panning. You can still zoom by pressing the Shift key. When active, this button is highlighted.

Zoom is default graph interaction mode - no <shift> key press required

When checked, Zooming (see below) is the default mode on the selected graph. You can still Pan by pressing the Ctrl key. When active, this button is highlighted.

Enables interactive panning/zooming in both the Y and X axes

When selected, the Pan and Zoom actions are active on both the X- and Y-axes. When active, this button is highlighted. This is the default mode.

Limits interactive panning/zooming to X axis only

When selected, the Pan and Zoom actions are only active on (limited to) the X-axis. When active, this button is highlighted.

Limits interactive panning/zooming to Y axis only

When selected, the Pan and Zoom actions are only active on (limited to) the Y-axis. When active, this button is highlighted.

Panning and Zooming notes

For the panning and zooming modes to work, you must select a graph by clicking on it. If both Graphs 1 and 2 are visible, then the edge of the active graph is highlighted.

Click the **Panning** button to make panning the default graph interaction mode. In this mode, clicking and dragging in the graph will move the traces, grid lines and tick labels with the mouse cursor, depending on whether only the X-axis or the Y-axis or both the X- and Y-axes are selected.

If panning is not the default graph interaction mode, you can press and hold the <Ctrl> key and click and drag the mouse in a graph to pan the graph.

Or, you can press and hold the <Ctrl> key and press and hold the left or right arrow key to pan horizontally or press and hold the up or down arrow key to pan vertically. In this case, the graph will pan 20 % of the horizontal or vertical range of the graph with each arrow key click, and repeatedly if the key is held down.

Click the **Zooming** button to make zooming the default graph interaction mode. In this mode, click and drag the mouse cursor in the graph to create a selected area in the graph, depending on whether only the X-axis or the Y-axis or both the X- and Y-axes are selected. Release the mouse button to zoom to the selected area.

If zooming is not the default graph interaction mode, you can press and hold the <Shift> key and click and drag the mouse in a graph to zoom the graph. To zoom proportionally in the horizontal and vertical directions, press and hold the <Shift> and <Alt> keys, and click and drag the mouse to create a proportional selection in the plot area of the graph.

To zoom around a point in the graph area, press and hold the <Shift> key and click a point in the plot area. This will zoom in to 50 % of the horizontal and vertical ranges, centered about the selected point.

You can use the up and down arrow buttons as keyboard shortcuts for zooming around the center of the plot area. Press and hold <Shift>, then press the up arrow button to zoom in or the down arrow button to zoom out around the center of the plot area. Note: this zoom mode zooms both X- and Y-axes. Clicking the up arrow key zooms in to 5/6 of the graph area per click, or repeatedly if the key is held down. Clicking the down arrow key zooms out to 6/5 of the graph area per click, and repeatedly if the key is held down.

 **Toggle Data Cursor**

Toggles the Data Cursor ON/OFF on the active graph. When active, this button is highlighted. For the Data Cursor to be visible, a visible trace must be selected in the legend. Selecting a hidden trace in the legend hides the Data Cursor. The Data Cursor highlights a data point as a circle at the intersection of a horizontal and a vertical line, and shows the X- and Y-coordinates in parentheses. When it is first toggled on, the Data Cursor is placed on the trace at the midpoint of the X-axis. To move the Data Cursor, place the mouse cursor on the vertical or horizontal line. The mouse cursor changes to a double-headed arrow. Click and drag the arrow to move the vertical line along the X-axis. Moving the horizontal line up or down causes the Data Cursor to jump to whichever data point on the trace has the next highest or lowest Y-value, respectively.

 **Toggle Visibility**

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

 **Show/Hide Event Marker on Graphs**

Toggles the visibility of event markers on the active graph. When active, this button is highlighted. **Note**: Event Marks are hidden by default. Clicking on the Edit Event Marker opens that dialog, but does not display event markers until this button is toggled ON.

 **Add Event Marker**

Event Markers show as a light blue vertical line in the graph with an arrow pointing from the caption to this line.

To add an event marker at a user defined position on a trace **during a scan**:

1. Click on Graph 1 to make it the active graph. Event markers will show only in Graph 1.

2. Select the trace you wish to mark in the trace legend by clicking once on the trace name, so that it becomes highlighted.

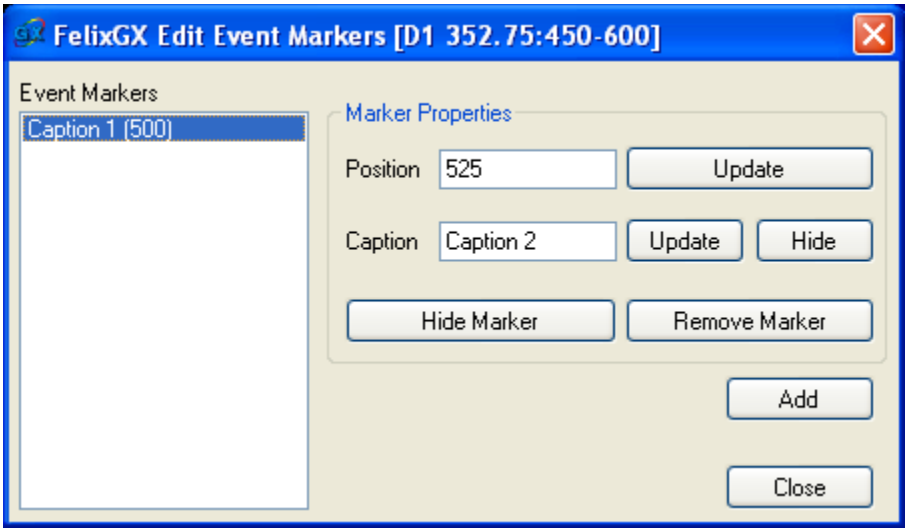
3. Make this trace visible. You can only add an event marker to a visible trace.

4. Click on the **Add Event Marker** button to put an event marker on the graph at the data point when the key is pressed. The event marker is placed on the graph halfway up the vertical range, no matter where the trace data is. The default name of an event marker added during a scan is "Event#N", where N is the Nth marker created on that trace.

To add an event marker at a user defined position on a trace **after a scan** click on the trace and then click on the **Add Event Marker** or **Edit Event Marker** icon on the toolbar. This opens the "Edit Event Marker" dialog where events can be added or edited. See the **Edit Event Markers** icon description for details.

The currently selected trace is shown in the dialog title bar.

Edit Event Markers



Click on a visible trace and then click on the Edit Event Markers button on the toolbar. This opens the "Edit Event Markers" dialog where events can be added, edited, hidden or deleted. Event Markers show as a vertical line in the graph area with a caption attached to the line by an arrow. The event marker is placed on the graph halfway up the vertical range, no matter where the trace data is.

Note: Event Marks are hidden by default. To make them visible, the **Show/Hide Event Marker on Graphs** button,  , must be turned ON.

The currently selected trace is shown in the dialog title bar.

Event markers: Shows a list of the current event markers.

Marker Properties

Position: The X position where the event marker will be placed in the graph area. You can enter a new value for the X position of an existing marker by first selecting it from the list.

Update: Updates the new X position of the selected event marker in the list and in the graph area.

Caption: Enter the caption label here. For event markers added after a scan has been acquired, the default caption label is "Caption N" where N is the Nth marker created on that trace. You can enter a new name for an existing marker by first selecting it from the list.

Update: Changes the name of the selected event marker in the list and in the graph area.

Hide: Hides the selected event marker caption in the graph area. When a caption is hidden, this button changes to **Show** to allow you to make it visible again.

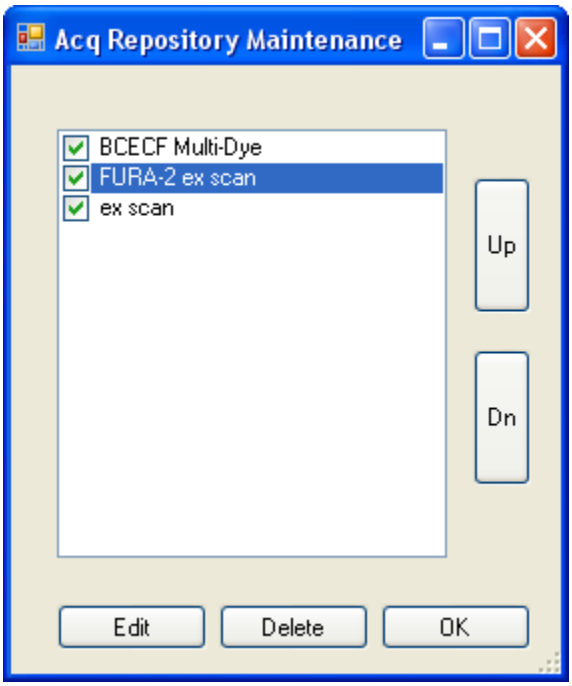
Hide Marker: Hides the selected event marker and caption in the graph area. When an event marker is hidden, this button changes to **Show Marker** to allow you to make it visible again.

Remove Marker: Removes the selected event marker from the list and the graph area.

Add: Adds a new event marker to the list and the graph area. You can then edit the X position and caption. The default position is halfway up along the visible Y-range.

Close: Close the Event Marker dialog window.

Manage Acquisition Setups



Shows a list of all acquisition setups, whether or not they are shown on the Acquisition Bar. To make an acquisition setup visible on the Acquisition Bar, check the box in front of the acquisition setup name. The order of configurations shown on the Acquisition Bar can be changed by selecting a configuration and clicking on **Up** or **Down**: The configuration at the top of the list is shown farthest to the left on the Acquisition Bar.
Edit: Select a setup name then clicking on the Edit button shows a dialog where you can change the acquisition name.
Delete: Deletes the selected setup from FelixGX.
OK: Closes the dialog.

Export Data

Opens a Windows dialog to save a session, group, or trace as a txt file in the format detailed at **File, Import**. If more than one session, group, or trace is selected only the last such selected item will be exported. Same as the legend menu commands Export Session, Group, or Trace.

Post Process

Same as the **File, Open** command. Shows a Windows dialog to open a a Compressed Record (*.gxz), Record (*.gxr), Session (*.gxs), Group (*.gxg), or Trace (*.gxt) file stored on disk or on the network.

 **Calibration**

Shows a dialog to allow calibration of wavelength, intensity or other instrument performance.

Legend Commands

Right clicking on a session, group, or trace in the legend opens a menu with links to commands found in the main menus. The following is an overview of the listed commands found in each menu by right clicking on a session, group, or trace. Some options can only be accessed from these menus and they are described in more detail below.

Right-clicking in an empty legend will not show any commands. Data must be present to show commands.

Commands common to two or three of the legend menus:

Rename: This command opens a dialog to change the name of a session, group, or trace as it appears in the legend.
Note: Trace names of acquired data are set in the **Acquisition Setup, Traces** tab, and may be based upon labels assigned to signal channels in the Hardware Configuration.

Save … As …: Opens a Windows dialog to save a session, group, or trace as a binary file (.gxs, .gxg, or .gxt file, respectively).

Toggle Visibility: Show/hide all the traces in a session or group or individual traces. Traces that were hidden will become visible and traces that were visible will be hidden.

Plot Mode: Use this command to change the way the selected group or trace is plotted in graph mode. The traces can be displayed as enlarged individual data points or as lines, or as lines plus enlarged data points. The default display is lines.

Cut …: This command cuts the selected sessions, groups, or traces from the active workspace and places the data on the FelixGX clipboard. **Note** that, unlike the **Delete** command, the selected data is not lost because it is transferred to the clipboard. However, any data that was previously on the clipboard will be lost. **Note** that the FelixGX clipboard is internal to FelixGX. Data placed on the FelixGX clipboard is not available to the Windows clipboard.

Copy …: This command copies the selected sessions, groups, or traces to the FelixGX clipboard. Any data that was previously on the clipboard will be lost.

Paste: This command pastes the sessions, groups, or traces that are currently on the FelixGX clipboard to the selected location in the legend. Pasted groups and traces will show " (copy)" appended to the group and trace names. This command is not shown in session, group or trace menus if the clipboard does not have sessions, groups or traces, respectively.

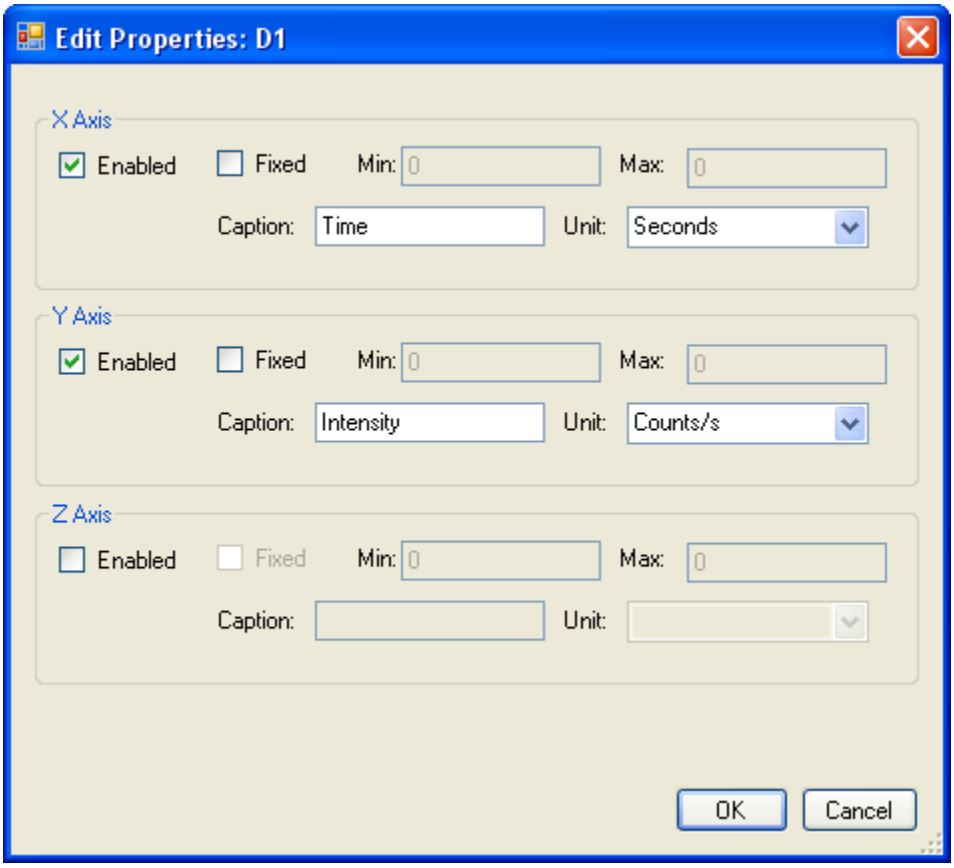
Delete …: This command deletes the current group or trace from the workspace. Note that, unlike the Cut command, this command does not copy the data to the FelixGX

clipboard. Deleted data is permanently removed from the workspace and cannot be recovered. No warning is given that the data will be lost when this command is used on a group or trace. If this command is used on a session a prompt will appear stating "Data has not been saved. Close anyway? Yes No".

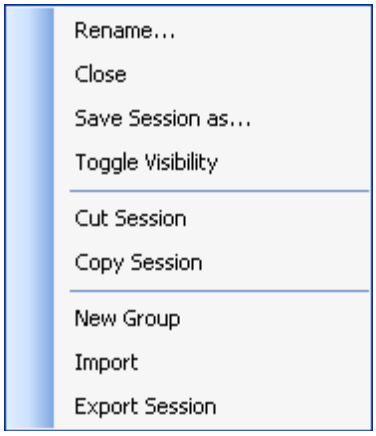
Import: Opens a Windows dialog to search for a txt file from the hard drive to import. These txt files must conform to the format detailed at **File, Import**. If the file contains a session, then a new session will be shown. If the file contains one or more groups, then they will be appended to the current session, or open as a new session if the legend is empty. If the file contains one or more traces, then they will be appended to the current group, or open as a new session if the legend is empty.

Export …: Opens a Windows dialog to save a session, group, or trace as a txt file in the format detailed at **File, Import**.

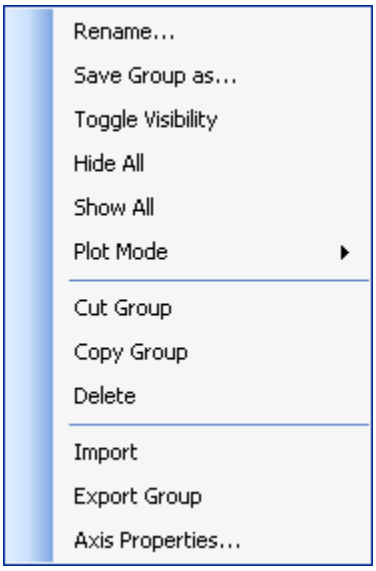
Axis Properties…



This window lets the user rename the axes labels and change the units with which the selected groups or traces are plotted against. By checking the appropriate checkbox the following caption and unit will be applied to that axis. The text in the Caption box will appear on the screen. The Unit is chosen from a list and is displayed in parentheses after the caption.

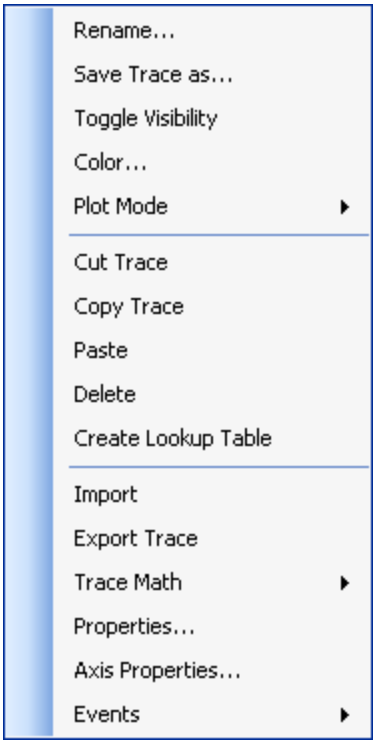


Session:
Close: Closes the current session. If the data has not been saved or has been modified since it was saved, a prompt will appear stating, "Data has not been saved. Close anyway? Yes No".
New Group: Creates a new empty group in the current session in the legend.



Group:
Hide All: Hides all of the traces within the selected group.

Show All: Shows all of the traces within the selected group.



Trace:
Color: This command opens a Windows dialog to change the color of the selected trace.

Create Lookup Table: Opens a dialog to create a Lookup Table from the selected trace. Enter a name and choose the LUT type.

Trace Math: Shows the list of Trace Math commands. Clicking on a Trace Math name opens the dialog for that command.

Properties…: Shows the properties of the trace and the summary of the acquisition used to acquire the trace. If the trace has been imported into the workspace from a txt file, then there are no useful properties shown (then Time Executed is the time the file was imported into the workspace).

Events, Align: Not working properly at this time.

Graph Commands

Right-clicking anywhere in the graph area opens a menu for changing the graph display, using commands also found on the Toolbar or main menus.

Plot Mode: Use this command to change the way the selected session, groups or trace are plotted in graph mode. The traces can be displayed as enlarged individual data points or as lines, or as lines plus enlarged data points. The default display is lines. This command is also found as Legend group and trace commands.

Default interaction: Use these commands to set the default interactive mode to panning or zooming. These are the same as the toolbar buttons.

Pan & Zoom Interaction Mode: Use these commands to limit the active axes for panning and zooming. These are the same as the toolbar buttons.

Toggle Visibility: Toggles the visibility OFF/ON of the selected traces, groups, or session. This is the same as the toolbar button.

Axes Scaling: Same as the commands in the Axes menu.

Chapter 5

File Commands

Data Storage

The fluorescence data that you acquire with FeliX is saved on your hard drive. It is important that you save your data in a consistent manner so that you can retrieve it when necessary with a minimum of effort. There is no preset folder for saving data files. You should set up your own file folder structure for saving data files. If your system has multiple users, you may want to segregate these files into different directories.

The best way to keep track of your data files and to copy or move them from place to place is to use the Windows Explorer utility. For more information on creating directories and copying, moving or deleting files, launch Explorer and click on Help.

Data files have two general formats. Files with a .gx? extension have a proprietary format and can only be saved and opened using FelixGX.

Files with a .txt extension have an ASCII file format. FelixGX recognizes two different ASCII file formats; one type is for data exported from earlier PTI programs (FeliX, TimeMaster, and FeliX32), a second closely related file format is for data exported from FelixGX. These two formats are explained in the **File, Import** section for FelixGX .txt files, and in **File, Import from FeliX32** for FeliX32 .txt files.

Files with a .rep extension are repository files created by FelixGX to save Acquisition Setups (acq.rep), Control Panel Configurations (cpc.rep), Hardware Configurations (hwc.rep), Lookup Tables (lut.rep), and Program Preferences (pref.rep). With FelixGX build 3.1.6.51 and later, these files are stored by FelixGX in the folder: **<My Documents>\PTI\FelixGX\Repository\#.#.#.#** (where #.#.#.# is the build number of your copy of FelixGX). If you want to save them elsewhere, copy these files and paste them where you want them. If you remove them from the <**My Documents>\PTI\FelixGX\Repository\#.#.#.#** folder, then the next time you open FelixGX, the saved setups, configurations, LUTs, and preferences, respectively, will not be available. In addition there is a file hwc.rep.txt that saves descriptions of the hardware configurations in an ASCII txt file, but is not necessary for FelixGX operation.

Extension	Contents
.gxz	Compressed FelixGX record
.gxr	FelixGX record
.gxs	FelixGX session
.gxg	FelixGX group
.gxt	FelixGX trace
.rep	FelixGX repository
.txt	Text file (for importing or exporting ASCII data)

Open…

Use the **File, Open** command to open a Compressed Record (*.gxz), Record (*.gxr), Session (*.gxs), Group (*.gxg), or Trace (*.gxt) file 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.

File name: Type a file name in the text box, or select the file name from the list. Only one file can be opened at a time.

Files of type: You can choose to show FelixGX Compressed Record (*.gxz), Record (*.gxr), Session (*.gxs), Group (*.gxg), or Trace (*.gxt) files. All of these file types are saved in a proprietary binary format. *.gxz is the default choice shown.

Save Record As…

Use this command to name and save the contents of the active window (all sessions, groups and traces shown in the legend). 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 **Save 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: Records can only be saved as *.gxz or *.gxr types. *.gxz is the default choice shown.

Save: Saves the file.

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

Close Record

Closes all sessions in the active window. If the data has not been saved or has been modified since it was saved, a prompt will appear stating, "Data has not been saved. Close anyway? Yes No".

Import…

Use the **File, Import…** command to open an ASCII data (*.txt) file exported from FelixGX and 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.

File name: Type a file name in the text box, or select the file name from the list. Only one file can be opened at a time.

Files of type: With this command, you can only open *.txt files exported from FelixGX.

Open: Click this button or double-click on the file name in the list to open it in FelixGX.

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

Format

Traces, groups and sessions may be exported from FelixGX as tab-delimited ASCII (*.txt) files, and have similar formats. Tab characters are the only acceptable field delimiters.

Trace files

LINE 1: shows the string "<Trace>" followed by a carriage return/line feed.

LINE 2: number of data pairs (N) in the trace followed by a carriage return/line feed.

LINE 3: the trace name followed by carriage return/line feed.

LINE 4: "X", tab character, "Y", tab character followed by a carriage return/line feed.

LINES 5 to N+4: X value, tab character, Y value followed by a carriage return/line feed.

LINE N+5: shows the string "</Trace>" followed by a carriage return/line feed to indicate the end of the file.

If the contents of a sample trace file were viewed in a word processor with formatting turned on, it would appear as follows: · represents a character space (ASCII code 20), → represents a tab character (ASCII code 09), and ¶ represents a paragraph character (carriage return/linefeed, i.e., ASCII codes 10 13) in the .txt file; all other spacing is for illustration only and does not represent characters in the file.

```
<Trace>¶
3¶
ramp·1¶
X  →  Y  →  ¶
0  →  0¶
1  →  1¶
2  →  2¶
</Trace>¶
```

Group files

Groups exported as .txt files have a format similar to trace *.txt files.

LINE 1: shows the string "<Group>" followed by a carriage return/line feed.

LINE 2: the group name followed by a carriage return/line feed.

LINE 3: number of traces in the group followed by a carriage return/line feed.

LINE 4: number of data pairs for the first trace (N1), two tab characters, number of data pairs for the second trace (N2), two tab characters, … This line ends with two tab characters and a carriage return/line feed after the number of data pairs for the last trace. Let N_{max} = maximum number of any trace's data pairs = max(N1, N2, …)

LINE 5: first trace name, one tab character, first trace T-number, one tab character, second trace name, second trace T-number, one tab character, … This line ends with one tab character and carriage return/line feed after the trace name for the last trace. The T-numbers are unique identifiers for each object (trace or event marker) and should not be edited.

LINE 6: "X", tab character, "Y", tab character, repeated for the number of traces, followed by a carriage return/line feed.

LINES 7 to N_{max}+6: first trace X value, tab character, first trace Y value, tab character, second trace X value, tab character, second trace Y value, tab character… These lines end with one tab character and carriage return/line feed after the Y values for the last trace in the lines.

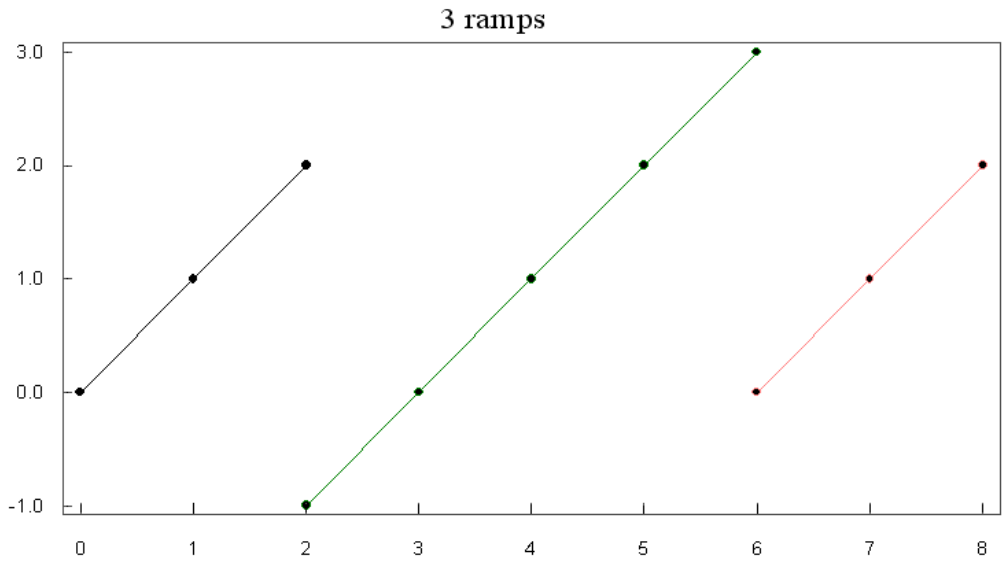
Note: All unused data fields are padded with tab characters. For example, if the first trace has fewer data pairs than the second, tab pairs are used prior to the remaining second trace values. If latter traces have fewer data points than preceding traces, these data fields are also padded.

LINE N_{max}+7: shows the string "</Group>" followed by a carriage return/line feed to indicate the end of the file.

If the contents of a sample group file were viewed in a word processor with formatting turned on, it would appear as follows: · represents a character space (ASCII code 20), → represents a tab character (ASCII code 09), and ¶ represents a paragraph character (carriage return/linefeed, i.e., ASCII codes 10 13) in the .txt file; all other spacing is for illustration only and does not represent characters in the file.

```
<Group>¶
3·ramps·group¶
3¶
3 →    →  5 →    →  3 →    →  ¶
ramp·1  →  T633604416880443355 →  ramp·2  →  T633604416880443356 →  ramp·3
  →  T633604416880443357 →  ¶
X →  Y →  X →  Y →  X →  Y →  ¶
0 →  0 →  2 →  -1 →  6 →  0 →  ¶
1 →  1 →  3 →  0 →  7 →  1 →  ¶
2 →  2 →  4 →  1 →  8 →  2 →  ¶
  →    →  5 →  2 →    →    →  ¶
  →    →  6 →  3 →    →    →  ¶
</Group>¶
```

This group is shown in the following graph (the grid lines have been removed from the picture for clarity).



Session files

Sessions exported as .txt files have a format similar to group *.txt files.

LINE 1: shows the string "<Session>" followed by a carriage return/line feed.

LINE 2: the session name followed by a carriage return/line feed.

Following this are all the lines for a group file as described above from "<Group>" to "</Group>". This is repeated for each group in the session.

The last line in the session .txt file shows the string "</Session>" followed by a carriage return/line feed to indicate the end of the file.

Import from FeliX32…

Use the **File, Import from FeliX32…** command to open an ASCII data (*.txt) file exported from FeliX32, FeliX, or TimeMaster and stored on disk or on the network. Note that in FeliX32 only group files allow export as .txt files. 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.

File name: Type a file name in the text box, or select the file name from the list. Only one file can be opened at a time.

Files of type: With this command, you can only open *.txt files exported from FeliX32, FeliX or TimeMaster.

Format

LINE 1: number of traces, followed by a carriage return/line feed.

LINE 2: number of data pairs for the first trace (N1), two tab characters, number of data pairs for the second trace (N2), two tab characters, … This line ends with one tab character and a carriage return/line feed after the number of data points for the last trace. Let N_{max} = maximum number of any trace's data pairs = max(N1, N2, …).

LINE 3: first trace label, two tab characters, second trace label… This line ends with one tab character and a carriage return/line feed after the trace label for the last trace.

LINE 4: "X", tab character, "Y", tab character, repeated for the number of traces, followed by a carriage return/line feed.

LINES 5- N_{max}: first trace X value, tab character, first trace Y value, tab character, second trace X value, tab character, second trace Y value… ending with a carriage return/line feed.

Note: All unused data fields are padded with tab characters. For example, if the first trace has fewer data pairs than the second, tab pairs are used prior to the remaining second trace values. If latter traces have fewer data points than preceding traces, these data fields are also padded.

If the contents of a sample group file exported from FeliX32 were viewed in a word processor with formatting turned on, it would appear as follows: · represents a character space (ASCII code 20), → represents a tab character (ASCII code 09), and ¶ represents a paragraph character (carriage return/linefeed, i.e., ASCII codes 10 13) in the .txt file; all other spacing is for illustration only and does not represent characters in the file.

```
3¶
3 →    →  5 →    →  3 →  ¶
ramp·1  →    →  ramp·2  →    →  ramp·3  →  ¶
X  →  Y  →  X  →  Y  →  X  →  Y¶
0  →  0  →  2  →  -1 →  6  →  0¶
1  →  1  →  3  →  0  →  7  →  1¶
2  →  2  →  4  →  1  →  8  →  2¶
   →    →  5  →  2  →    →  ¶
   →    →  6  →  3  →    →  ¶
```

Export to FeliX32

You must select a single group in the legend to make this command active. It opens a Windows dialog to save a group as a txt file in the format detailed at **File, Import** from FeliX32….

Send to Data Analysis

You must select a single group in the legend to make this command active. It launches the Legacy Data Analysis module for analyzing Fluorescence Decay data with the group showing in the legend and graph area. See the FelixGX Legacy Data Analysis Manual.

Export to JPG

Opens a Windows dialog to save a screen shot of the graph area as a .jpg file.

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.

Print Preview

Use this command to display the active workspace as it would appear when printed. When you choose this command, the main window will be replaced with a print preview window.

Refer to Windows documentation and online help for details on using this dialog box.

Print Setup…

Use this command to select a printer and a printer connection. The options allow you to select the destination printer, its connection, paper size and layout, and other printing related options.

Refer to Windows documentation and online help for details on using this dialog box.

Exit

Use this command to end your FelixGX session. If the data has not been saved or has been modified since it was saved, a prompt will appear stating, "Data has not been saved. Close anyway? Yes No".

Shortcut: Click the Window Close button (X) in the top right corner.

Chapter 6

Math Commands

The results of a fluorescence experiment are usually fluorescence emission intensity values that have been measured at specific wavelength or time increments. A contiguous group of data points is a trace, and the trace(s) resulting from an experiment are displayed in one or more groups in a session.

The commands in the Math menu allow specific mathematical functions to be carried out on single traces or selected regions of a trace. 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 trace (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 **Toggle Range** icon,  , from the graphing toolbar and use the mouse to click and drag within the graph display over the desired region of the trace. 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 trace will be created. The original (source) data will be preserved.

Replace Old Data

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

Label

Type the name of the new trace in the text box. If the label text box is made empty so that no label is specified, then the Execute button will be made inactive until text is entered into the box.

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 trace, even if you then select another trace.

Close

Closes the math function dialog box.

Antilog

Calculates the antilogarithm to base 10 of the selected trace. The maximum value created by this command is 1.0e+20.

Average

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

The sample standard deviation is also determined using the equation:

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

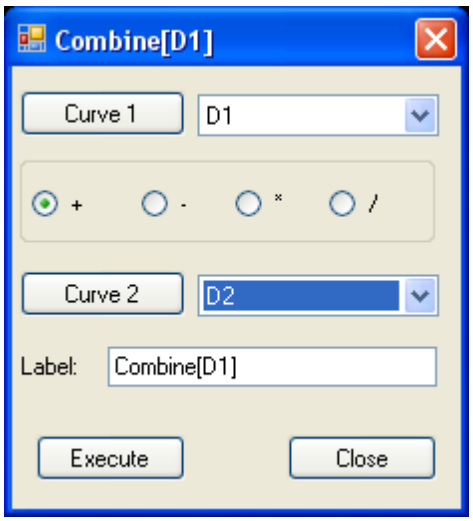
Where x_i is a data point and n is the total number of data points in the selected region of the data trace being averaged.

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 trace to another, subtract a trace from another, multiply a trace by another, or divide a trace by another. The math is performed in a point-by-point fashion. Only the portions of the traces that overlap are combined.

Curve 1, Curve 2

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

Operation

Select an operator to add (+), subtract (-), multiply (x), or divide (/) Curve 1 by Curve 2. If the operation is divide and the selected denominator trace contains any Y-values = 0,

the Combine function is not performed and no trace is created. A popup message will state this.

The resultant curve will be placed in whichever group is selected in the legend (or by virtue of having a curve selected within a group) when the Execute button is used. Using a choice list to select a curve does not mark that curve in the legend as being selected. If no group (or curve) is selected in the legend, then a new session will be created (with name *New Session*) in the legend.

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.

Curve 1 (X)

Use the choice list to select the trace from which to create the X data. Alternatively, select a trace from the legend and click on the Pick icon beneath the Source trace with X data header.

Curve 2 (Y)

Use the choice list to select the trace from which to create the Y data. Alternatively, select a trace from the legend and click on the Pick icon beneath the Source trace with Y data header.

The resultant trace will be placed in whichever group is selected in the legend (or by virtue of having a trace selected within a group) when the Execute button is used. Using a choice list to select a trace does not mark that trace in the legend as being selected. If no group (or trace) is selected in the legend, then a new session will be created (with name *New Session*) in the legend.

Differentiate

Differentiate takes the derivative of the selected trace. 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 trace. 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 trace.

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. FelixGX projects a line between the points where the boundaries of the range intersect the trace. Peak Area is the

integrated area above that line. If most of the trace data lies below this line, then the Peak Area will be a negative number.

Linear Fit

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

Linear Scale

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

Y and X Value

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

Divisor: Divides all Y values in the trace by the specified divisor. Divide by zero is not allowed.

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

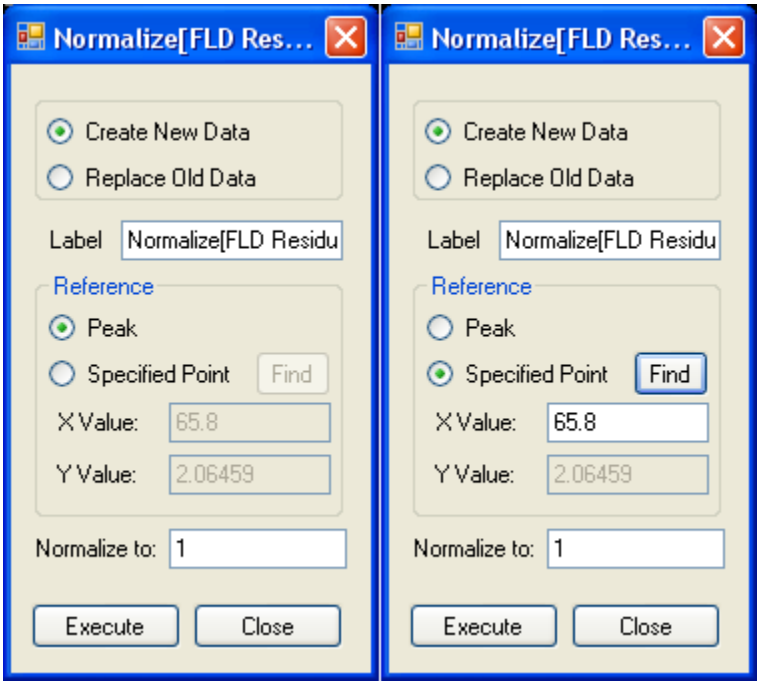
Select Range

Applies the transformation only within the region selected by the user. The range is selected using the Toggle Range toolbar icon,  , and clicking and dragging the mouse over the desired area in the workspace. Note that if you perform an X offset within a selected range, the resultant trace can have pairs of data points with the same X-values.

Logarithm

Calculates the base 10 logarithm of the selected trace. If the selected trace contains any Y-values # 0, the logarithm function is not performed and no trace is created.

Normalize



Normalizes a trace to a set value. The normalization function reference Y-value may be either a peak or a specified point within the selected range.

Reference

Peak. A new trace will be created based on the peak point within the selected range.

Specified Point. Enter the X value of the specified point in the text boxes, and then click on **Find** to obtain the corresponding Y-value, to be used as the reference value.

Normalize to:

Enter the value to which the trace will be normalized.

Reciprocal

Calculates the reciprocal (1/Y) of the Y-axis data in the selected trace. If the selected trace contains any Y-values = 0, the reciprocal function is not performed and no trace is created.

Smooth

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

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 trace. The selected region of the trace is preserved and all X values above and below this region are permanently

deleted. The region may also be selected using the **Toggle Range** icon in the toolbar and clicking and dragging the mouse over the desired range in the workspace.

Baseline

Baseline suppression causes a selected region of a trace to be set to a constant Y value (commonly zero). The region 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.

Peak Finder

This function 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 Toggle Range.

Mark peak on graph

Shows a crosshair at the peak position on the graph.

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.

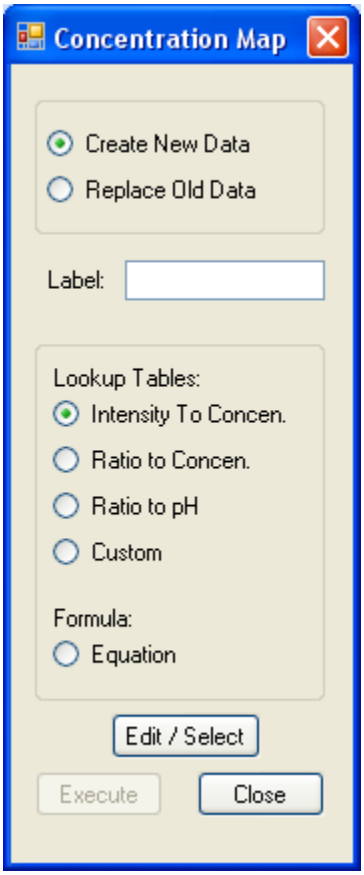
Legacy Data Analysis

Clicking on this command opens the FelixGX Legacy Data Analysis module for analysis of fluorescence lifetime decay data. See the FelixGX Legacy Data Analysis manual for instructions in using this manual.

Transform Commands

Concentration Map

This dialog is used to convert acquired or saved data to concentration or pH using lookup tables or equations, or to construct or modify lookup tables. The experimental data may be intensity or the ratio of two intensities.



Create New Data, **Replace Old Data**, **Label**, **Execute** and **Close** are the same as described for **Common Math Controls**.

Label: Be sure to enter a label. Otherwise a trace with a blank label will be created and will be difficult to manipulate.

Lookup Tables

Lookup tables can be constructed to calculate the concentration in several different ways.

Intensity to Concentration: For most steady state experiments, the intensity is related directly to concentration.

Ratio to Concentration: For most ratio fluorescence experiments, the ratio of two intensities is related to concentration.

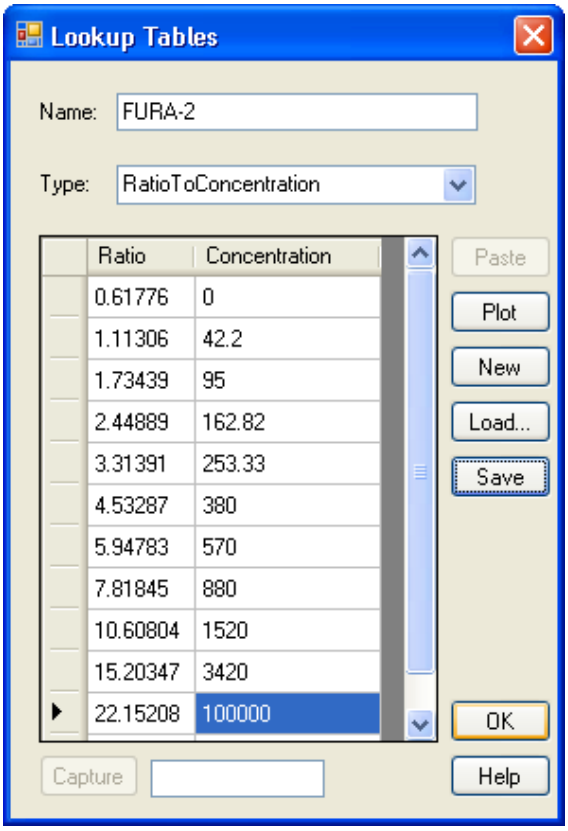
Ratio to pH: Converts ratio values to pH.

Custom: Create a new LUT based on a parameter that varies with successive traces vs. the average values of the traces.

Formula: The concentration of intracellular ions can be calculated directly from the ratio of intensities through the equation from Grynkiewicz, Poenie, and Tsien.

Edit/Select: Click this button to open a dialog to choose/modify/create a lookup table or equation that contains the calibration curve. Excitation and emission correction, GFactor and Custom lookup tables can also be constructed or modified in this way.

Execute: Select the desired trace in the legend, select the type of lookup table or formula to be used for the transformation, then click on the **Execute** button to create the transformed data.



Lookup Tables

This dialog is used to construct or edit a lookup table to relate intensities or ratios to concentration or pH; or wavelength to emission or excitation correction or GFactor; or a user defined Custom LUT. At least two values must be entered to constitute a valid LUT.

Click on a cell in the table and enter a numeric value. You can press the tab key to move to the next cell.

Note. A LUT can be created from a trace by using the **Create Lookup Table** command in the Trace menu in the legend.

Name: Enter a name for the lookup table.

Type: Select the type of lookup table from the list.

Paste: Enters values from a trace in Grid mode. In the Grid click and drag to highlight some cells, right-click and choose **Copy**. Then in the LUT dialog click on the **Paste** button. The values will be appended to the table. **Note** that these **Copy** and **Paste** commands use an internal clipboard, not the Windows clipboard. I.e., these commands will not work with a spreadsheet from a different program.

Plot: Puts the LUT data in the legend and graph in a new session. **Note**: that such data is not a trace and cannot be used in the graph and legend as a normal trace – i.e., if you right-click on the LUT name in the legend only the **Toggle Visibility** command works.

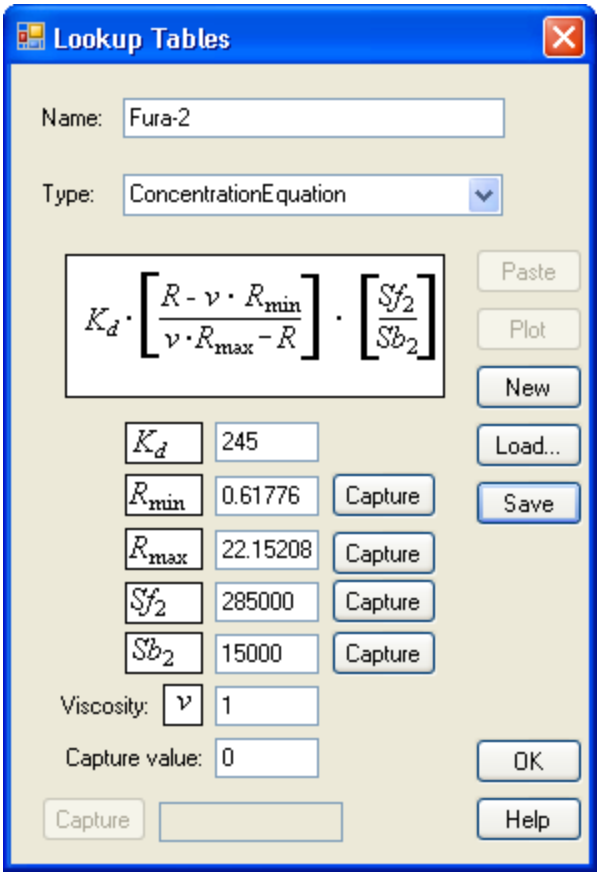
New: Clears the data and name in the LUT dialog so that a new lookup table can be constructed. See Polarizer Calibration for an example of using Custom and New.

Load: Opens a saved LUT of the type shown.

Save: Saves any changes to the current LUT values.

OK: Selects the displayed LUT for transformation use and closes the LUT dialog. If you were constructing or modifying the LUT, then such changes will not be saved. You must save the Lookup Table if it has not already been saved. No warning is given that any changed values will not be saved.

Capture: Not implemented at this time.



Lookup Tables – Concentration Equation

Name: Enter a name for the lookup table.

Type: Select the Concentration Equation from the list.

New: Clears the name and resets the equation values to defaults in the LUT dialog so that a new lookup table can be constructed.

Load: Opens a saved LUT of the type shown.

Save: Saves any changes to the current LUT values.

OK: Selects the displayed LUT for transformation use and closes the LUT dialog. If you were constructing or modifying the LUT, then such changes will not be saved. You must save the Lookup Table if it has not already been saved. No warning is given that any changed values will not be saved.

K_d: Enter this value manually. The units for K_d will be the units used to for the resulting ion concentration.

R_{min}, R_{max}, Sf_2, and **Sb_2**: Enter these values manually or capture them from the selected region of a trace. Toggle Range On and select a region. The average of the Y-values for this range is shown in the Capture value text box. Click on the **Capture** button beside the respective variable.
R_{min}, R_{max} = $F_{\lambda 1}/F_{\lambda 2}$ ratios of the ion-free and ion-saturated sample, respectively.
Sf_2 = $F_{\lambda 2,min}$ of the ion-free sample.
Sb_2 = $F_{\lambda 2,max}$ of the ion-bound (saturated) sample.

Viscosity: The intracellular viscosity. For water this is close to 1. Enter this value manually.

Axes Commands

These commands allow the user to control the scaling and axes in the graph area. A large dot will appear next to the current X- and Y-scaling mode.

Quick Full Scale X & Y

Displays the full X- and Y-ranges of data. During acquisition, the X-axis is set to be at least as wide as the View Window.

Shortcut:  Use the toolbar icon.

Autoscale X

Displays the full X -range of data. The Y-range of data is left as is.

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.

Autoscale Y

Displays the full Y -range of data. The X-range of data is left as is.

Autoscale from 0

Scales the Y-axis to show the maximum space from Y = 0 to the largest positive Y-value of the visible data. Negative Y-values are not shown. The X-range of data is left as is.

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.

Logarithmic Y-Scale

Makes the Y-axis logarithmic. The log scale is automatically selected. Zero or negative values in the displayed trace(s) will be displayed as near vertical lines going down as far as the number of log decades allowed (to a maximum of 23 decades).

Visible log decades

This command is only active when the Y-axis is shown using the logarithmic scale. You can choose an automatic display of log decades or a specified number of decades. This rescales the Y-axis to show the number of full log decades down from the nearest decade above the maximum Y-value of the visible data points.

Action

Calibration

Wavelength

Click on a button to display a graph showing the principal lines in the spectra of various standards such as DYAG excitation, DYAG emission, or Mercury emission. You can use these displays as references when comparing data using these samples.

Accessories

Click on a button to show a dialog to calibrate an instrument accessory. These dialogs are displayed even if they are not enabled in the Hardware Configuration. These dialogs are also available in the **Edit hardware Configuration** window and are described there. For instruments that have been assembled and tested at the factory, or installed by a PTI Service technician on site, these calibrations have already been done. If you feel the accessories need to be done again, you should first contact PTI Service.

Macros

Shows the Macro Control dialog where automation jobs can be selected, run, and display status reports.

When **Action, Macro…** is initially opened, the dialog shows a toolbar, a blank Status report, and a blank Saved Macro(s) choice list. Click on the Create new Macro button to open the Macro Editor dialog, or on the down arrow on Saved Macro(s) to show a list of saved macros. If the list is not empty, you can Load a macro from the list and play it.

When a macro is not running, only the Create new Macro, Delete Macro(s), Edit current Macro, and Play current macro buttons are active. When a macro is running, only the Pause and Stop buttons are active.

Create new Macro: Opens the Macro Editor dialog.

Delete one or more Macro(s): Opens the Delete Job dialog where you can select and delete saved macros.

Edit current Macro: Opens the Macro Editor dialog to modify the current macro.

Play current Macro: Plays the current macro. A listing of the macro actions performed is shown in the Status box.

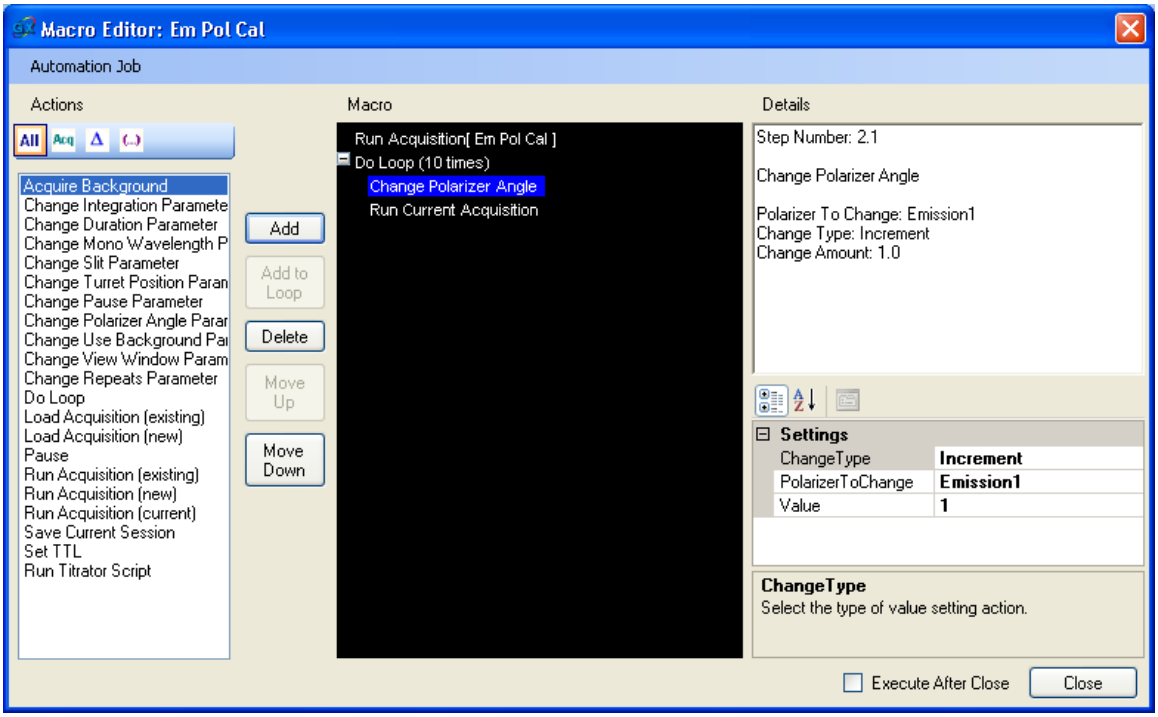
Clear All Clears the Status box.

Pause running Macro: Pauses the currently running macro.

Stop running Macro: Stops the currently running macro.

Load Loads the macro selected in the Saved Macro(s) list.

Macro Editor



This dialog is where the user can create, edit, save or export, open or import and execute macros. Below the title bar is the menu. Below this is the editing area. On the left is the list of available actions. Next to the right are buttons to place actions into the job, delete actions from the job, or move actions up or down in the job list. In the center is the macro listing. Nested actions (Do Loops) can be condensed or expanded. On the far right are the details of individual actions and a table where the action settings and values can be set or changed.

The dialog window can be resized by clicking and dragging an edge or corner. Also, the dividers between the Macro listing and Details, between Details and the Action Settings, and between the Action Settings and the box below it can be moved.

Automation Job

New: Clears the macro listing of commands.

Open: Opens the list of automation jobs saved in the automation repository (aut.rep). This list is the same as the Saved Macro(s) list on the Macro Control dialog. Select a job from the list and click **OK** to place this job in the Macro listing. **Cancel** closes the Open dialog. **Help** does nothing at this time.

Delete: Same as the Delete one or more Macro(s) button on the macro Control dialog. **Cancel** closes the dialog. **Help** does nothing at this time.

Save: Saves the current macro into the repository using the same name.

Save As…: Saves the current macro to the macro repository (aut.rep). Enter a name for the macro. Click on **OK** to save the acquisition. Click **Cancel** if you do not want to save the current macro.

Import…: Use this command to open a macro file (*.gxa) exported from FelixGX and 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. The imported macro will overwrite the current macro.

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. Only one macro file can be opened at a time.

Files of type: With this command, you can only open *.gxa files exported from FelixGX.

Open: Click this button or double-click on the file name in the list to open it in FelixGX.

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

Export …: Opens a Windows dialog to save a macro file (*.gxa) on a drive or on the network

Actions: Shows lists of the actions that can be added to an automation job.

All: Shows all the actions.

Acquisition Choices: Shows **Load** or **Run** existing, new, or current acquisitions. Acquisitions created by the Acquisition Setup command or in the Automation Maintenance dialog are interchangeable, but in either case depend upon the current hardware configuration.

Change Acquisition Choices: Shows actions to set or alter acquisition choices that are shown on the Acquisition Settings tab in Acquisition Setup.

Miscellaneous Choices: Other actions.

Placement buttons

Add: Select an action and then click on this button to add an action to bottom of the code main level.

Add to Loop: Select an action, click on a Do Loop action, and then click on this button to add an action to the bottom of the actions within that Do Loop. Actions within a Do Loop are indented. **Note**: there is no 'End Loop' or similar action – the first action after a Do Loop is flush with the Do Loop action. Do Loops can be nested.

Delete: Select an action in the automation job and then click on this button to remove it from the job.

Move Up: Select an action in the automation job and then click on this button to move it up in the job. Selecting a Do Loop will move the nested actions with the Do Loop action.

Move Down: Select an action in the automation job and then click on this button to move it down in the job. Selecting a Do Loop will move the nested actions with the Do Loop action.

Details

Click on an action in the Macro listing to see details in the top box. The first line shows the step number. Nested actions are designated by appending a decimal number to the step number of the Do Loop. If the action is Load Acquisition or Run Acquisition as either New or Existing then the Details box will show all the details of the loaded acquisition setup, similar to the Acquisition Summary tab. Run Current Acquisition (i.e., based on changes to acquisition parameters) will show only the text 'Run Current Acquisition' in the Details box. If the action is anything else, then the Details box will give a summary of the action properties and values. Numerical values show only one decimal place in the Details box.

Table

The table shows the action's properties and values with the property names in alphabetical order. Most changes to initial acquisition parameter settings have some of the following options. Initial values may be shown as Unknown and must be changed to a valid property value to be able to run or save the job.

ChangeAmount, NewValue, **Pause Time**: Enter a numerical value (decimal values allowed).

Loop Count, TTLNumber: Enter an integer value.

NewValue, **Graph1**, **Graph2**: Select True or False.

ChangeType: Unknown, Increment, SetParameter, ChangeToValue. I.e., increment a parameter setting by a positive or negative value, or set a new fixed value.

MonochromatorType or **PolarizerToChange**: Select the relevant monochromator.

ParamType: IntegrationTime, PointsPerSecond, or Stepsize for QuantaMaster (Steady State) or RatioMaster hardware configurations.

WavelengthType: SingleWavelength, ScanStart, or ScanEnd

SlitToChange: Entrance, Exit, or Both. Motorized slits have a backlash. This applies to motorized slits only. Whenever changing motorized slits, always change from a wider width to a narrower width. If going from a narrow width to a wider width, first use a command to go to an extra wide width and then a second command to go the desired wide width.

PauseType: Indefinite, IndefiniteWithUserMessage, or Timed. Indefinite and IndefiniteWithUserMessage wait for the user to click the Start button, or if set up, for a TTL In signal. If set to Timed, the action will end after the PauseTime has elapsed.

TriggerMode: Low or High

TTLTypeAction: In or Out.

SaveCurrentSession: Enter a filename. Click on the Browse button at the right end of the Filename row to select a location.

Execute after Close: Runs the current job after clicking the Close button returns to the Macro Control dialog..

Close: Closes the Macro Editor dialog. A prompt will be shown if the current job has not been saved since it was created or modified.

Chapter 10

Configure Commands

Preferences

Shows a dialog where the user can set program preferences.

Background Black/White: Choose the background for the graph area. The default is black. The background is white only when there is a session showing in the legend. **Note:** there is currently a bug that when the background is set to white by this preference, then printed output will not show the graph title, axes captions, or tick labels.

Acquisition Defaults, Include all repeats in one trace: Sets the default for this acquisition preference. Checking the box turns the default acquisition preference ON.

Logging: Gives information about the ptilog.txt file. This file records hardware events and exceptions to proper program flow. This file may help in troubleshooting some problems.

Language: Changes the language for some program commands, text, etc. The default is English.

Preserve space on bottom for Windows task-bar: If checked, then space for the Windows task-bar will be preserved when launching FelixGX.

ASOC with swapped XDAC0 / XDAC2:
For systems with DeltaRAM excitation, make sure this check box is checked, and that the BNC from the DeltaRAM Position connector goes to the XCORR GAIN (ASOC-10 boxes with S/N from 1000 to 1999) or the Ex. Cor. Gain (ASOC-10 boxes with S/N greater than or equal 3000) connector on the back panel of the ASOC-10. For the older ASOC-10 boxes, the TTL I/O jumpers need to have been reconfigured to allow high-speed DeltaRAM operation with FelixGX version 4.0.2 or higher. Contact PTI Service to check if this has been done.

For systems without a DeltaRAM, this check box should not be checked, and the Excitation Correction BNC cable labeled “G” goes to the XCORR GAIN (ASOC-10 boxes with S/N from 1000 to 1999) or the Ex. Cor. Gain (ASOC-10 boxes with S/N greater than or equal 3000) connector on the back panel of the ASOC-10.

Hardware

This command opens a dialog window where components of the desired hardware are selected and their properties defined.

The current hardware configuration is shown when this dialog is first opened. If no hardware configuration has been created so far, the diagram and properties will be empty.

To start a new configuration, click on **Configuration**, **New** to show the **Open New Hardware Configuration** list of templates. The available templates are QuantaMaster (Steady State) and Photometry. Choose a template from the list to show it in the diagram area.

The diagram shows the active components in a configuration with bright lines. Optional components that are not currently active are shown with faint lines. To make a component active or inactive, right-click on it and toggle **Active** on or off. In some cases, e.g., lamp or detector type, clicking on a property value will activate the component and set that property as well. In the diagrams, the most recent component that has been selected is highlighted in yellow, while moving the mouse cursor over a different component without selecting it will highlight it in brown. A component must be selected to change any of its properties. In the gray area to the left of the diagram, choose the relevant properties of the component. Some properties are for information only, such as steady state lamp type or filters. Other properties need to be set correctly for the instrument to work as desired, such as pulsed lamp sources, detectors, all motorized components, and Peltier Cuvette Temperature Controllers.

If you want to alter one of the existing configurations that are stored in the hardware configuration repository (hwc.rep), click on **Configuration**, **Open** and choose a name from the list, **OK**.

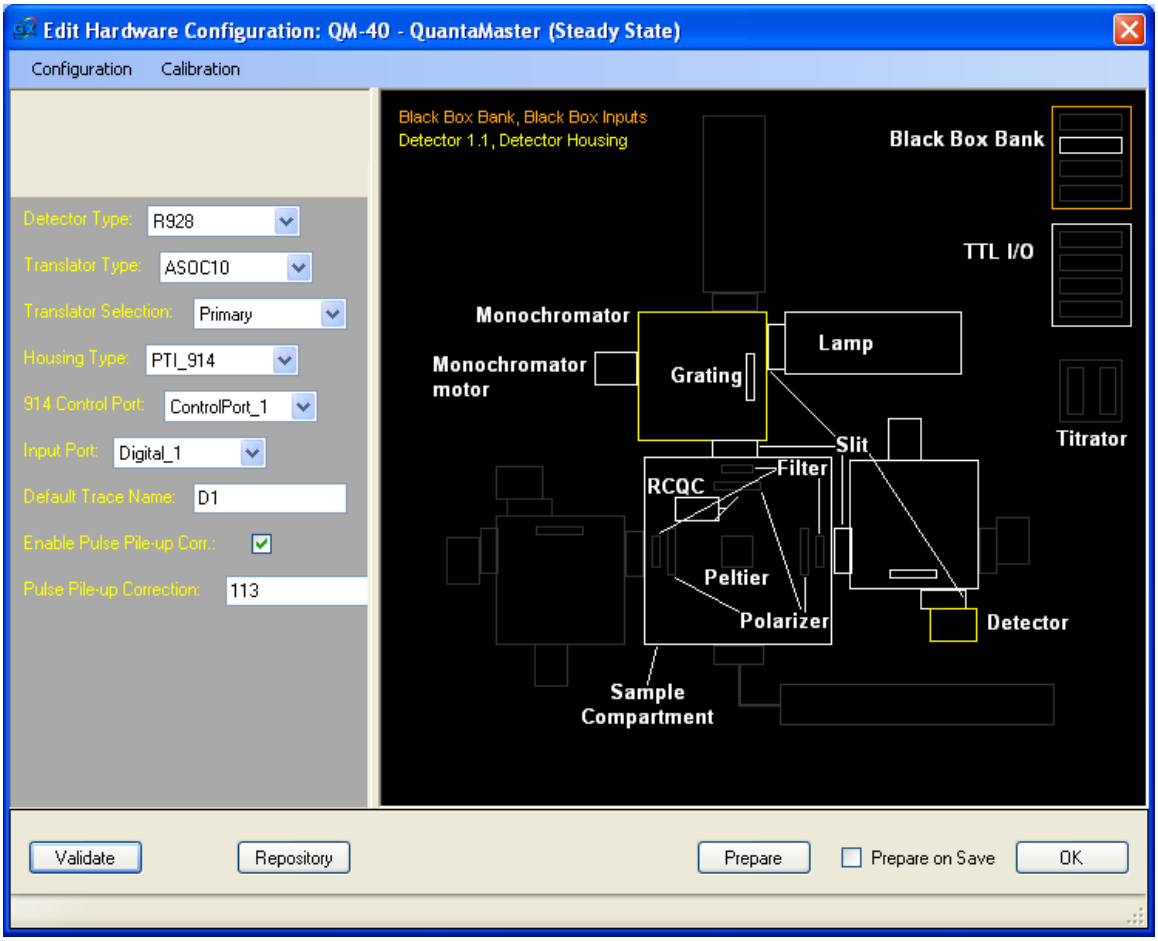
Once all the desired components have been made active and their properties set or altered, click on the **Validate** button at the bottom of the dialog window. This performs a simple check on the validity of the configuration and either shows on the Status Bar "Validation succeeded" or a message that needs attention before the configuration can be saved and used.

Next, click on **Configuration**, **Save As…** and enter a name for the configuration. Check the **Display** box if you want the configuration to show on the Acquisition Bar. If you want a name displayed on the Acquisition Bar different than the name saved in the Repository, enter this name in the **Display Name** text box. Click on **OK** to save the configuration in the hardware configuration repository (hwc.rep).

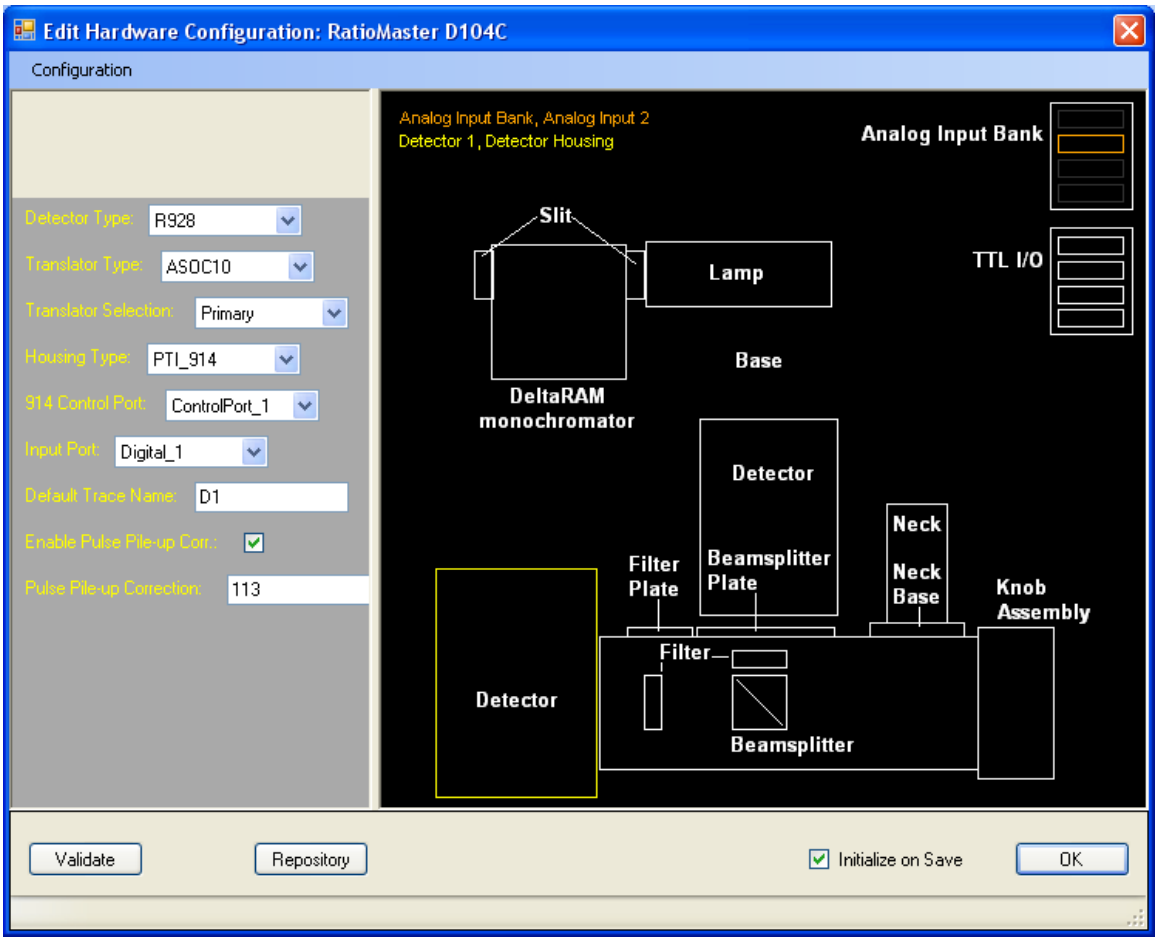
If you want to save any changes you have made to an existing hardware configuration without changing the configuration name, click on **Configuration**, **Save**. The configuration will be saved with no warning that you are overwriting an existing configuration.

The **Repository** button at the bottom of the **Edit hardware Configuration** window shows a list of all hardware configurations, whether or not they are shown on the Acquisition Bar. To make a hardware configuration visible on the Acquisition Bar, check the box in front of the hardware configuration name. The order of configurations

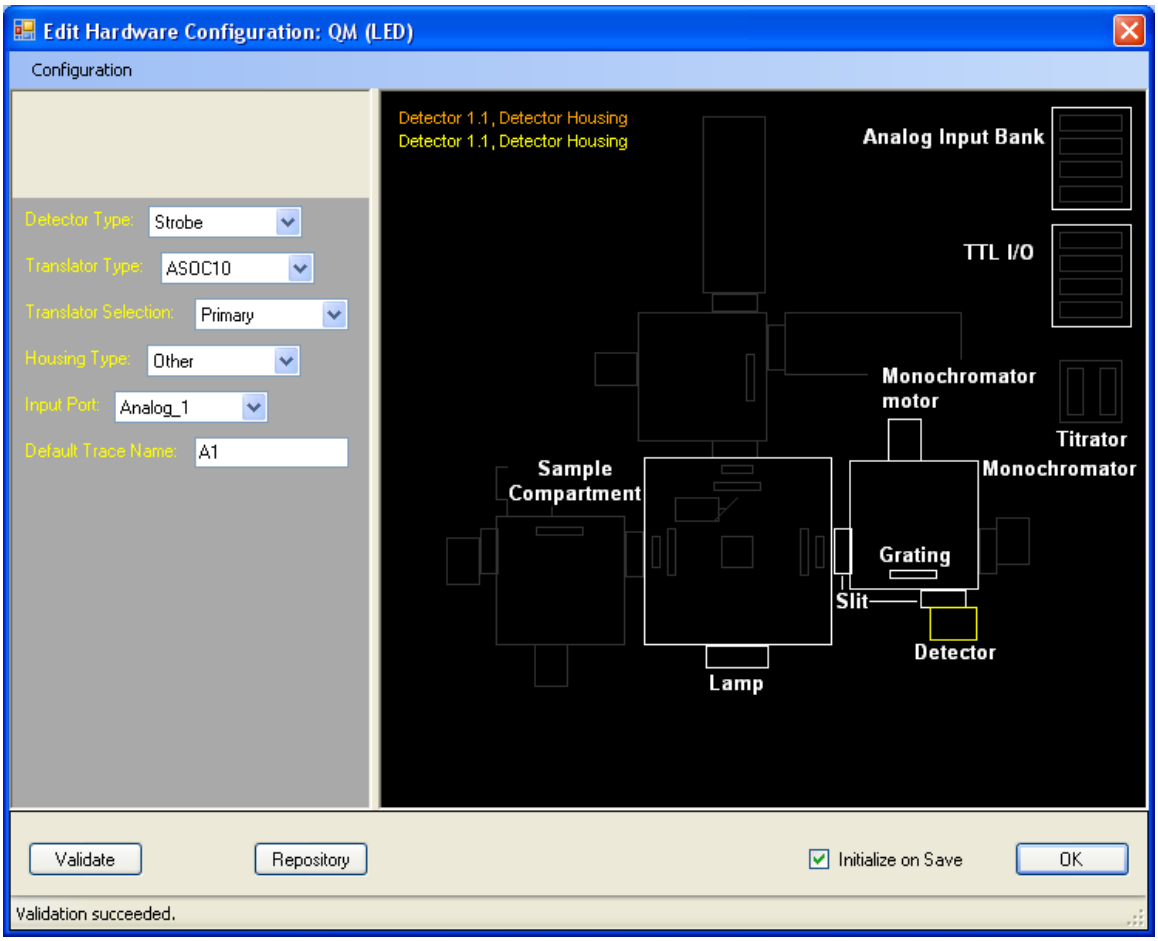
shown on the Acquisition Bar can be changed by selecting a configuration and clicking on **Up** or **Down**. The configuration at the top of the list is shown farthest to the right on the Acquisition Bar.



The above picture shows an annotated L-format QuantaMaster (Steady State) or QuantaMaster (Phosphorescence Lifetime) hardware configuration. FelixGX requires that the first emission wavelength device (monochromator or filter), slits, and detector be on the right of the sample compartment in the HWC diagram. A T-format system requires a second emission wavelength device (monochromator or filter), slits, and detector to be added to the left of the sample compartment in the above configuration.



The above picture shows an annotated RatioMaster with dual channel photometer hardware configuration. A Photometry hardware configuration is the same as the emission portion of a RatioMaster configuration (I.e., a RatioMaster hardware configuration without the lamp and excitation device).



The above picture shows an annotated QuantaMaster (Fluorescence Lifetime) or QuantaMaster (TCSPC) hardware configuration. For the Fluorescence Lifetime configuration select LED for the lamp type and Strobe for the detector type. For the TCSPC configuration select LED for the lamp type and TCSPC for the detector type.

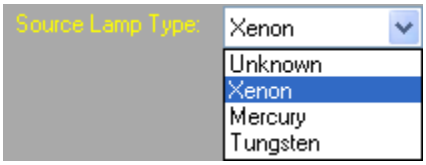
Hardware Components

Following the light path from the lamp, the various components and their properties shown in the area to the left of the diagram are listed below.

Note that a hardware configuration may not have more than one motorized device with the same Motor Drive Position and Motor Drive Channel combination, and may not have the same input port assigned to different digital or analog inputs. This will be noted in the Validation message.

Motorized devices (monochromators, slits, polarizers, and the PTI 4 position turret) receive power and control from MD-4000 and/or MD-8000 boxes. The box the USB cable is connected to defines the Master Motor Driver Position. If more than four motor channels are required, additional MD-4000(s) may be supplied, or an MD-8000 box may be substituted for two MD-4000 boxes. When more than one MD-4000 box is required, a cable is supplied to connect Extension OUT on the Master box to Extension IN on a Slave box, or similarly from Slave box i to Slave box i+1. An MD-8000 box is internally wired and is configured in FelixGX as two successive MD-4000 boxes (e.g., as Master and Slave 1, or Slave 1 and Slave 2).

Lamp Types



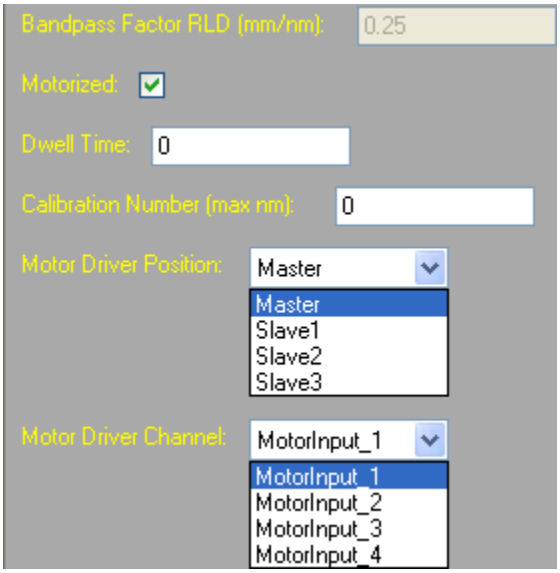
The following system types show the listed source lamp types. In some cases a particular lamp type must be selected. Photometry systems do not include a lamp.

System type	Source Lamp Type
RatioMaster	Xenon
QM (Steady State)	Xenon, Mercury, Tungsten
QM (Phosphorescence)	Pulsed Xenon, Nitrogen Laser, YAG Laser[1]
QM (VCI)	Pulsed Xenon[1]
TM (Fluorescence Lifetimes)	LED, LaserDiode
TM (LaserStrobe)	Nitrogen Laser, YAG Laser[2]
TM (TCSPC)	LED

1 For QM (Phosphorescence) or QM(VCI), select PulsedXenon, even if using a Nitrogen or YAG laser.

2 Select a Nitrogen laser instead of a YAG laser. The latter selection does not work.

Slit



If the slit is manual (default), only an unchecked **Motorized** box will be shown. Checking this box shows further properties.

Calibration Number: set at the PTI factory.

Bandpass Factor (mm/nm): Shows the conversion between the physical slit opening in mm and the bandpass in nm. The grating groove density and single or double monochromator are taken into account.

Dwell Time (s): Used only when auto-calibrating the slit. The slit will move to the closed position and hold there for this amount of time before opening.

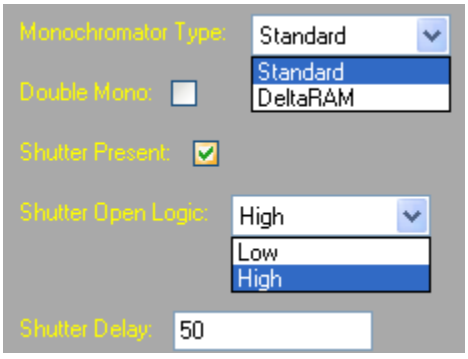
Calibration Number (max nm): This value is set at the factory for each slit. You can check the calibration number by using the command **Calibration, Slit…** from the Edit hardware Configuration menu. A value of 0 causes the slit to be re-calibrated each time the hardware configuration is re-initialized.

Motor Driver Position: this is the motor driver box the slit motor cable is attached to.

Motor Driver Channel: this is the connector on the motor driver box the cable is attached to.

Note: motorized slits automatically use a backlash to always go from a wider opening to a smaller opening.

Monochromator (Standard)



Monochromator Type: Choose between Standard or DeltaRAM for the monochromator type. A standard monochromator allows a shutter only for the excitation position and then must use motor channel 1 on the MD-4000. If a shutter is not used, then any motor channel on the MD-4000 may be used.

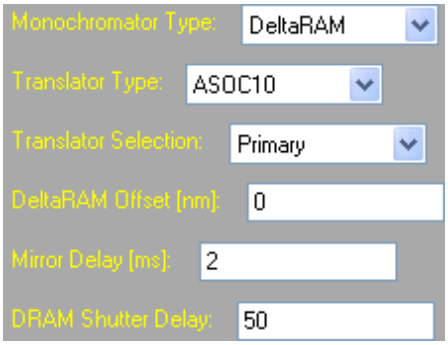
Double Mono: check this check box to indicate that a double monochromator is being used. This affects the bandpass calculation (see the bandpass value shown in **Slit** dialog).

Shutter Present: check this check box to indicate that an active shutter is present, and to show the **Shutter Open Logic** and **Shutter Delay** fields.

Shutter Open Logic: Specify whether a high or a low signal will open the shutter.

Shutter Delay: enter the delay in milliseconds. Data acquisition will start after this delay.

DeltaRAM



Monochromator Type: Choose between Standard or DeltaRAM for the monochromator type. At this time a DeltaRAM is not practical as an emission monochromator.

Translator Type: The interface between the computer and the instrument. Use ASOC10.

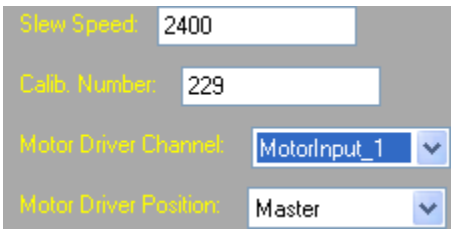
Translator Selection: Use Primary.

DeltaRAM Offset (nm): Allows an offset of the DeltaRAM wavelength. Run an excitation scan of a standard and enter a value if the trace near 450 nm is different than the expected position. The DeltaRAM is adjusted at the factory so that this value is 0 nm.

Mirror Delay (ms): Also called dead time. During this time the mirror is moving and the signal is not acquired.

DRAM Shutter Delay: enter the delay in milliseconds. Data acquisition will start after this delay.

Monochromator motor



Slew Speed: Microsteps/second. This is the maximum speed at which the monochromator moves from one wavelength position to another. On a standard monochromator with 1200 grooves/mm grating, 1 nm = 16 microsteps. Use 2400 for a standard monochromator. Using too fast a speed can cause skipped steps and possibly jam the monochromator at the lower or upper mechanical limit.

Calibration Number: Enter the value on the monochromator wavelength dial when the monochromator is initialized.

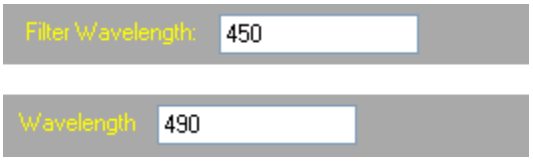
Motor Driver Channel: this is the connector on the motor driver box the cable is attached to.

Motor Driver Position: this is the motor driver box the motor cable is attached to.

Sample Compartment

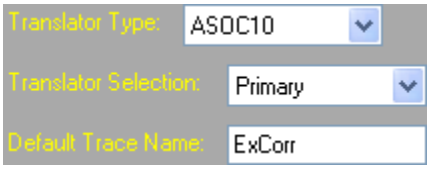
In a new QuantaMaster hardware configuration the sample compartment only has the box active, other components in the sample compartment are inactive. To change the properties of those components, right-click on the individual components.

Filter or Beamsplitter



Enter the wavelength for the filter or beamsplitter. The beamsplitter is used in a dual channel photometer. **Note** that if a beamsplitter is activated then detector 2 and its filter must also be activated.

RCQC

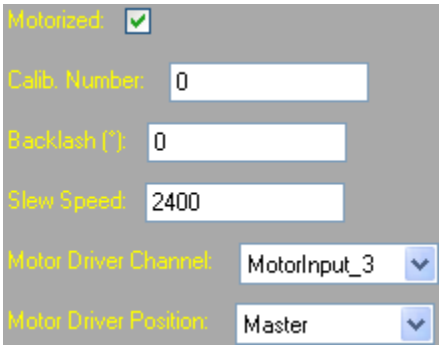


Translator Type: The interface between the computer and the instrument. Use ASOC10.

Translator Selection: Use Primary.

Default Trace Name: The name assigned to acquired RCQC traces.

Polarizer



If the polarizer is manual (default), only an unchecked **Motorized** box will be shown. Checking this box shows further properties.

Calib. Number: enter the calibration value (the calibration procedure is described at **Accessories, Polarizers Control** in **Chapter 12 Acquisition Control**).

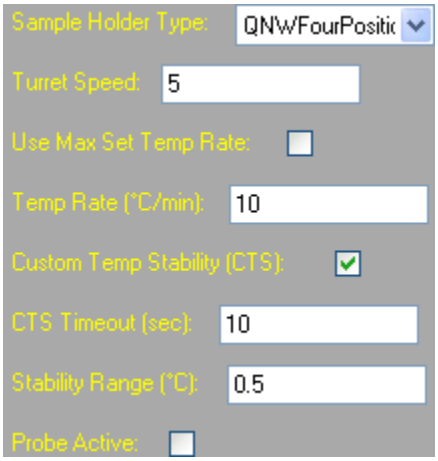
Backlash: Enter a value for the backlash. When returning to a smaller angle, the polarizer will move this value beyond the angle and then advance to the angle. This removes any gear backlash.

Slew Speed: Microsteps/second. This is the maximum speed at which the polarizer moves from one angle to another. Use 2400. Too fast a speed can cause missed steps.

Motor Driver Channel: this is the connector on the motor driver box the cable is attached to.

Motor Driver Position: this is the motor driver box the motor cable is attached to.

Peltier



Sample Holder Type: Choose among QNW Single Cuvette Holder, QNW 4 Position Turret, and PTI 4 Position Turret.

Turret Speed: This field is seen only for the QNW Four Position Turret. Set the speed at which the turret rotates from slow (250) to fast (5).

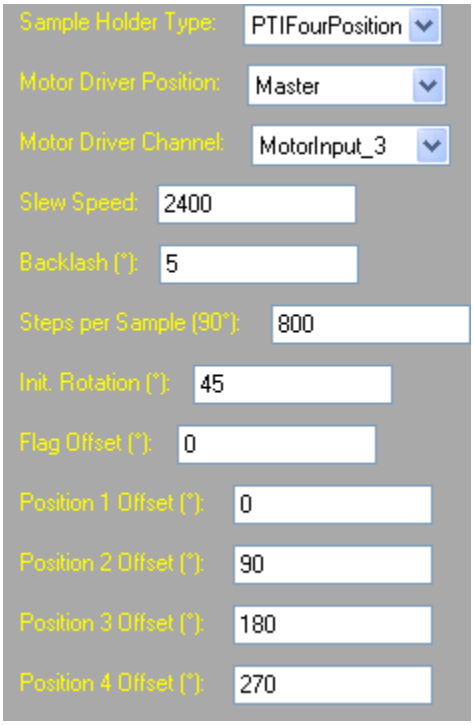
Use Max Set Temp Rate: Sets the default temperature ramp rate to the maximum value of 20° C/minute.

Custom Temperature Stability (CTS): check this box to enable FelixGX control of CTS Timeout and Range (if disabled, then the QNW values of 5 minutes Timeout and 0.02° C range are used).

CTS Timeout (sec) and Stability Range (°C): when setting single or multiple (e.g., repeat) temperature points, FelixGX will wait until the temperature has reached the target temperature within the Stability Range for the CTS Timeout period before starting or continuing onto the next temperature point. If after a scan has started to acquire data, the temperature moves outside the CTS range, the data acquisition will continue without waiting for the temperature to get back inside the CTS range again.

Probe Active: If this is disabled, then temperature will be measured using a sensor in the cuvette holder. If this is enabled, then temperature will be measured using a probe inserted into the sample cuvette.

PTI 4 Position Turret



Sample Holder Type: Choose among QNW Single Cuvette Holder, QNW 4 Position Turret, and PTI 4 Position Turret.

Motor Driver Position: The motor driver box the slit motor cable is attached to.

Motor Driver Channel: The connector on the motor driver box the cable is attached to.

Slew Speed: Microsteps/second. This is the maximum speed at which the polarizer moves from one angle to another. Use 2400. Too fast a speed can cause missed steps.

Backlash: Enter a value for the backlash. When returning to a lower number position, the turret will move this angle beyond and then advance to the position. This removes any gear backlash.

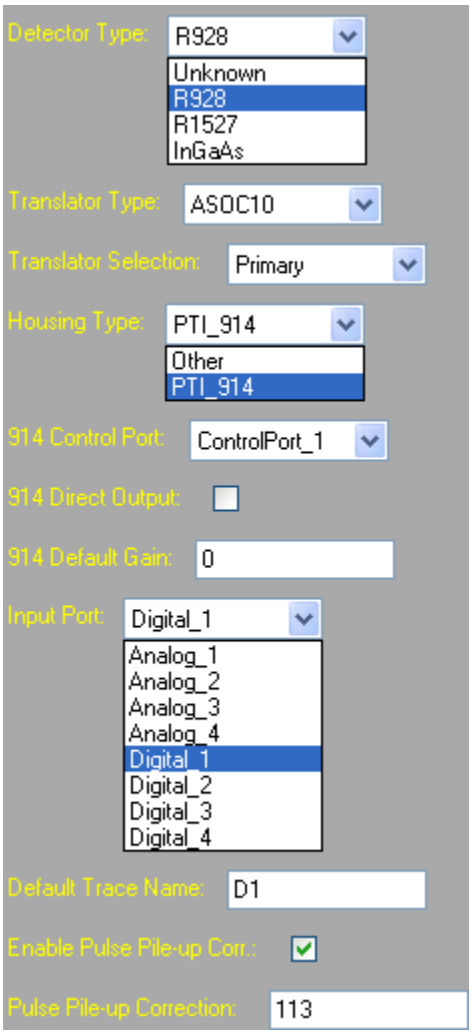
Steps per Sample (90°): Leave this value = 800.
Init. Rotation: The angle the turret first moves during system or turret initialization.

The Flag Offset and Position Offsets should be set using the **Calibration, Turret…** dialog.

Flag Offset: The offset of position 1 from the autocalibration flag. The other position offsets are relative to the flag position. Position 1 Offset is left at 0°.

Position 2, 3 and 4 Offsets: The angles of these positions relative to Position 1.

Detector (QM Steady State, QM Phosphorescence, RatioMaster, and Photometry systems)



Detector Type: Select the PMT or detector type. The detector types are for information only. It makes no difference to the software control. NIR detectors (e.g., InGaAs) only have analog output.

Translator Type: The interface between the computer and the instrument. Use ASOC10.

Translator Selection: Use Primary.

Housing Type: Select **914**, or **Other** for a non-914 housing (610 or 710).

914 Control Port: This text box is only visible for the 914 housing type. This is the number of the 5-pin DIN connector on the side or back panel of the ASOC-10 used for power and control of the 914 housing.

914 Direct Output: Check this box only if using the Direct Output mode on a 914 detector.

914 Default Gain: Sets the default gain for the 914 in analog mode. Not used in digital mode. Enter 900 for analog mode.

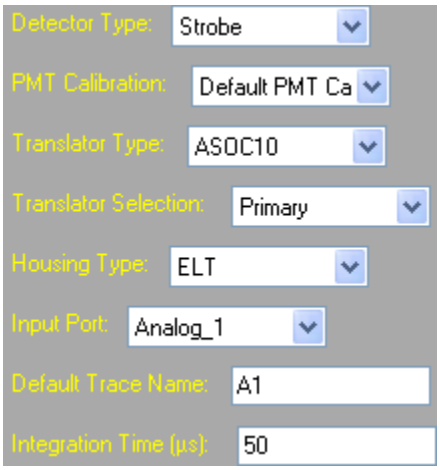
Input Port: Select the connector on the ASOC-10 that the signal cable is attached to. PMT 1 and 2 refer to digital PMT detector inputs. PMT 3 and 4 do not exist on the ASOC-10 at this time. AN1 – AN4 refer to analog PMT detector inputs. **Note** – Input ports here cannot be set to the same ports used by black box inputs.

Default Trace Name: The name assigned to acquired traces for a signal from this detector. Enter a name.

Enable Pulse Pile-up Correction: Check this box to correct the digital detector signal for pulse pile-up.

Pulse Pile-up Correction: This text box is only visible for digital detectors. Enter 113 for R1527 and R928 PMTs in a 914 detector. Enter 520 for R1527 and R928 PMTs in other detectors. Other PMTs may require different pulse pile-up correction values.

Strobe Detector (TM Fluorescence Lifetime and LaserStrobe systems)



Detector Type: Use **Strobe**.

PMT Calibration: For LaserStrobe systems select the PMT calibration from the list.

Translator Type: The interface between the computer and the instrument. Use **ASOC10**.

Translator Selection: Use **Primary**.

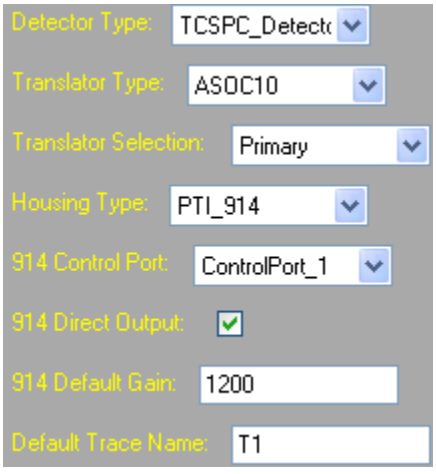
Housing Type: Use **ELT**. **Other** will be invalid.

Input Port: Select the Analog connector on the ASOC-10 that the BNC cable from **a** on the electrometer is attached to.

Default Trace Name: The name assigned to acquired traces for signal from this input.

Integration Time (us): Use the default value of 50 µs.

TCSPC 914 Detector



Detector Type: Use **TCSPC Detector**.

Translator Type: The interface between the computer and the detector. Use **ASOC10**.

Translator Selection: Use **Primary**.

Housing Type: Use **914**.

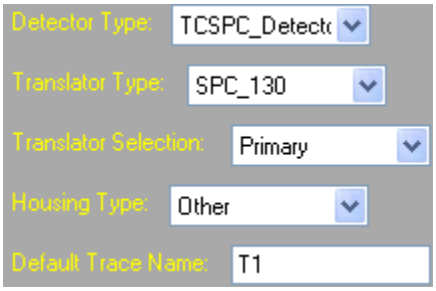
914 Control Port: Use the number of the 5 pin DIN connector on the side or back panel of the ASOC-10 used for power and control of the 914 housing.

914 Direct Output: Check this box.

914 Default gain: Enter 1200.

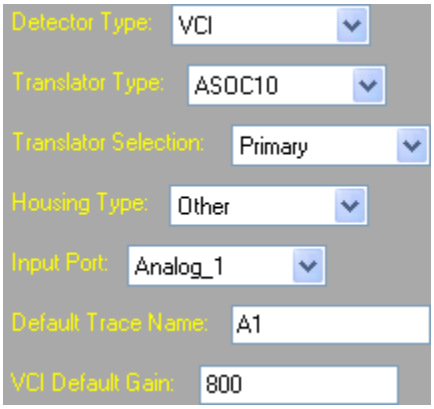
Default Trace Name: The name assigned to acquired traces for signal from this input.

TCSPC B&H Detector



Translator Type: The interface between the computer and the detector. Use **SPC_130**.
Housing Type: Use **Other** (B&H detector).

VCI Detector



Detector Type: Use **VCI**.

Translator Type: The interface between the computer and the instrument. Use **ASOC10**.

Translator Selection: Use **Primary**.

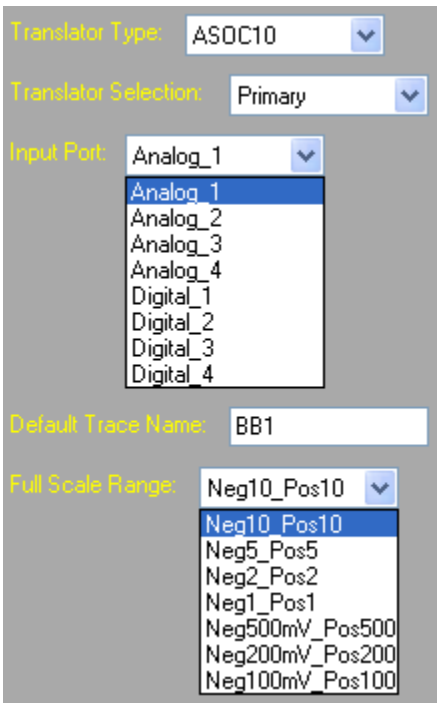
Housing Type: Use **Other**.

Input Port: Select the Analog connector on the ASOC-10 that the BNC cable from **a** on the electrometer is attached to.

Default Trace Name: The name assigned to acquired traces for signal from this input.

VCI Default Gain: Set the default gain for the VCI.

Black Box Inputs



Black Box Inputs allow additional analog or digital inputs from external devices, not under control by FelixGX.

Translator Type: The interface between the computer and the instrument. Use ASOC10.

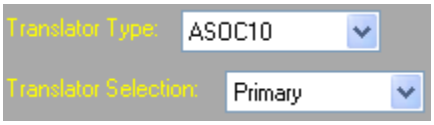
Translator Selection: Use Primary.

Input Port: Select the connector on the ASOC-10 that the signal cable is attached to. Use Analog 1 to 4 for analog signals and Digital 1 to 4 for digital signals. No pulse pile-up correction is applied to digital signals. **Note** – Input ports here cannot be set to the same ports used by detectors.

Default Trace Name: The name assigned to acquired traces for signal from this input.

Full Scale Range: Choose the full-scale range that analog data will be displayed in. 16-bit Analog to Digital conversion will be applied to the signal at each scale. Therefore more precision will be displayed for smaller full-scale ranges.

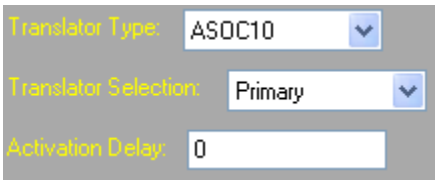
TTL In. In the hardware configurations, TTL In 1 and 2 and TTL OUT 1 and 2 are all disabled by default.



Translator Type: The interface between the computer and the instrument. Use ASOC10.

Translator Selection: Use Primary.

TTL Out



Translator Type: The interface between the computer and the instrument. Use ASOC10.

Translator Selection: Use Primary.

Activation Delay: Enter a value to create a delay (in seconds) after the time the TTL Out is initiated.

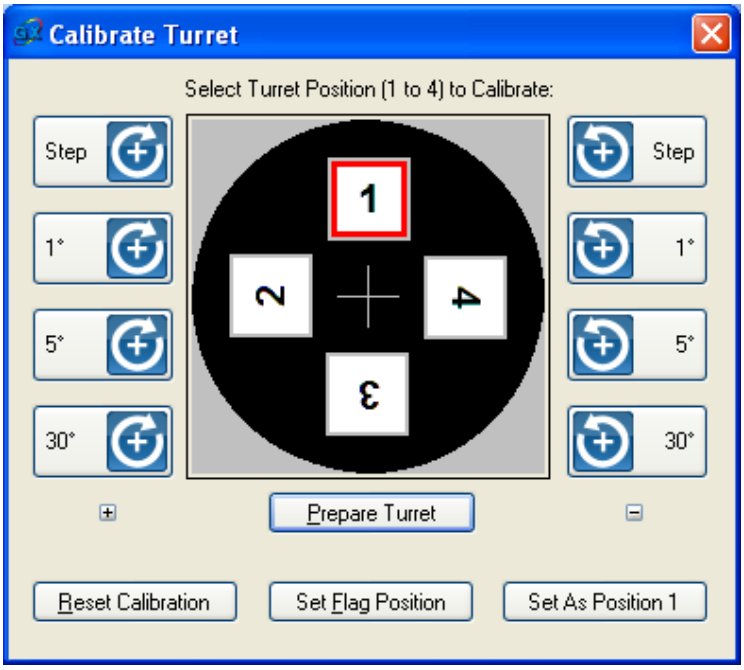
Calibration

Click on a button to show a dialog to calibrate a hardware accessory. These dialogs are displayed even if they are not enabled in the Hardware Configuration. These dialogs are also available in the **Action, Calibration, Accessories** window. For instruments that have been assembled and tested at the factory, or installed by a PTI Service technician on site, these calibrations have already been done. If you feel that calibrating an accessory needs to be done again, you should first contact PTI Service.

Monochromators

The monochromators with active text boxes depend on the current hardware configuration. For a particular monochromator, enter a value in the text box to the left of a **Go to** button, and then click the **Go to** button. Read the actual value displayed on the monochromator wavelength dial. Enter this value in the text box to the left of the **Set** button. Clicking the **Set** button will save this value to the hardware configuration.

Turret



When opened the Calibrate Turret dialog does not highlight any of the turret positions and the **Set Flag Position** and the **Set as Position 1** buttons are inactive. Click on the **Prepare Turret** button. This initializes the turret and moves it to any saved flag offset position, and selects position 1 as the current position and indicates this by outlining the button in red. Observe if position 1 is aligned parallel to the excitation axis of the sample compartment. E.g., place a cuvette in position 1, place a straight edge against the side of the cuvette, and check that the straight edge is parallel to a line of screw holes in the floor of the sample compartment. If the turret needs adjustment, click on the angle adjustment

buttons to rotate the turret by the displayed amounts until position 1 is aligned with the excitation axis. (800 steps = 90° so 1 step = 0.1125°.) Click on **Set Flag Position**. This sets an offset value for the calibration flag from its position found at turret initialization. Since this was determined using a cuvette in position 1, this step also defines the offset for Position 1 with respect to the flag position to be equal to 0.0°.

Click on **2** on the turret in the **Calibrate Turret** dialog. This will move the turret 90° clockwise from position 1's default offset of 0.0°. Put the cuvette in position 2 and check the alignment of position 2. If it is not aligned parallel to a line of screw holes in the floor of the sample compartment, click on the angle adjustment buttons until the side of the cuvette is aligned. Then click on **Set As Position 2** to save its offset value.

Repeat this for positions 3 and 4. The default offsets for these two positions are 180.0° and 270.0° from the flag position respectively.

Clicking **Reset Calibration** sets the flag offset and the position offsets all to 0.0°.

The **+** and **–** buttons below the angle adjustment buttons do nothing. They just declare clockwise rotation as positive and counterclockwise rotation as negative.

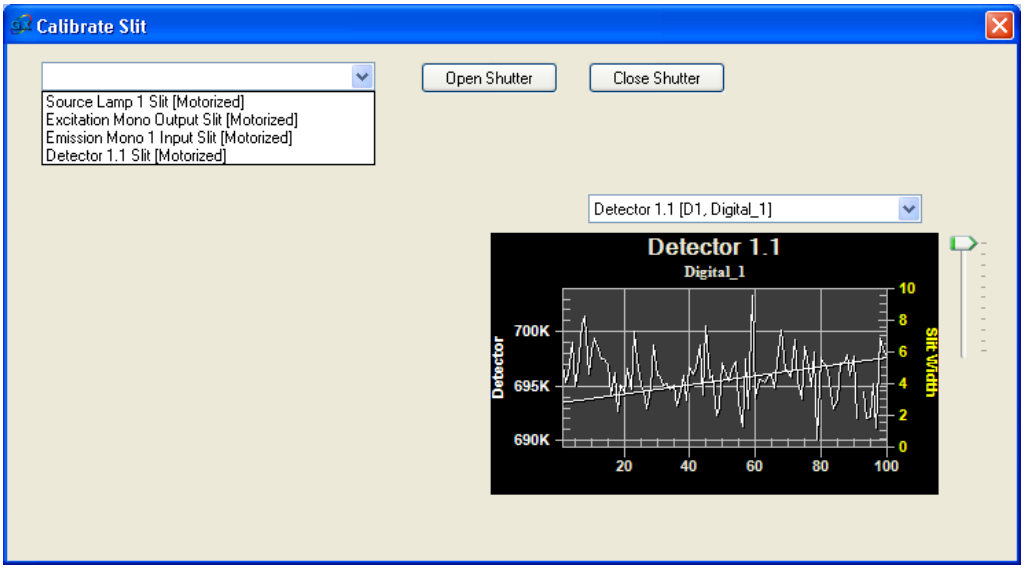
Click on the  button in the upper right corner to close the dialog. The offset values will be saved to the current hardware configuration.

Click **Configuration, Save** to overwrite the Hardware Configuration. If you want to save this configuration under a different name, use **Save As.**

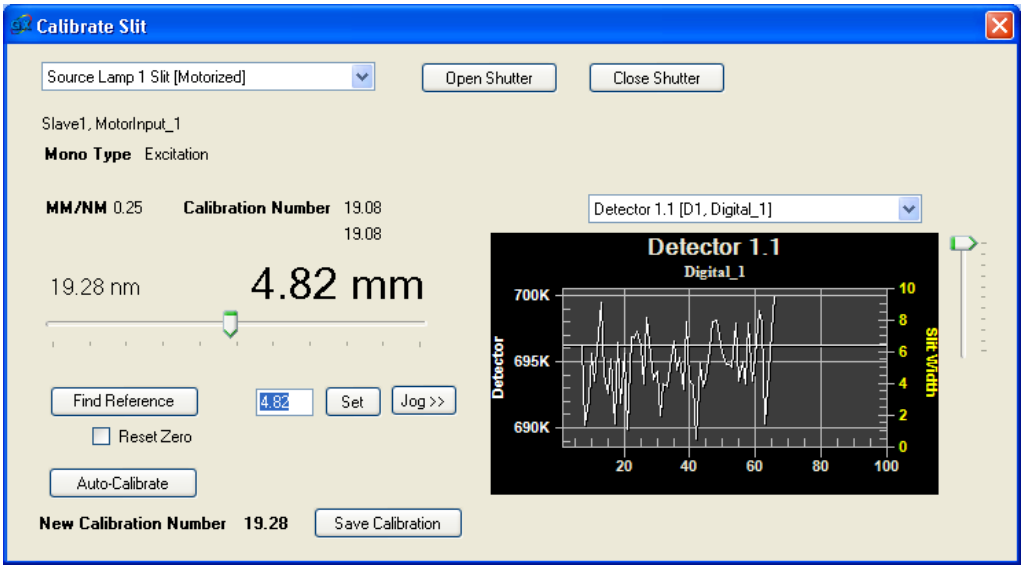
Slit

A motorized slit has a reference switch to mark where the slits are open to their maximum value. The calibration value is the bandwidth distance in nm between the fully closed position and the reference switch position and depends on the grating and single vs. double monochromator.

This dialog allows you to check and reset the calibration of the motorized slits. When first opened, the slit choice list is blank. Click on the down arrow to show a list of the motorized slits.



Select the slit, and then click on the **Auto-Calibrate** button. The slit will open and close twice and report the New Calibration Number in the dialog. Click **Save Calibration** to save the slit calibration. Repeat this process for each motorized slit you want to re-calibrate.



The horizontal slider, **Find Reference**, **Set**, and **Jog** buttons are manual tools to find the distance the slit jaws must travel between the reference position and fully closed slits by observing the detector signal graph. This procedure is only useful when:

- the lamp is on
- the shutter and all sliders are open
- all the other slits are open
- there is a sample in the sample compartment
- the monochromators are at suitable wavelengths for that sample
- there is nothing else blocking or minimizing the detector signal such as polarizers or other filters

Utilize the Auto-Calibrate button in the Hardware Configuration (HWC), Calibrate Slit dialog, and then click on the Save Calibration button to save that value to the HWC. Note that the Auto-Calibrate value in the Calibrate Slit dialog and HWC Motorized Slit parameter list is a bandwidth value (units of nm) and also depends on the grating and single vs. double mono.

Check the **Reset Zero** check box before clicking on the **Find Reference** button. Click on the **Find Reference** button to move the slit jaws to the reference switch position. Then, while watching the detector signal graph, move the slider to the right to make the slit narrower, or you can enter a slit width value in mm in the text box and click the **Set** button to move that distance in mm from the reference position toward the closed position. Keep moving the slider to the right, or clicking the **Jog** button to move it 0.01 mm per click until the signal stops decreasing. As you move toward the closed position, the bandwidth will be shown on the bottom as **New Calibration Number**. When satisfied with the closing of the slit, clicking the **Save Calibration** button will save this value to the Hardware Configuration. E.g., if the previous calibration value was correct, then converting the calibration value to mm and entering that value in the text box and clicking the **Set** button should close the slit.

If the **Reset Zero** check box is unchecked, then clicking the **Find Reference** button will move the slit jaws to the fully open position and show the difference from the last saved calibration value.

LaserStrobe PMT

Clicking this opens the **Calibrate PMT** dialog. The LaserStrobe PMT has been calibrated and the corresponding values entered into this Lookup Table (LUT) by PTI. If you feel the calibration needs to be done again, you should first contact PTI Service.

Control Panels

The Control Panels have text boxes and buttons to set and control data acquisition and hardware operation. The current hardware configuration determines which panels will be visible and where as the Default Control Panel Configuration.

Right-clicking on the double bar at the top of the control panel shows a list of all control panels and the option to show all or hide all control panels. Click in front of a control panel's name to toggle its visibility.

If all Control Panels have been hidden, then clicking on the hardware configuration on the Acquisition Bar will initialize the hardware and show the control panels relevant to that hardware configuration. Clicking **Configure, Control Panel…** shows the Control Panel Configuration dialog. Clicking on a saved Control Panel Configuration and then on the **Set** button will also make the control panels visible again.

The control panels are dockable. A group of control panels can be moved by clicking and dragging on the double bar at the top of the control panel. A single control panel can be moved by clicking and dragging on the tab at the bottom of that control panel. While dragging a single control panel or group of control panels, new location indicators appear on the screen giving options of the four edges of the FelixGX window, or an edge of the top or bottom half of the FelixGX window, or to the left or right or between other visible control panels. Dropping a control panel or group of control panels on an indicator icon places the control panel(s) there. Dropping on an indicator showing overlapping label tabs adds to that control panel group. Dropping away from an indicator leaves the control panel(s) floating. When several control panels are overlapped in a group click on a label tab below the group to show the desired control panel.

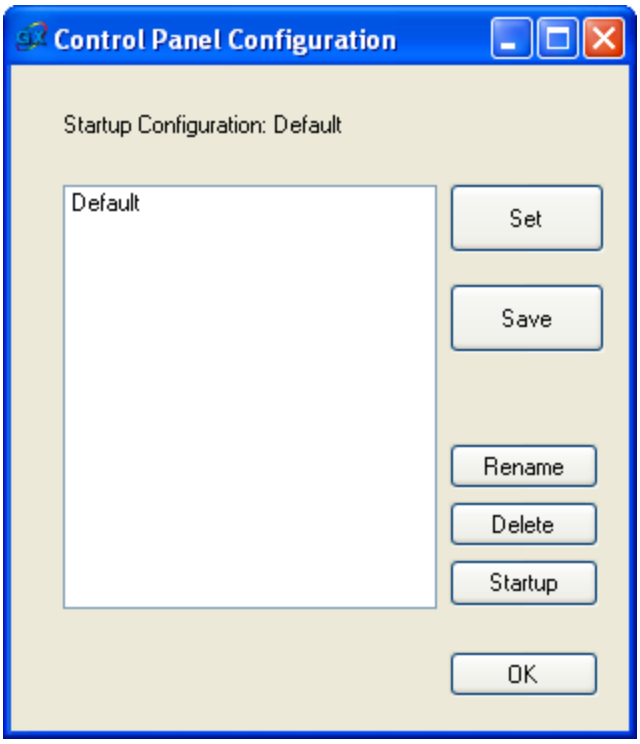
At the right end of the double bar on top of a visible control panel are three controls for changing a control panel's display: an arrow, a push-pin, or an X. If two or more control panels are in the same row or column, clicking a left-facing arrow, ◂, or an up-facing arrow, ▴, expands that control panel across the column. In such an expanded control panel, clicking a right-facing arrow, ▸, or a down-facing arrow, ▾, reduces the control panel to show other control panels or groups of control panels in the same row or column.

Clicking the vertical pushpin, 📌, will minimize the control panel to a label tab at one corner of the row or column. Clicking on this label tab expands the control panel to fill the row or column. Clicking on the horizontal pushpin, 📌, reduces the control panel to share the row or column.

Clicking on the × button will hide the control panel. The **Acquisition Control Panel** cannot be hidden by clicking on the × button.

The **Acquisition Control** panel is described in Chapter 12 Acquisition Control.

If you have changed the current Control Panel Configuration and want to save it, click on **Configure**, **Control Panel….** This opens the **Control Panel Configuration** dialog.



Click on a Control Panel Configuration from the list, then click on **Save** and enter a name for the new configuration.

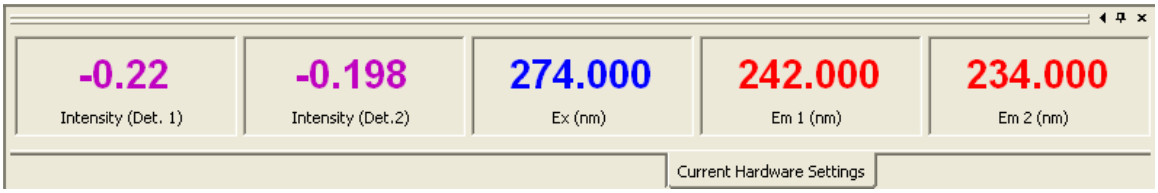
To make this or any saved configuration the configuration seen on launching FelixGX, click on the name in the list and click on **Startup**. This will be the Control panel Configuration seen the next time FelixGX is launched.

To change the current Control Panel Configuration, open the Control Panel Configuration dialog, click on the name in the list, click on **Startup**, click on the name in the list again, and click on **Set**. The control panels then change to the new configuration. **OK**.

Rename: To change the name of an existing Control Panel Configuration, select a name in the list, click **Rename**, enter a new name, **OK**.

Delete: To delete an existing control panel configuration from the list, select a name from the list and click **Delete**. This does not change the current configuration. However, if you deleted the name of the current Control Panel Configuration, then the Default configuration will be used when FelixGX is next launched.

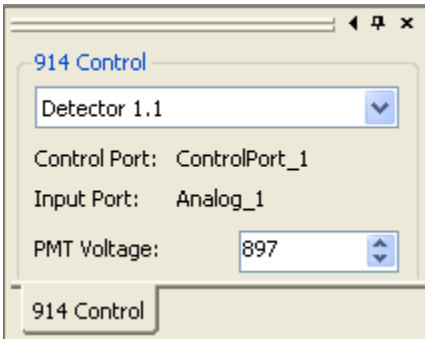
Current Hardware Settings



The Current Hardware Settings panel only gives information about the detector's signal intensity and the monochromator positions. The picture above shows signal intensities from T-format analog detectors.

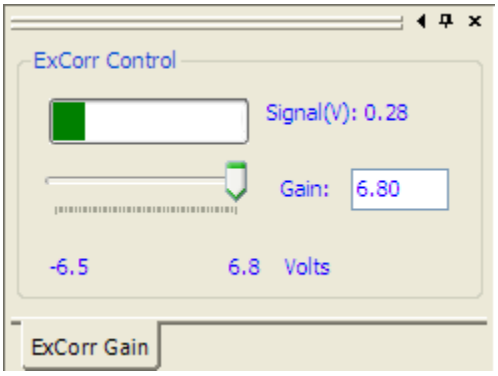
All the other control panels are used to change particular acquisition parameters for the current acquisition setup. These changes are not permanently saved. As soon as a different acquisition setup is selected from the Acquisition Bar or by using the menu command **Acquisition, Open** from within the acquisition setup dialog, any changes made to previous acquisition setup and not saved are lost.

914 Control



This control panel is only shown for systems that use a 914 detector in analog mode.
Detector #: If the system has more than one 914 housing, select the detector number from the choice list. The number refers to the detector position shown on the hardware configuration drawing. The Control Port and Input Port will automatically change with the detector number.
PMT Voltage: Change the voltage applied to the PMT.

ExCorr Gain



This control panel only affects the Current Hardware Settings and running acquisitions. It does not change the ExCorr **Gain** for the next time the current or other acquisition is started.

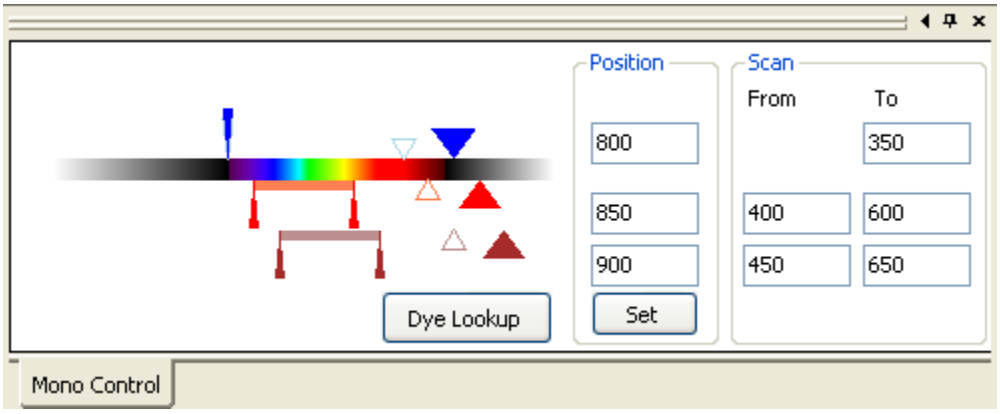
To adjust the **Gain** for a phosphorescence (QM-30) system, start a Phosphorescence Timebased scan with the excitation monochromator at the wavelength of interest or for

maximum excitation correction signal if that wavelength is already known. While the scan is running, click and drag the **Gain** slider (or click on the **Gain** slider and press the left or right arrow key) until a satisfactory signal is observed. When clicking and dragging the **Gain** slider, the signal is updated only when the mouse button is released.

Note the **Gain** value. You can open the current or other acquisition setup and set this Gain value.

You can also use this procedure to adjust the ExCorr **Gain** for steady state acquisitions (you may also have to use the Shutter control panel to open the shutter), but that is better done on the Real-time Corrections tab.

Mono Control



This picture shows a Monochromator Control Panel for an emission scan on a T-format system (a system with two emission monochromators). Excitation wavelengths are on top of the spectrum bar and emission wavelengths are on the bottom of this bar.

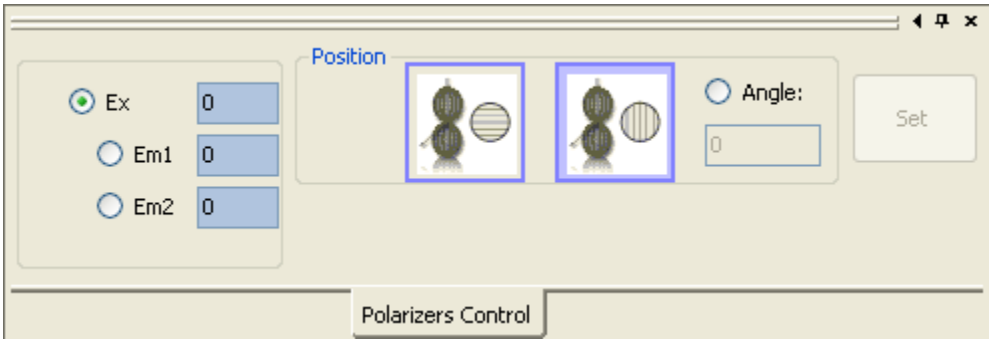
The vertical bars show the wavelengths set by the acquisition setup. During a wavelength scan the filled triangles move with the current positions of the monochromators.

You can move the monochromators by clicking and dragging the filled triangles to new positions (clicking and dragging only changes the values in increments of 4 nm). The current positions will be shown as triangle outlines. Or, you can enter values into the **Position** text boxes. Clicking the **Set** button will move the monochromators to the new positions.

You can change the acquisition settings by clicking and dragging the vertical bars or entering values in the **Scan** text boxes and clicking the **Set** button.

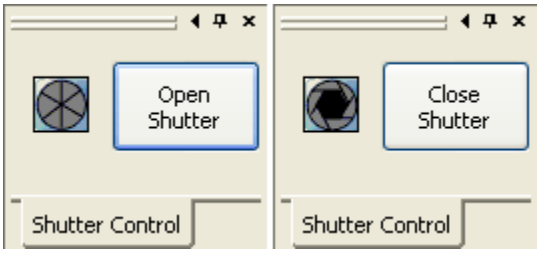
Clicking on the **Dye Lookup** button shows a library of dyes and their excitation and emission wavelengths. Selecting a dye from this list enters those values into the excitation and emission text boxes. The emission value will be entered into both emission text boxes for a T-format system. Only one dye can be entered. A multi-dye setup with more than one dye is not supported by the Mono Control Panel.

Polarizer Control



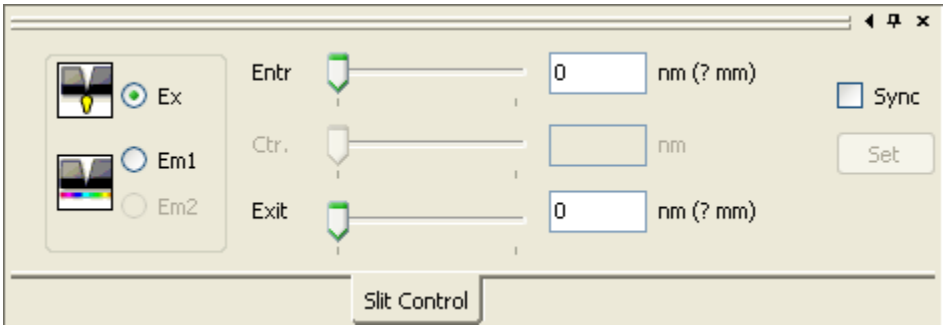
This control panel is only useful if there are motorized polarizers in the current hardware configuration. Click on the radio button for a specific polarizer, click on the horizontal or vertical polarization icon or click on the Angle radio button and enter an angle, and then click on the **Set** button to rotate the polarizer to the desired angle. For an L-format system, Em2 is inactive (dimmed).

Shutter Control



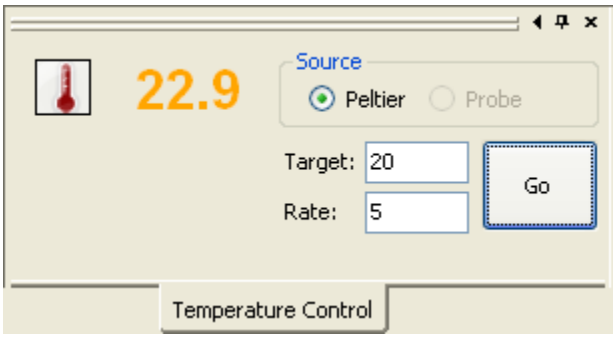
The icon shows the current state of the shutter. Click on the **Open Shutter** or **Close Shutter** button to open or close the shutter.

Slit Control



This control panel is only shown if there are motorized slits. Click on the radio button for a specific slit, move the slider or enter a bandpass value (nm) into the text box, and then click on the **Set** button to adjust the slit. If the **Sync** box is checked, then both slits of a monochromator (or all three slits if a double monochromator has a center motorized slit) shown will go to the same slit width.

Temperature Control



This control panel is only shown if there is a Peltier cuvette holder. The number to the right of the thermometer icon shows the current temperature in degrees Celsius. If the number is orange then the temperature is outside the current target range. When the temperature is within the target range or under control during a temperature ramp, then the number is shown in blue. The source can be either Peltier or Probe, if both are active. Enter numbers into the **Target** and **Rate** text boxes and click on the **Go** button to send the temperature to a desired temperature.

TCSPC Control

SYNC bar

The SYNC bar graph should show a value equal approximately to the LED repetition rate. Note that this is a logarithmic scale. If the SYNC bar graph on the TCSPC Control Panel shows a value significantly larger than the LED repetition rate, see the procedure under TCSPC - Acquisition Setting Parameters – SYNC Threshold.

ADC / TAC / CFD bar

The bar graph under the SYNC bar graph shows values equal to the pulse rate at the output of the Analog-to-Digital Converter (ADC), Time-to-Amplitude Converter (TAC), or Constant Fraction Discriminator (CFD), depending which radio button below the bar graphs is selected. The ADC rate represents the rate of pulses received from all channels used in the graph display and is the rate to use to check if the data rate is at a proper level.

Once the TCSPC Acquisition Settings parameters have been set, insert filters or adjust the slits so that the ADC rate is about 1 – 5 % of the SYNC rate (= lamp trigger rate). 5 % is OK for rough operation, 1 - 3 % is better for more precise operation in accord with Poisson statistics (i.e., better for avoiding pulse-pile up). Test for a light leak: turning the room lights OFF/ON will change the ADC rate if there is a light leak.

Photon Rate Type

Click on a radio button to change the name and counts displayed on the bar below the SYNC bar graph.

User settable parameters

You can use the two choice lists to select TAC (Range, Limit Low, Limit High, and Gain), SYNC (Frequency Divider, ZC Level, and Threshold), and CFD (ZC Level and Limit Low) parameters. Select or enter a value in the text box below, then click the Set button to set the new parameter value. These parameters are also settable in the Acquisition Setup and are described there.

Displayed values

The values of various parameters are displayed on the right side of the TCSPC Control Panel. TAC parameters are shown in violet, CFD parameters in blue, and SYNC parameters in red. Channel Count, Collect Time, and Overflow Count are set in the Acquisition Setup.

Channel Count

The number of time channels (data points) for a trace.

Collect Time

When the Stop Method is set to Time, the Duration value is shown here.

Overflow Count

When the Stop Method is set to Peak Channel Count, the Peak value is shown here.

Delay

The displayed Delay is that entered on the Acquisition Settings tab if ‘Use Delay’ is checked, or an automatically calculated value dependent on the TAC Range.

Channel Width

The time-width per channel.
Channel Width = Range (exact value – see below)/(Channel Count – 1)

Range

The exact value of the range.

Chapter 11

Help Commands

Help Topics

Opens the FelixGX Help utility.

Shortcut: Press the F1 key.

The Table of Contents is in the left panel and a menu containing a general overview of FelixGX opens in the right panel. Press the plus sign in front of a book label or double-click on the book label to show the related topics. Press the minus sign in front of a book label or double-click on the book label to condense the list. Selecting any of the labels from the Table of Contents will make that topic appear in the right panel.

You can also use the Search function, which is listed as a tab above the Table of Contents. Enter a keyword into the text box. Entering more than one keyword will only search on the first keyword. Click on the **List Topics** button to show a list of topics containing the keyword. Either double-click on a topic name or click on the topic name and then on the Display button to show that topic.

About FelixGX

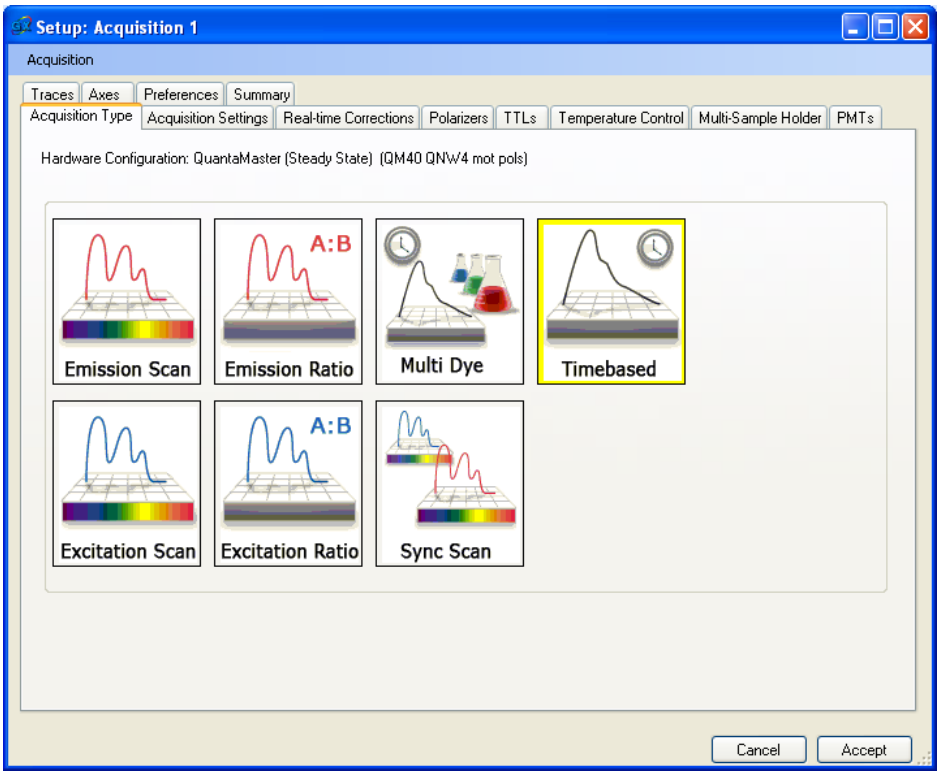
Use this command to display the copyright notice and version number of your copy of FelixGX.

Acquisition Setup

On the Acquisition Control Panel clicking on the **Setup** button shows the Setup window with the Acquisition Type tab. Click on the **Start** button to start acquiring data. The **Setup** and **Start** buttons will dim and the **Stop** and **Pause** buttons become active. Click on the **Stop** button to stop acquiring data. The **Stop** button will dim and the **Start** button will become active. During acquisition, clicking on the **Pause** button will pause acquisition, and the **Start** button will change to **Continue**. Click on the **Continue** button to continue the current acquisition.

Acquisition Types – Steady State, RatioMaster, or Photometry

QuantaMaster (Steady State), RatioMaster, or Photometry hardware configurations are based on a continuous light source, or no light source in the case of bio- or chemi-luminescence. These hardware configurations allow the different acquisition types shown in the picture below. The icons and tabs shown will vary with the hardware configuration and accessories.



You must select an acquisition type to see the tab list. Click on an acquisition type and then click on the other tabs in turn to set the various acquisition parameters.

Acquisition: Shows the command list: **New, Open, Close, Save As….**

New: Creates a new acquisition setup. The acquisition type must be selected before saving or accepting the setup.

Open: Opens a saved acquisition for editing.

Close: Does nothing.

Save As…: Saves the current acquisition setup to the acquisition repository (acq.rep). Enter a name for the acquisition. Check the **Display** box if you want the acquisition to show on the Acquisition Bar. Click on **OK** to save the acquisition. When a new session is started, it is automatically named with the acquisition name plus the time stamp YYYY-MM-DD HH:MM:SS AM/PM.

Emission Scan

In an Emission Scan, the emission wavelength is scanned between two wavelengths while the excitation monochromator is fixed. The emission intensity is measured as a function of excitation wavelength. Due to the nature of fluorescence, the excitation wavelength should be set at a shorter wavelength than the emission wavelength range. Scanning the emission across the excitation wavelength or vice versa can result in excessively high signal intensity due to reflected light.

Excitation Scan

In an Excitation Scan, the excitation monochromator (or DeltaRAM) is scanned between two wavelengths while the emission monochromator is fixed. The emission intensity is

measured as a function of excitation wavelength. Due to the nature of fluorescence, the emission wavelength should be set at a wavelength that is longer than the excitation wavelength range (red-shifted).

Emission Ratio

Emission Ratio is used to set up and run experiments for intracellular ion determinations using emission-shifted probes such as Indo-1 for calcium and SNAFL for pH. In this experiment, a constant excitation wavelength is used and two emission wavelengths must be selected. This is normally done with two monochromators in a cuvette system, but one monochromator can be utilized. In a microscope-based system, the two emission wavelengths are selected using a dichroic assembly in the photometer. The emission intensity at both emission wavelengths is measured and the ratio of these intensities is calculated. The ratio can be converted to the concentration of the ion being determined.

Excitation Ratio

Excitation Ratio is used to set up and run experiments for intracellular ion determinations using excitation-shifted probes such as Fura-2 for calcium and BCECF for pH. In this experiment, the excitation source must alternate between two different excitation wavelengths that are characteristic of the probe. The emission intensity at both excitation wavelengths is measured at a longer emission wavelength and the ratio of these intensities is calculated. The ratio can be converted to the concentration of the ion being determined.

Multi-Dye

The Multiple Dyes function is used to set up and run experiments for intracellular ion determinations using several indicators in combination, such as Fura-2 for calcium and BCECF for pH.

Synchronous Scan

In a Synchronous Scan, the excitation and emission monochromators are scanned simultaneously at identical scan rates with a constant wavelength difference between them. A synchronous scan often results in the simplification of complex excitation or emission scans.

Timebased

In a Timebased experiment, the excitation and emission wavelengths remain fixed throughout the experiment. The emission intensity is measured as a function of time. Timebased experiments typically involve kinetic measurements.

Common Acquisition Setting Parameters (General)

Some of the following acquisition parameters appear in most acquisition types, for both steady state and pulsed lamp sources. **Note**: The contents of any given control panel or tab may vary depending on the particular hardware configuration of your instrument.

Wavelength scans

Enter the beginning and end wavelengths in the text boxes. The value of the length is automatically calculated and entered in the Length text box. If the length is changed the corresponding end wavelength is automatically calculated and entered into its text box.

If your instrument has two emission monochromators, emission scans and synchronous scans will show two emission wavelength ranges of the same length. Excitation and timebased scans will show two fixed emission wavelengths.

Fixed wavelengths

A fixed wavelength will have one text box. If your system has two emission detectors, two emission wavelength text boxes will be shown.

Timebased scans

The excitation and emission wavelengths are fixed. Enter the wavelengths in the appropriate text boxes.

Ratio scans

Excitation Ratio scan setups will show two text boxes separated by a forward slash. The excitation wavelength device (standard monochromator or DeltaRAM) will move back and forth between the two excitation wavelengths.
With a single emission monochromator and detector, Emission Ratio scan setups will show two text boxes separated by a forward slash. The emission monochromator will move back and forth between the two emission wavelengths. With two emission detectors, an Emission Ratio scan setup will show one text box for each emission detector. The ratio will be calculated as the ratio of intensities (detector 1 / detector 2) during acquisition. This is the same as a Timebased acquisition.

Slit Widths

Enter the monochromator entrance and exit bandwidths in nm into the text boxes. These values are for information only for manual slits, but control the opening of motorized slits. For motorized slits, the slit widths can also be adjusted using the Slit Control panel. Motorized slits have a backlash. When changing motorized slits, FelixGX will always change from a wider width to a narrower width. If going from a narrow width to a wider width, FelixGX will first go to the maximum width and then go the desired width.

Background Acquire & Use (QuantaMaster Steady State or RatioMaster)

You may want to acquire and use background values to subtract PMT dark count or solvent blank intensities from the traces. A separate background value will be acquired for each detector in the system.

> **Duration**: Click the radio button and enter a value or use the up/down arrows to change the value. Any decimal values will be rounded to the nearest integer. If you do not click the Duration radio button, then clicking **Acquire**, will show a time

counter and the Acquire button will change to a Stop button. Click Stop to stop acquiring the background. The average of the acquired data for each detector will be shown on the traces tab.

Acquire: Click on this button to acquire background values. For wavelength scans, these values will be acquired at the start wavelengths. If the hardware configuration does not have a shutter, then a prompt to close the excitation slider will be shown before acquiring the background and another prompt to open the slider will be shown after the background has been acquired. If the hardware configuration has a shutter, these prompts will not be shown.

Use: After the background is acquired, this check box will be checked by default. The values will then be shown as the background values in the traces tab and trace properties.

Clear: Click on this button to clear the background values from memory and from the Traces tab.

The background values will remain in effect for subsequent measurements until cleared in the Traces tab, or by removing the check mark in the **Background Use** checkbox located beside the Background Acquire button, or by clicking the **Background Acquire** button, which will clear the previous value and force a new background to be acquired. Toggling the Use box keeps the background value in memory for future use. Care must be taken when using scanning dialogs since the background may change as a function of wavelength. Thus for these types of acquisitions a separate scan may be required of a blank sample that will be manually subtracted from the experimental sample to produce an accurate background measurement.

Acq

Shows the data and time that the background was acquired as MM/DD/YYYY HH:MM:SS AM/PM.

Use Maximum Sampling Rate

This box should be checked when using a PMT detector in analog mode.

Points/sec (pps)

Enter the number of data points/second to be displayed. The maximum rate is 1,000,000 points per second. The more data points that are collected per second, the greater the peak-to-peak noise associated with the signal. Conversely, the fewer data points that are collected per second, the better the signal-to-noise ratios obtained.

If a DeltaRAM is used as the excitation device, Excitation Ratio and Multi-Dye setups use Points/sec. If a standard monochromator is used as the excitation device, then these setups use Integration as the timing parameter.

There is no practical limit in FelixGX to the total number of points that can be taken during an experiment. The real limit is the available space in your RAM. Avoid taking an excessive number of data points though, because the processing and analysis time takes longer. Currently anything over 500 pps will cause the “strip chart” mode. When in this “high speed” mode, the view window is temporarily set to 10 seconds and as you are acquiring, only the last 10 seconds collected will be shown.

Integration

The time during which the signal is integrated for each data point. Since FelixGX signals are normalized as counts per second for digital signals, or volts for analog signals, changing the integration time does not change the signal amplitude, but does change the noise in the signal. A longer integration time will reduce the noise but extend the time of the measurement.

Duration

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.
Note: The Duration value is not used for Temperature Ramp acquisitions, as these will take as long as required to reach the end temperature.

Repeats

Enter the number of times the experiment will be repeated. Maximum # Repeats = 1000.

Step Size

For wavelength scan experiments, the step size value controls the scan rate. A smaller step size increases the resolution of the measurement, but also increases the time of the experiment. Allowable values depend on the monochromator used.

Pause

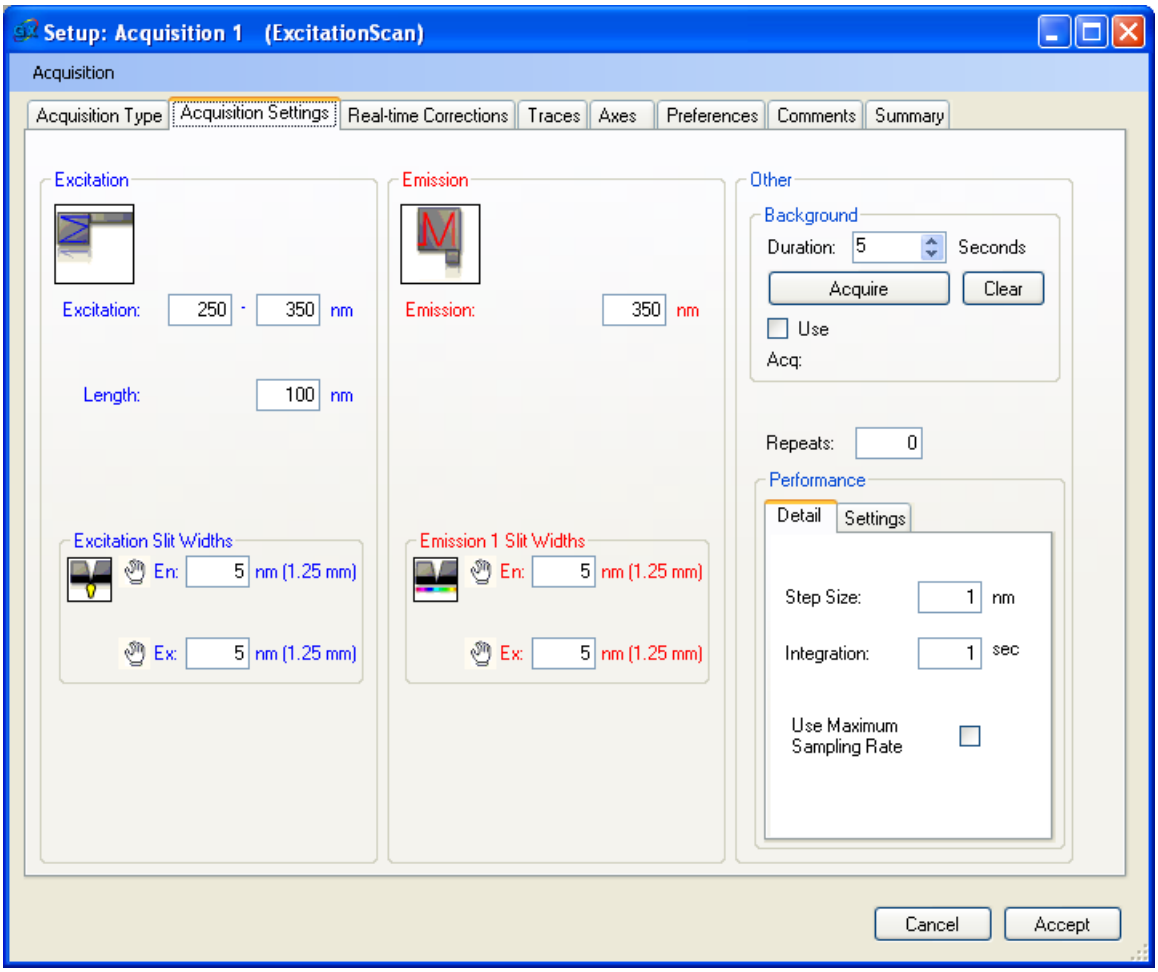
Enter the time to pause between repeated experiments. This is not available for wavelength scanning or temperature ramping acquisitions.

View Window

Enter the time segment that will be displayed on the X-axis. If the scan is longer than View Window, then the X-axis (time) will be scrolled as data is acquired. If the Repeats are set to a value greater than zero, and the Acquisition Preference "Include all repeats in one trace" is ON, then the default View Window is equal to the sum of all repeated durations, plus Pause times.
If a motorized polarizer or four position turret with multiple sample position is used, the polarizer or turret slewing time is not factored into the default View Window time.
During Timebased acquisitions, anything over 500 points per second will cause the "strip chart" mode. When in this "high speed" mode, the view window is temporarily set to 10 seconds and as you are acquiring, only the last 10 seconds collected will be shown.

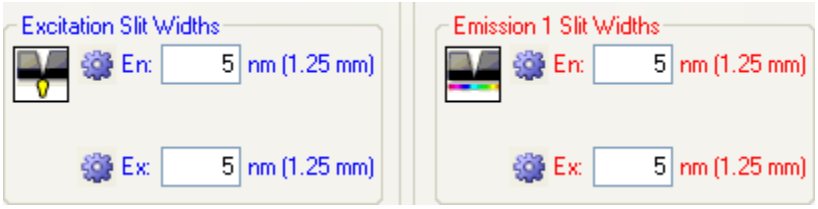
Acquisition Settings Examples (Steady State and RatioMaster)

QuantaMaster Excitation Scan

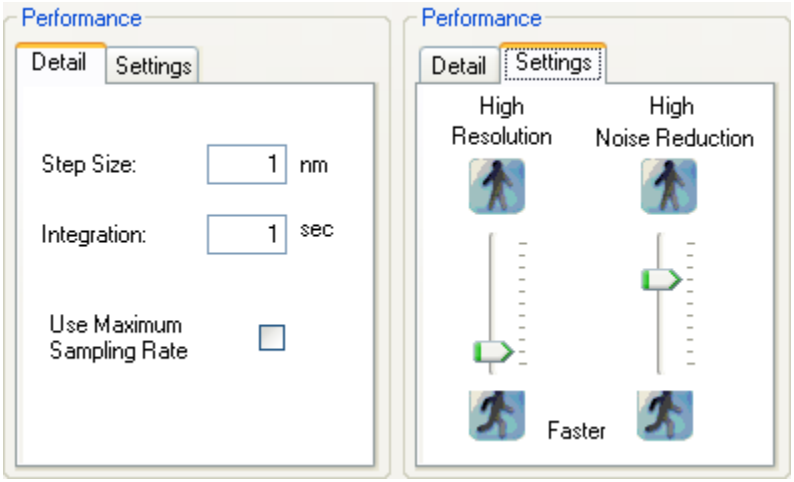


The above picture shows the acquisition settings for an excitation scan on a QuantaMaster system with an excitation monochromator, one emission monochromator, and manual slits.

If the system has motorized instead of manual slits, the slit icons will be shown as:

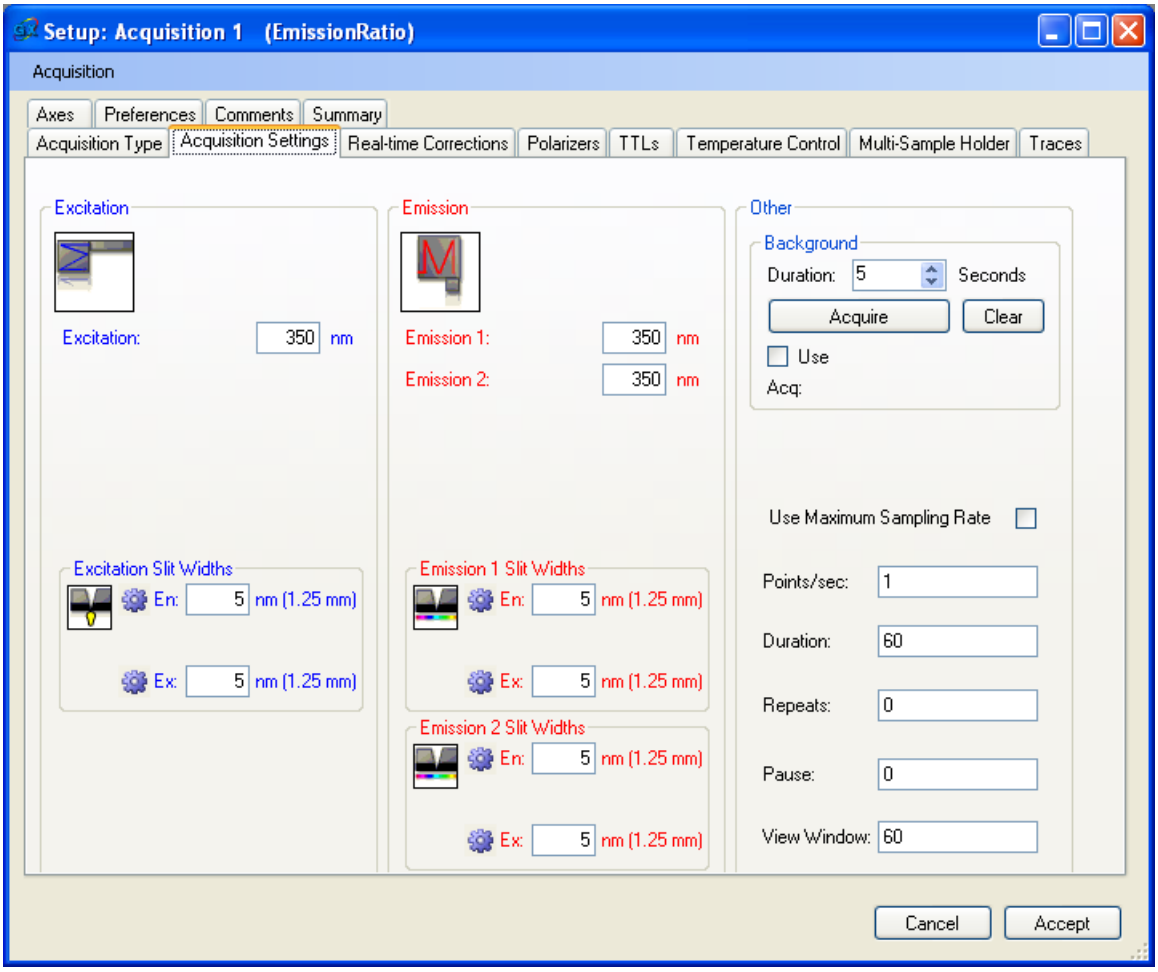


The Performance can either be set by the sliders or by text boxes.



Finer granularity (i.e., step size) will extend the time of the measurement. A longer integration time will reduce the noise but extend the time of the measurement.

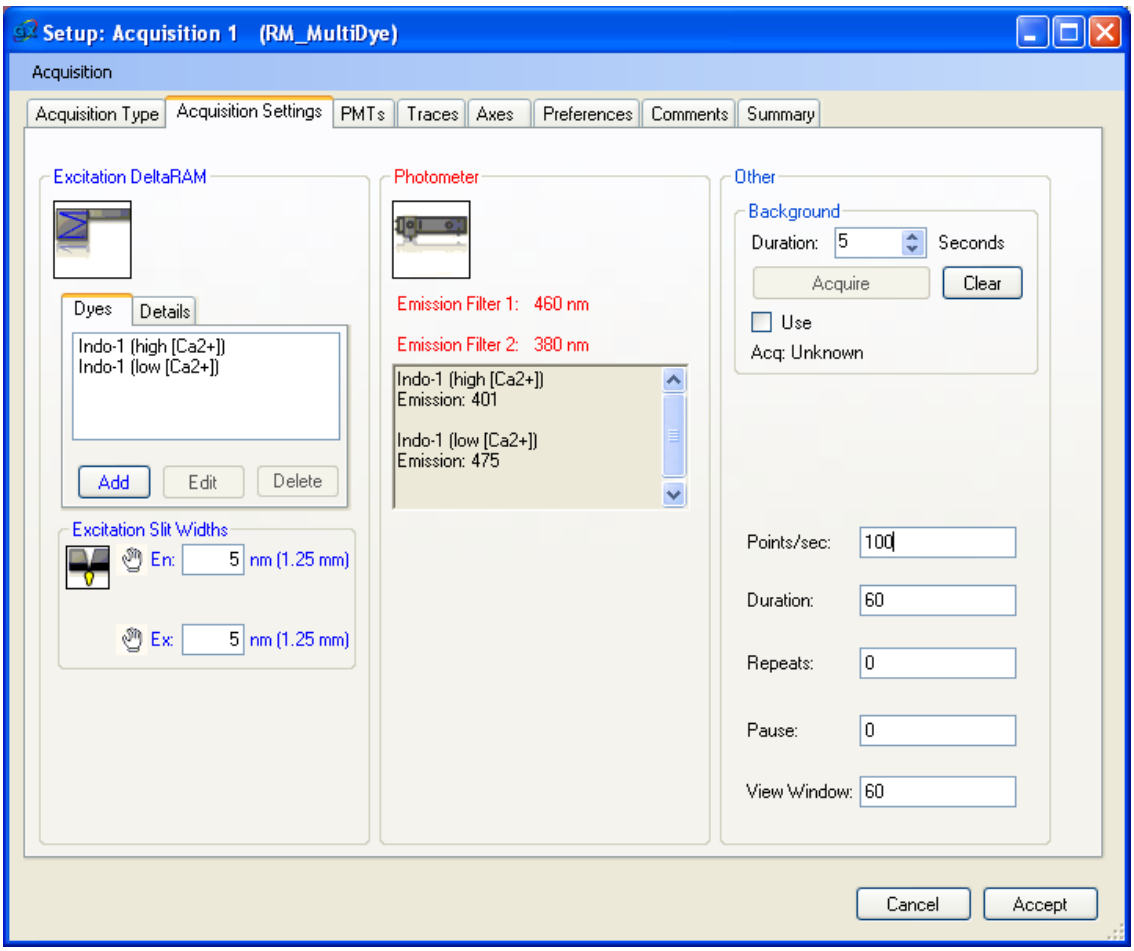
QuantaMaster Emission Ratio



The above picture shows the acquisition settings for an emission ratio scan on a QuantaMaster system with two emission monochromators and motorized slits.

In a T-format system the emission monochromator does not have to move during emission ratio operation and Points/sec is shown. This is identical to a T-format Timebased setup. In an L-format system the emission monochromator must move during emission ratio operation and Integration Time (in seconds) is shown.

DeltaRAM RatioMaster Multi-Dye



The above picture shows the acquisition settings for a MultiDye acquisition on a RatioMaster system with a DeltaRAM and a dual photometer. The emission filter wavelengths are set in the hardware configuration.

Dyes: Shows the names of the selected dyes.

Details: Shows the name and peak excitation wavelength for the selected dyes.

Add: Clicking on this button shows a list of dyes. Click on a dye name and click OK, or double-click on a dye name to add the dye name and peak excitation and emission wavelengths to the Acquisition dialog. Pressing a letter key will jump to the place in the list following that letter. The list can also be scrolled. Only one dye can added at a time.

Edit: Clicking on a dye name in the excitation Dyes list activates the Edit and Delete buttons. Clicking on Edit shows a dialog where you can change the dye name and the excitation and emission wavelengths in the Acquisition dialog.

Delete: When this button is active, clicking on it will delete the selected dye from the Acquisition dialog.

Acquisition Types – Pulsed Light Sources

In pulsed light source systems, the light source is repeatedly pulsed, with pulse widths of sub-nanosecond with LaserStrobe systems, nanoseconds with LED Strobe systems, and microseconds with Phosphorescence single shot or VCI systems. The pulse frequency also depends on the lamp source. The detector is gated so that data is acquired in a specific time window delayed relative to the onset of the excitation pulse. The delay of this window can be precisely advanced in time to build up a decay curve. Alternatively, the delay can be fixed so that the detector intensity is measured against other parameters such as excitation or emission wavelength, or time.

TCSPC setups are described in a separate section.

Decay

In a decay experiment, the excitation and emission wavelengths remain fixed throughout the experiment while the delay is scanned in time. Decay experiments are used to measure the lifetimes of samples.

Timebased

In timebased mode, the excitation wavelength, the emission wavelength and the delay window remain fixed throughout the experiment. The detector intensity is measured as a function of time. Timebased experiments ususally involve kinetic measurements but are also useful in maximizing intensity when adjustments are being made to the instrument.

Time Resolved Excitation Scan

In a time resolved excitation scan, the delay window and the emission wavelength remain fixed throughout the experiment while the excitation wavelength is scanned. Time resolved excitation spectra are used to investigate the spectral properties of various decay mechanisms in samples with complex decays.

Because the LEDs operate at fixed wavelengths, and changing the dye laser wavelength cannot be done by FelixGX, Time Resolved Excitation Scan is not possible for TimeMaster (Fluorescence Lifetimes) and TimeMaster (LaserStrobe) systems.

Time Resolved Emission Scan

In a time resolved emission scan, the delay window and excitation wavelength remain fixed throughout the experiment while the emission wavelength is scanned. Time resolved emission spectra are used to investigate the spectral properties of various decay mechanisms in samples with complex decays.

Common Acquisition Setting Parameters (Pulsed light sources)

In addition to the acquisition parameters seen for setups based on QuantaMaster (Steady State) hardware configurations, the following acquisition parameters are different from the general setting parameters or are used systems with for pulsed light sources.

Lamp Frequency

QuantaMaster (Phosphorescence) or QuantaMaster (VCI) systems using **Pulsed Xenon** as the **Source Lamp Type**. The frequency at which the xenon lamp is pulsed. The allowable range is from 1 Hz to 1/(End Time or Window End Time) up to a maximum of 300 Hz. For very long-lived samples, the phosphorescence signal from one pulse may not have completely decayed before the next pulse arrives. At least ten sample lifetimes should be allowed between each lamp pulse.

(Lamp) Frequency

A nitrogen and dye laser can be used as the light source in a TimeMaster(LaserStrobe) system (use **Nitrogen Laser** as the **Source Lamp Type** in the hardware configuration) , as well as a QuantaMaster (Phosphorescence) or QuantaMaster (VCI) system (use **PulsedXenon** as the **Source Lamp Type** in the hardware configuration). Shown as **Lamp Frequency** for QuantaMaster (Phosphorescence) and, QuantaMaster (VCI) setups, or **Frequency** for TimeMaster (LaserStrobe) setups. This parameter determines the frequency of laser firing and may be set up to 20 Hz for a nitrogen laser. Higher frequencies shorten the time required to acquire data and can improve time resolution. However, the consumption of nitrogen gas increases substantially at higher frequencies and the energy per pulse drops. Ten pulses per second is a reasonable choice for most experiments. When used with a QuantaMaster (Phosphorescence) or QuantaMaster (VCI) system, the TCM-1000 controller is not used.

Shots

QuantaMaster (Phosphorescence), QuantaMaster (VCI), or TimeMaster (LaserStrobe) systems. Enter the number of light source pulses to be collected and averaged together to produce displayed data. The user will not have access to individual shot data after the data has been acquired. A user can, however, get data for a single shot by setting shots to 1. Extra shots will improve the signal to noise ratio at the expense of additional acquisition time.

Delay

QuantaMaster (VCI), TimeMaster (Fluorescence Lifetimes), or TimeMaster (LaserStrobe) systems. Enter the delay at which the detection window will be opened. For QuantaMaster (VCI) systems this is in microseconds. For TimeMaster (Fluorescence Lifetimes) or TimeMaster (LaserStrobe) systems this is in nanoseconds. This parameter is used for Timebased, Time Resolved Excitation, or Time Resolved Emission Scans. If there are two detectors in the system, then these parameters will be labeled Delay 1 and Delay 2. At this time, only one QuantaMaster (VCI), TimeMaster (Fluorescence Lifetimes), or TimeMaster (LaserStrobe) detector is supported by FelixGX. VCI systems may be used to acquire fluorescence and phosphorescence spectra by choosing the

appropriate delay and integration time. See the discussion under Integration Time for details.

Start/End Delay

QuantaMaster (VCI), TimeMaster (Fluorescence Lifetimes), or TimeMaster (LaserStrobe) systems. Enter the delays at which data collection will start and end. For TimeMaster (Fluorescence Lifetimes), or TimeMaster (LaserStrobe) systems the delays are measured in ns. The excitation pulse is typically situated 50 to 100 ns for fluorescence after the beginning (0) of the delay allowing data to be collected before the excitation pulse to establish a baseline. The exact position of the pulse must be found from a scattering experiment. The start delay is normally chosen a little before the excitation and the end delay is some 5 to 10 lifetimes after the excitation.

For QuantaMaster (VCI) systems the delays are measured in μs. The excitation pulse is typically situated 100 μs after the beginning (0) of the delay allowing data to be collected before the excitation pulse to establish a baseline.

Channels

QuantaMaster (VCI), TimeMaster (Fluorescence Lifetimes), or TimeMaster (LaserStrobe) systems. For Decay scans only. Enter the number of data points to be collected for each scan. Although the limit imposed by the software is very large, the time taken to collect and analyze decays may become excessive for large numbers of points. A maximum of 1000 points is reasonable.

Collect Mode

A choice list allows 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

A choice list allows 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.

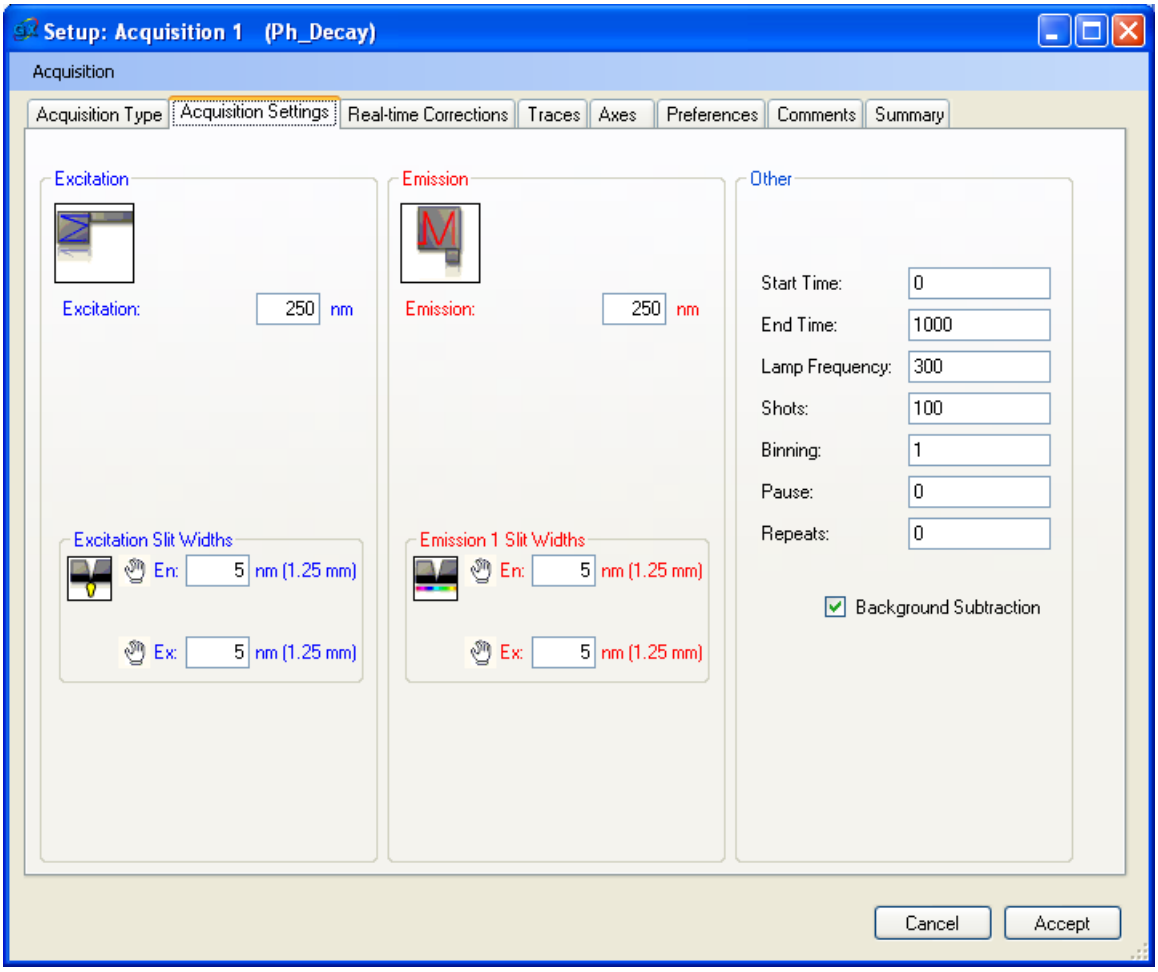
Logarithmic: Multiplies the previous time step by a constant factor to obtain the next time step. With this option, time between data points increases even faster than it does with the Arithmetic option.

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. With both Arithmetic and Logarithmic it is advisable to set the Start Delay very close to the onset of the IRF to maximize the resolution of the IRF.

Acquisition Types – QuantaMaster (Phosphorescence)

Phosphorescence Decay



The above picture shows the acquisition settings for a phosphorescence decay on a QuantaMaster (Phosphorescence Lifetime) system with an excitation monochromator, one emission monochromator, and manual slits.

The Phosphorescence Decay acquisition will measure and display a representation of the sample's response to a single pulse of light. This single decay curve may, however, be calculated by observing multiple shots and averaging the results. Each shot starts after a period of 100 µs, during which time the lamp is off and the background is observed.

Start Time

When to begin acquiring data to be shown in the graph area. This is measured in µs using the start of the background acquisition as 0. The allowed value is an integer between 0 and 10,000. A value below 100 will show part of the background acquisition. The default value is 0.

End Time

When to stop acquiring data to be shown in the graph area, also measured in µs using the start of the background acquisition as 0. The allowed value is an integer between 0 and 1,000,000. The default value is 1,000.

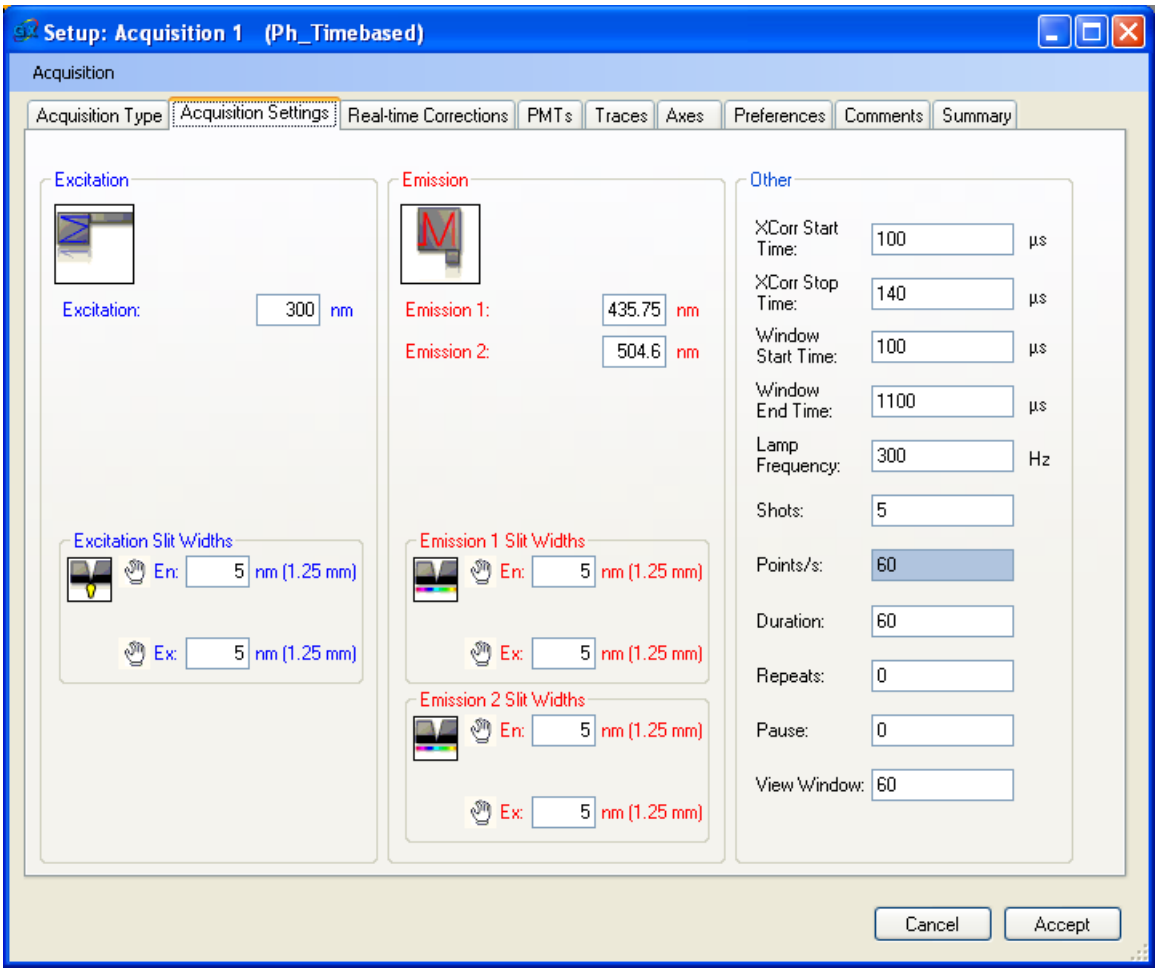
Binning

By default, phosphorescence data is acquired and displayed at 1,000,000 data points per second. Binning reduces the number of data points by summing groups of adjacent data points and displaying only the binned values. The binning value is the size of the group.

Background Subtraction

If Background Subtraction is turned off, then the background acquired during the first 100 µs is not subtracted and the decay trace is shifted away from Y = 0 by the background value. Background subtraction is on by default.

Phosphorescence Timebased



The above picture shows the acquisition settings for a phosphorescence timebased scan on a QuantaMaster (Phosphorescence Lifetime) T-format system with an excitation monochromator, one emission monochromator, and manual slits.

A Phosphorescence Timebased scan fixes the excitation and emission wavelengths and plots the average value of a window of the decay curve over time. Like the Phosphorescence Decay, this scan collects the background for 100 μs, then pulses the lamp, and then acquires the decay curve. The difference is that the program then forms an average value of a window of time and keeps only that value.

Xcorr Start Time and Xcorr Stop Time. Leave these at their default values.

These parameters appear for Timebased and Time resolved setups (not for Decay setups), and are shown whether or not the excitation correction device is enabled in the hardware configuration.

Window Start Time

The beginning of the time window.

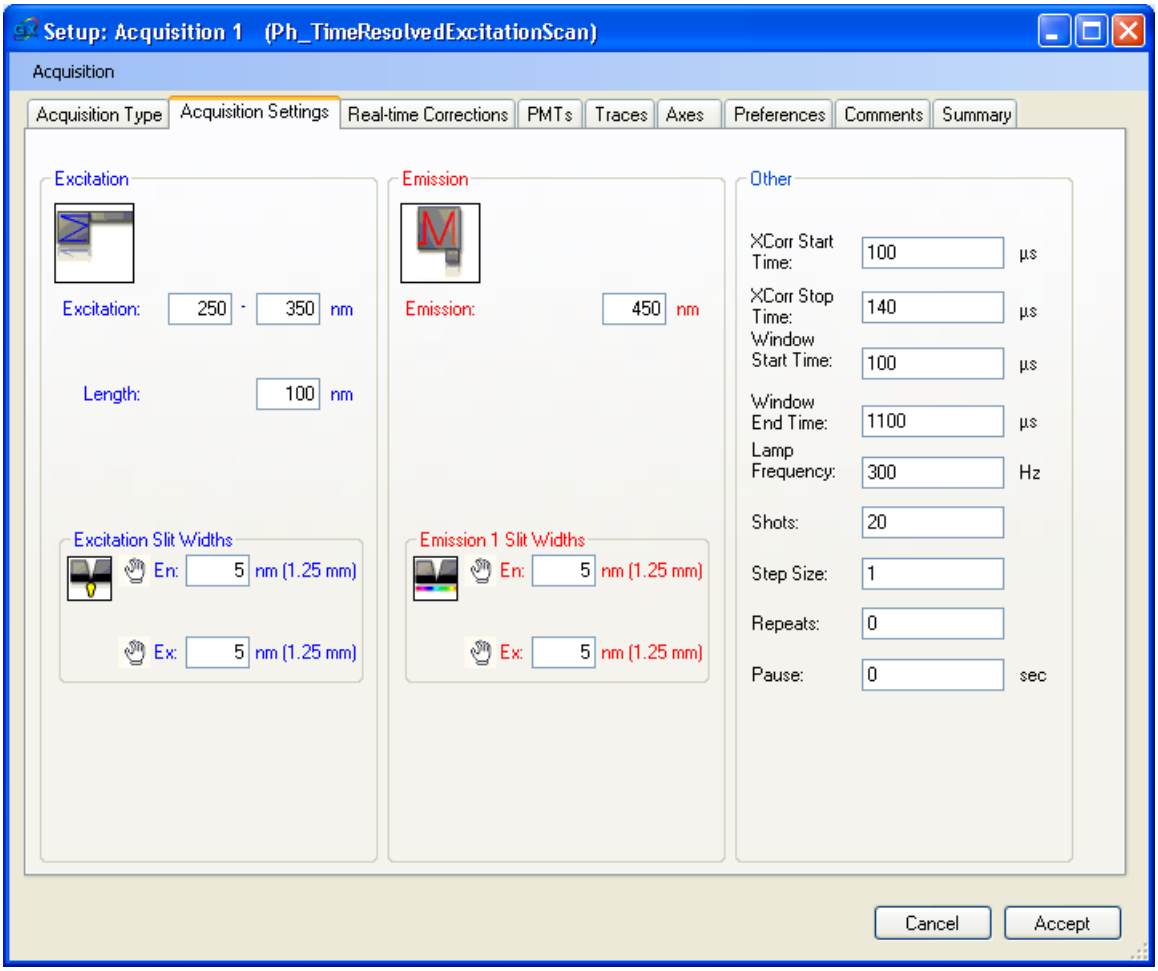
Window End Time

The end of the time window.

Points/s

= Lamp Frequency/Shots. This is how many data points per second will be displayed in the graph. This value is displayed in Timebased acquisitions and is for information only. It is not user settable.

Phosphorescence Time Resolved Excitation Scan



The above picture shows the acquisition settings for a Phosphorescence Time Resolved Excitation Scan on a QuantaMaster (Phosphorescence Lifetime) system with an excitation monochromator, one emission monochromator, and manual slits.

Similar to Phosphorescence Timebased scans, Time Resolved Excitation or Emission Scans look at the value of a part (the window) of the decay curve, but as the excitation or emission wavelength is changed instead of viewing the data versus time.

Acquisition Types – QuantaMaster (VCI)

Common Acquisition Setting Parameters (VCI)

In addition to the acquisition parameters seen for setups based on Steady State or Pulsed Light Source hardware configurations, the following acquisition parameters are different from the general setting parameters or are specific for VCI hardware configurations.

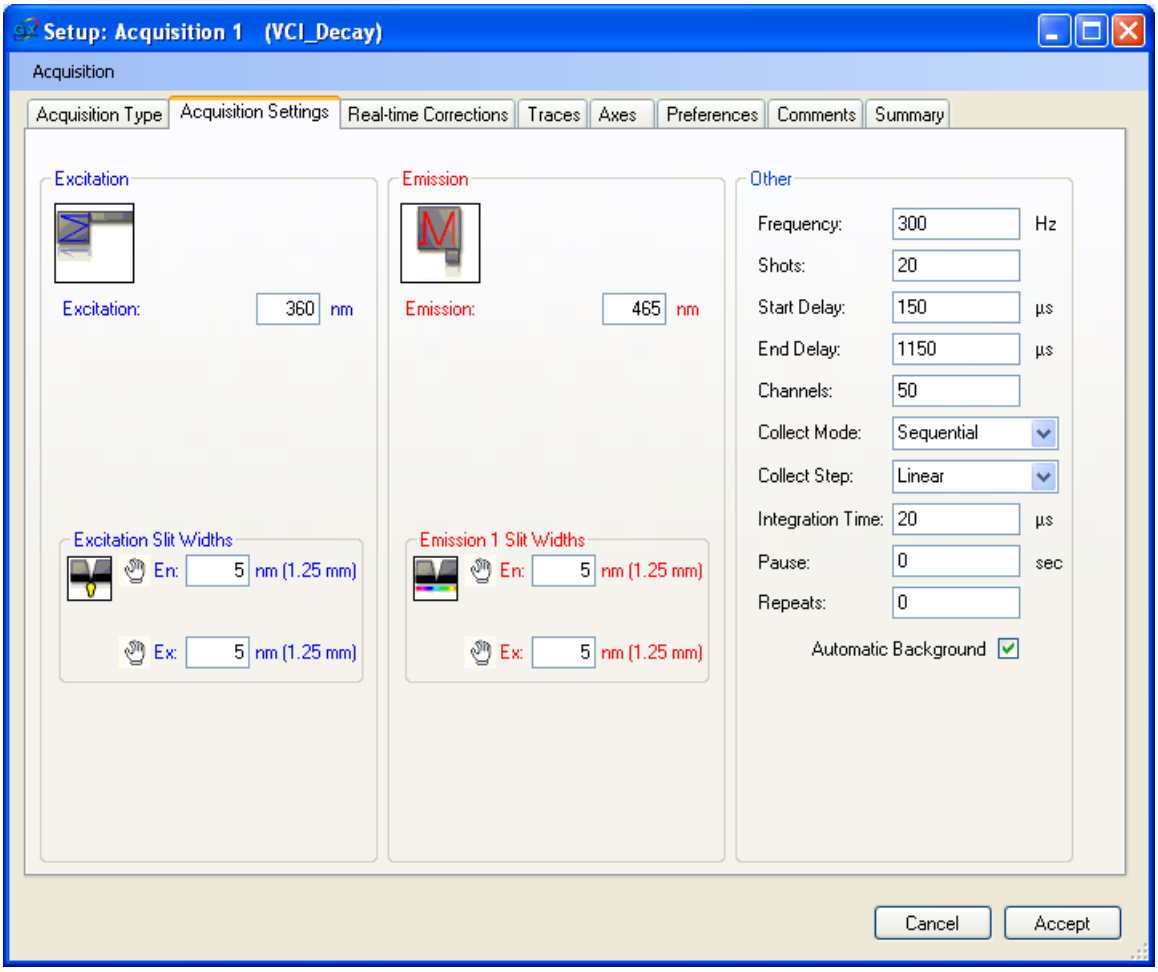
Integration Time

This parameter is used for VCI Timebased, Excitation, or Emission Scans. This is the time in microseconds for which the integration window is open for each lamp pulse. Since, in this case, the observation window is defined by the integration time, increasing the integration time will increase the signal at the expense of lifetime resolution while decreasing the integration time will increase the lifetime resolution at the expense of signal strength. In particular, when the instrument is used to separate fluorescence spectra from phosphorescence spectra, care must be used in selecting the integration time. Since fluorescence is essentially over in the first 5 to 10 μs after the excitation pulse, the delay should be set to the excitation peak and the integration time to 5 to 10 μs. Longer integration times will contaminate the fluorescence with phosphorescence. When collecting phosphorescence, the delay should be set 5 to 10 μs after the excitation pulse and the integration time chosen to be larger to maximize sensitivity.

Automatic Background

Keep this check box checked (default). The background value will be measured at the start of each scan.

VCI Decay



The above picture shows the acquisition settings for a VCI Decay scan on a QuantaMaster VCI system with one excitation, one emission monochromator, and manual slits.

Acquisition Types – TimeMaster (Fluorescence Lifetimes)

Common Acquisition Setting Parameters (Fluorescence Lifetimes)

In addition to the acquisition parameters seen for setups based on Steady State or Pulsed Light Source hardware configurations, the following acquisition parameters are different from the general setting parameters or are specific for Fluorescence Lifetime hardware configurations.

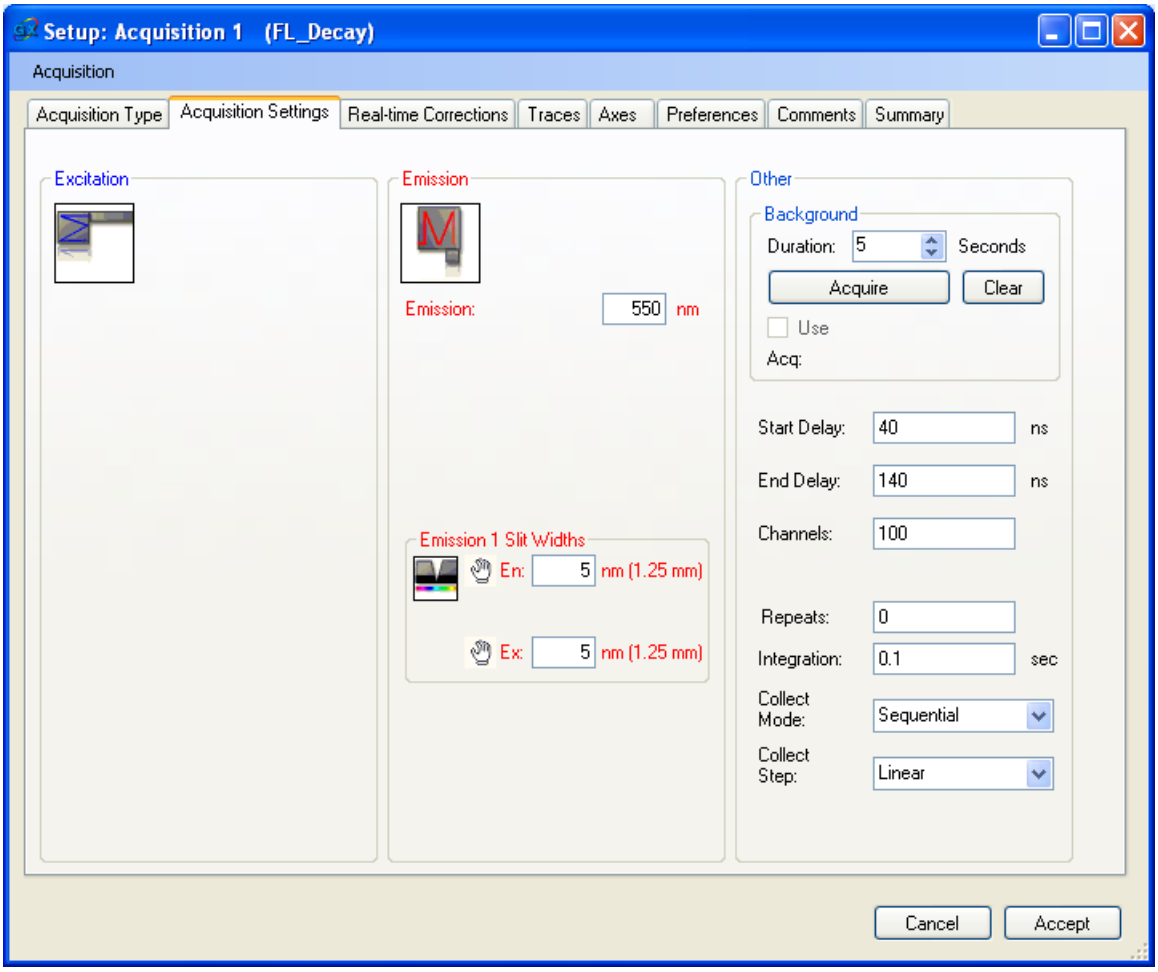
Background Acquire& Use (Lifetime)

For lifetime systems it is important to acquire and use the background value(s) for scans. A separate background value will be acquired for each detector in the system.

> **Acquire**: Click on this button to acquire background values. For wavelength scans, these values will be acquired at the start wavelengths. The background will be acquired at delay = 0, before the onset of any lamp pulse.
>
> **Use**: Check this box to use the acquired background values. The values will then be shown as the background values in the traces tab and trace properties.

The background values will remain in effect for subsequent measurements until cleared in the **Setup, Traces** tab, or by removing the check mark in the **Background: Use** checkbox, or by clicking the **Background: Acquire** button, which will clear the previous value and force a new background to be acquired. Toggling Use Background keeps the background value in memory for future use. It is important to measure the background during the first scan, otherwise the signals 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 fired. 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 is changed.

TimeMaster Fluorescence Decay



The above picture shows the acquisition settings for a fluorescence decay scan on a TimeMaster (Fluorescence Lifetime) LED system with one emission monochromator and manual slits.

Integration

Enter the time in seconds over which the signal will be averaged for each point of each scan. Extra integration time will improve the signal to noise ratio at the expense of additional acquisition time.

Acquisition Types – TimeMaster (LaserStrobe)

Common Acquisition Setting Parameters (LaserStrobe)

In addition to the acquisition parameters seen for setups based on Steady State or Pulsed Light Source hardware configurations, the following acquisition parameters are different from the general setting parameters or are specific for LaserStrobe hardware configurations.

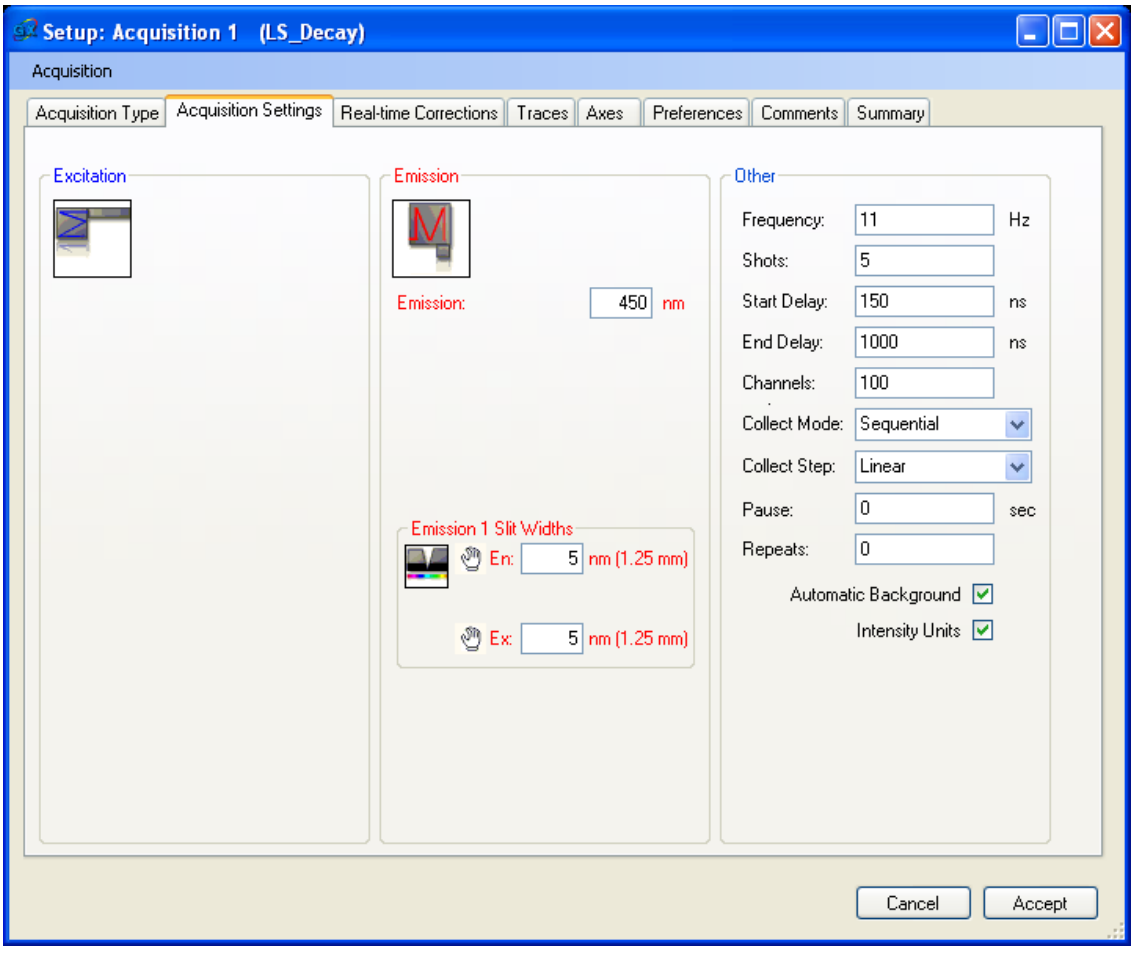
Automatic Background

Keep this check box checked (default). The background value will be measured at the start of each scan.

Intensity Units

Keep this check box checked (default). The intensity range is 0 to 65535.

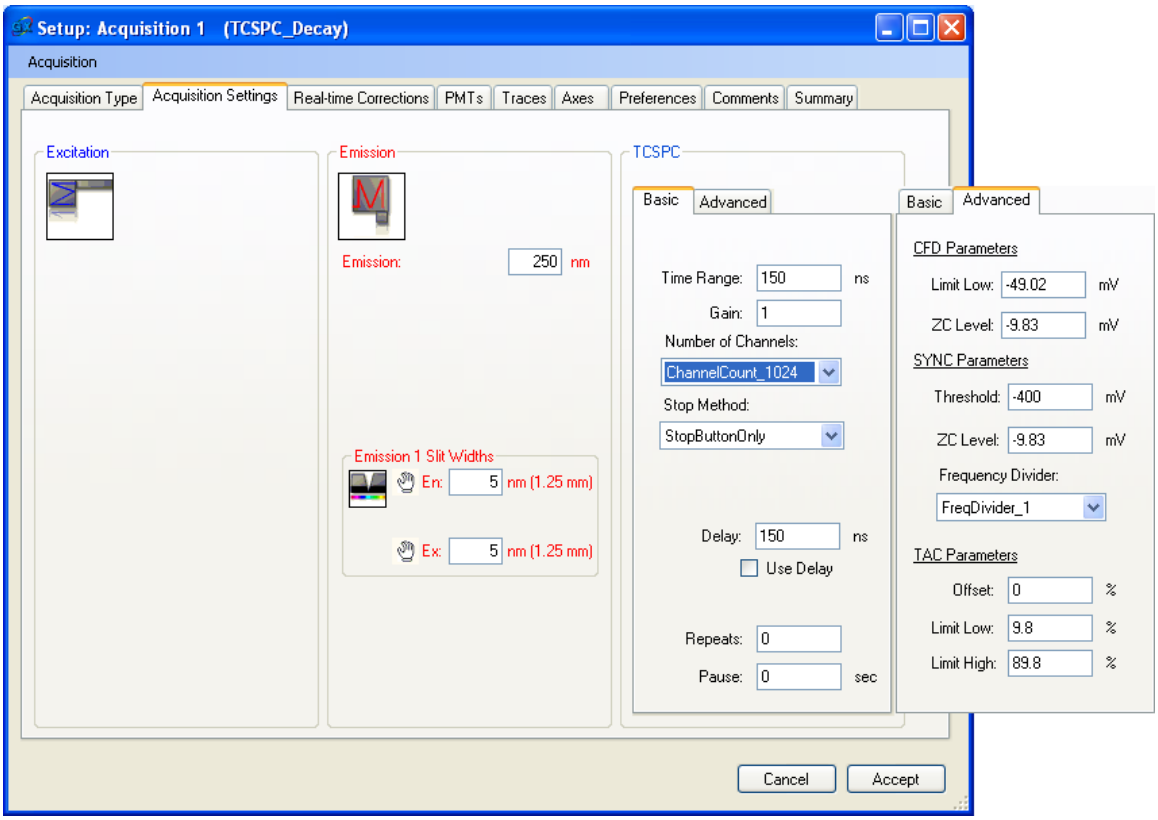
TimeMaster LaserStrobe Decay



The above picture shows the acquisition settings for a Decay scan on a TimeMaster LaserStrobe system with one emission monochromator and manual slits.

Acquisition Type – TimeMaster (TCSPC)

There is only one Acquisition Type for a TCSPC hardware configuration – Fluorescence Decay.



The above picture shows the acquisition settings for a Fluorescence Decay Scan on a TimeMaster (TCSPC) system with an LED excitation (no monochromator), one emission monochromator, and manual slits.

General principles of TCSPC

TCSPC measures the times between the single-photon pulses from a detector and subsequent reference pulses from a lamp control device or a second detector. In the TCSPC instrument, the reference pulse comes from the SYNC output on the LED control box EL-1000. Both the reference and single photon pulses are fed to Constant Fraction Discriminators (CFD) that allows rejection of pulses that are smaller than a threshold and produces a trigger at the time that a specific fraction of the pulse height is reached. This minimizes timing jitter that would be introduced by triggering on fixed amplitudes of the pulses.

A single photon pulse starts the Time-to-Amplitude Converter (TAC), generating a decreasing linear voltage ramp until the next reference pulse is received. A new reference pulse is received for each LED lamp pulse, whether or not a single photon is received by the detector. To stop the TAC with the correct reference pulse, the reference signal must be delayed so that it arrives after a photon pulse from the same period. The correct delay in the reference channel is the detector transit time, plus the width of the

recorded time interval, plus a few ns for the TAC start delay. The TAC output voltage signal is then inverted so longer arrival times correspond to larger voltages.

This voltage is sent to a Programmable Gain Amplifier that can rescale a section of the voltage into the measurement window. It can also reject any signals that are outside the window of interest. The Analog-to-Digital Converter converts this amplified voltage signal to a digital value that adds a single count to a corresponding memory address. In this way a histogram of counts vs. time between the reference and single photon pulses is built up, representing the probability of a single photon occurring at such times.

Basic Parameters

Time range

The maximum start-stop time of the linear-ramp generator, i.e. the total time interval in which photons can be measured. TAC ranges from 50 to 2000 ns are available.

Gain

This is the gain of the biased amplifier of the TAC. Gain stretches the time scale of the TAC. Values from 1 to 15 are available.

Number of Channels

Select the number of time channels (data points) for a trace. 64, 256, 1024 or 4096 channels can be selected.

Stop Method

Select the means to stop data acquisition:

StopButtonOnly – Click the Stop button on the Acquisition Control Panel

PeakChannelCount – Enter a value in the Peak text box. Data acquisition will stop when the number of counts in any channel reaches this value, or the Stop button is clicked. This value will be shown on the TCSPC Control Panel as Overflow Count. The channel with the most counts defines the Peak Channel. Note that the maximum number of counts in any channel is 65535. More counts in any channel will be clipped at this level.

Time – Enter a time in seconds in the Duration text box. Data acquisition will stop when this time has elapsed, or the Stop button is clicked. The allowed time range is 0.000 1 to 100 000 seconds. The time will be shown on the TCSPC Control Panel as Collect Time.

Delay

The displayed data is shifted to the left on the X-axis by the ‘Delay’ value. Increasing the ‘Delay’ shifts the displayed data further to the left. The reference (SYNC) pulse is received only after a single photon pulse starts the TAC. Adding a delay to the reference (SYNC) pulse produces a longer time after the single photon pulse shifting the data to the left on the X-axis. Data that is shifted off the left end of the TAC range (e.g., early photons) is not acquired.

Use Delay

Check this checkbox to use the entered delay time. The entered delay time will be shown on the TCSPC Control Panel. If this checkbox is unchecked, then an automatically calculated delay will be used and displayed on the TCSPC Control Panel.

Advanced Parameters

CFD Parameters

Limit Low

'Limit Low' is the lower discriminator threshold, the 'CFD threshold'. Single photon pulses with amplitudes smaller than 'Limit Low' are not counted. The parameter range for 'Limit Low' is 0 to -500 mV.

ZC Level (Zero Crossing Level)

'ZC Level' is the reference level of the zero cross trigger in the detector CFD. The value has a range of -100 mV to +100 mV.

SYNC Parameters

Threshold

Reference pulses with amplitudes smaller than the 'Threshold' value are not counted. The parameter range for 'Threshold' is -20 to -500 mV. If the SYNC bar graph on the TCSPC Control Panel shows a value significantly larger than the LED repetition rate, the Sync (reference) CFD is not properly rejecting noise pulses. In this case the SYNC Threshold must be increased (become more negative). Exit FelixGX. Open the file C:\temp\tcspc.ini and change the sync_threshold value to a more negative value (e.g., from –100 mV to –200 mV). File, Save to save the change to the tcspc.ini file. Launch FelixGX and repeat the steps above to show the TCSPC Control Panel after the TCSPC setup has been selected to observe any change in the SYNC bar graph value.

ZC Level (Zero Crossing Level)

This is the reference level of the zero cross trigger in the reference CFD. The value has a range of -100 mV to +100 mV.

Frequency Divider

Possible values are 1, 2 or 4. For the PTI LED source lamp operating at tens of kHz, use a value of 1.

TAC Parameters

Offset

The displayed data is shifted to the right on the X-axis by the TAC 'Offset' and the offset amount on the right is shifted off screen and not acquired. The remaining part of the TAC should be long enough to fill the display window for a given TAC Gain. The offset can be set from 0% to 100% of the overall TAC range.

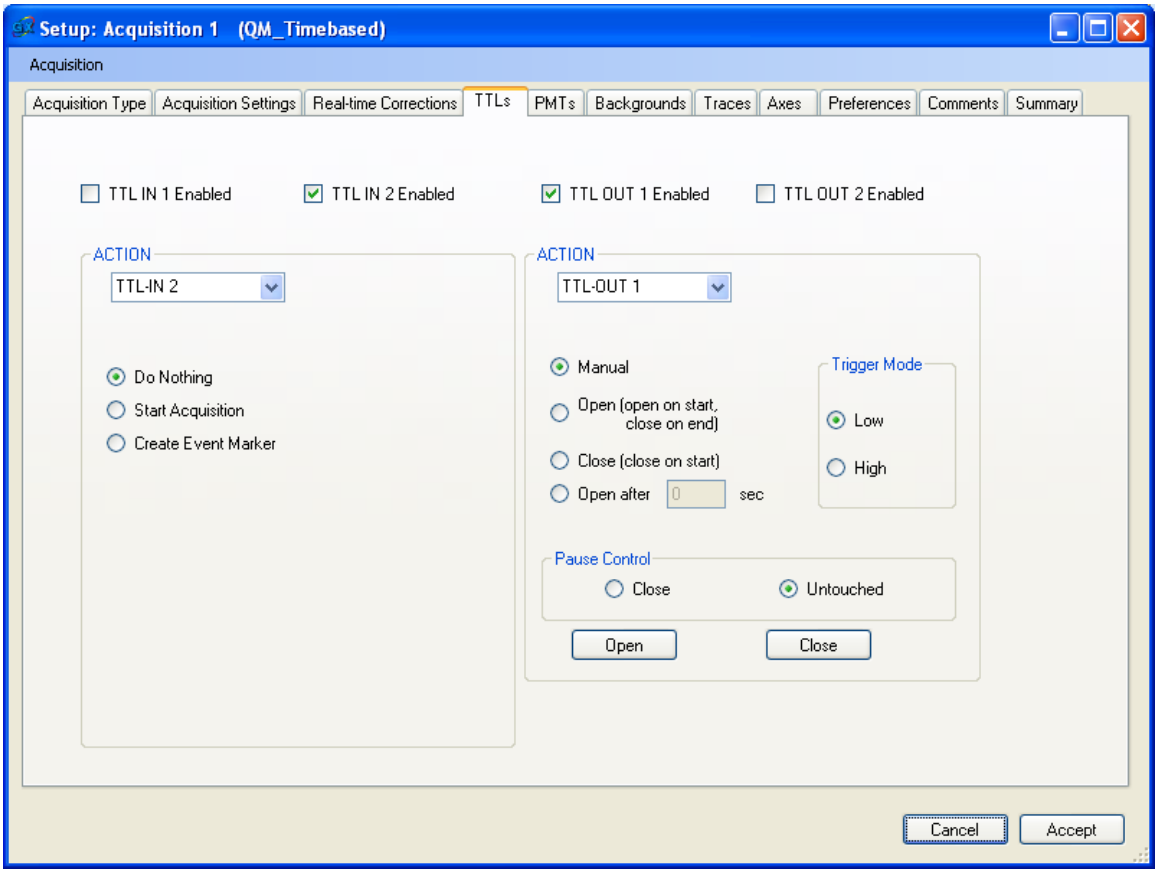
Limit Low

The TAC contains a discriminator to suppress events outside a selected time interval. 'Limit Low' sets the lower limit of this interval. The Limit Low can be set from 0% to 100% of the display window, but not higher than 'Limit High'. Typical Limit Low values are from 3 to 10%.

Limit High

The TAC contains a discriminator to suppress events outside a selected time interval. 'Limit High' sets the upper limit of this interval. The Limit High can be set from 0% to 100% of the display window, but not lower than 'Limit Low'. Typical Limit High values are from 90 to 97%.

TTL



The above picture shows the TTLs tab if all the TTL I/Os are enabled in the hardware configuration. Any TTL I/O not enabled in the hardware configuration is shown as that TTL I/O name and check box dimmed (inactive). If no TTL I/O are enabled in the hardware configuration, then the TTLs tab is not shown.

TTL (transistor-transistor logic) compatible logic levels are used to connect instrumentation together. The ASOC-10 recognizes TTL In levels between 0 and +0.8 V as Low (digital 0) and between +2.0 and +5.0 as High (digital 1). TTL OUT Low signals are < +0.8 V and High signals are > +2.0 V.

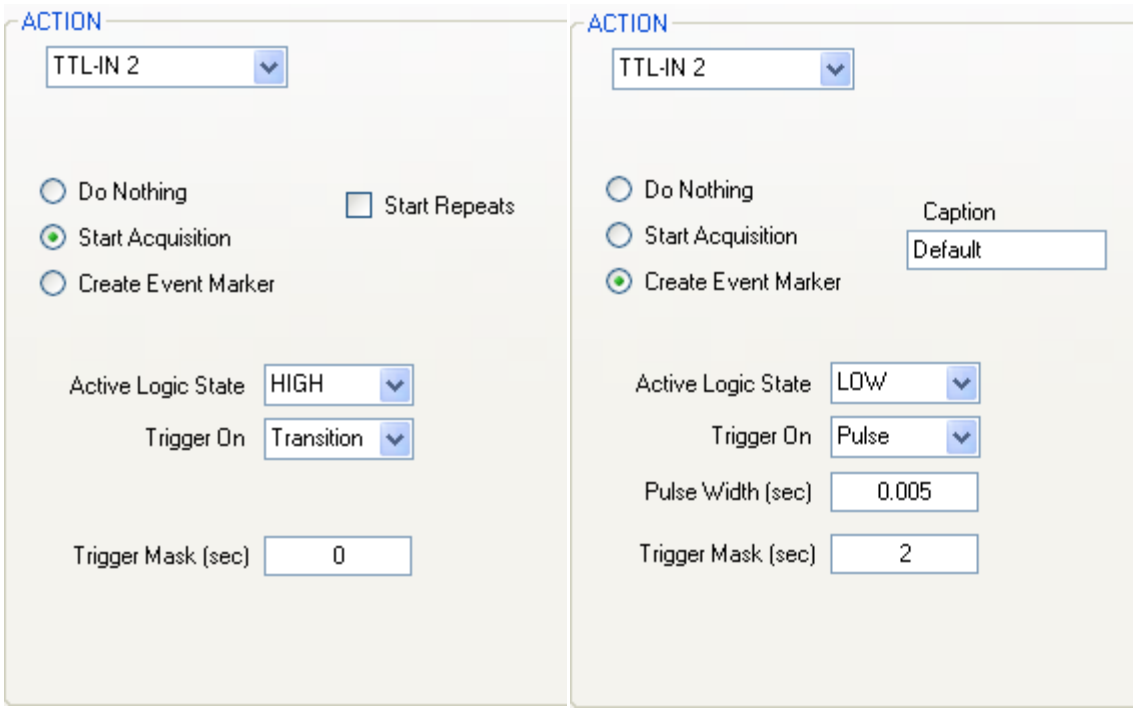
FelixGX suports two TTL In connections.

TTL IN 1 and **2 Enabled**: Checking one of these boxes activates the software and hardware functions to monitor the TTL input signals.

ACTION: TTL In 1 or **TTL In 2**: use the choice box to select which input to configure. If ACTION: TTL In 1 or TTL In 2 is selected when the respective enabled check box is not checked, then that action will have no effect.

The available actions are**:**

Do Nothing: the TTL Input is not monitored.



Start Acquisition: Click this radio button to activate the "Wait for TTL Input before starting acquisition" condition. Currently this condition is activated by default for a new acquisition. If the acquisition preference Auto-prepare is OFF, then the acquisition will not begin unless the conditions defined below are met. If Auto-prepare is ON, then either meeting these conditions or clicking the Start button will begin the acquisition.

> **Start Repeats**: Visible only for **Start Acquisition**. If this box is checked, then the TTL In put condition is also required to start repeat scans.

Create Event Marker: Click this radio button so that a TTL Input will attach an event marker to the active trace and display it in the graph area. Event Markers show as a light blue vertical line in the graph with an arrow pointing from the caption to this line.

> **Caption**: Visible only for **Create Event Marker**. Enter text that will be shown in the Event Marker caption.

Active Logic State: HIGH or LOW: This sets the trigger level to be met.

Trigger On:

> **Transition**: A transition to the Active Logic State level from the opposite TTL level is required.
>
> **Pulse**: A pulse from the Inactive to the Active Logic State level is required.
>
> > **Pulse Width (sec)**: The minimum width of the Active State required, measured from the leading edge transition. Pulses shorter than this width will not trigger an action.

Trigger Mask (sec): A subsequent TTL In trigger signal during this time will not trigger an action. The mask begins when a valid trigger has been recognized. For a transition the trigger mask begins at the time of the transition. For a pulse the trigger mask begins at the end of the Pulse Width time.

TTL OUT 1 and 2 Enabled: Selecting one of these activates the circuitry in the respective TTL Out 1 and 2 outputs. Depending on the output variables and trigger mode, the output will transition high or low, to trigger other connected TTL equipment. TTL Out pulses are not supported. Note that if only one transition is defined, then the TTL Out level will be left at that value. To trigger a subsequent action, the TTL Out may have to be reset to an initial state. This is the case for Manual.

TTL Out 1 and **TTL Out 2**: These radio buttons select the target output for editing the output parameters and trigger mode.

Manual: This option enables a TTL OUT button on the Acquisition Control Panel so the mouse can be used to initiate a TTL output transition. The TTL OUT button on the Acquisition Control Panel must be clicked again to reset the TTL Out level.

Open (open on start, close on end): This option automatically sends a TTL transition at the start of a scan and closes it at the end (this is commonly used to control a shutter).

Close (close on start): This option will switch the current logic state at the start of a scan.

Open after _____ seconds: This will activate the selected TTL Out to change states after the defined time in seconds (0 to 9999 seconds can be entered) has occurred. When the acquisition starts a timer is started that counts down. When the variable time has elapsed, the TTL Out will change states.

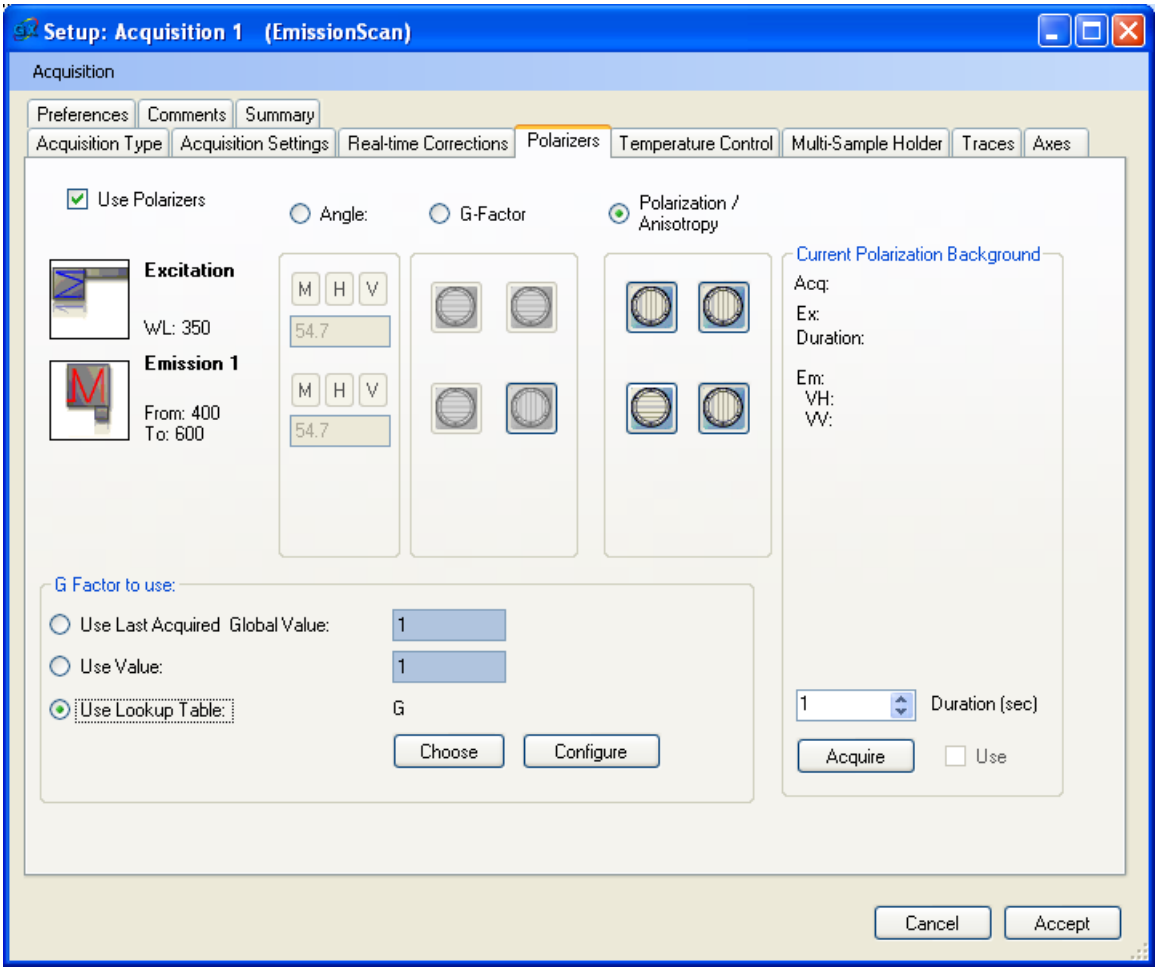
Pause Control Close: Selecting this will cause the selected TTL state to change when the user manually pauses the acquisition (useful for a shutter).

Pause Control Untouched: Selecting this will prevent the selected TTL Out from changing states during a user initiated pause (useful if TTL is driving a perfusion system).

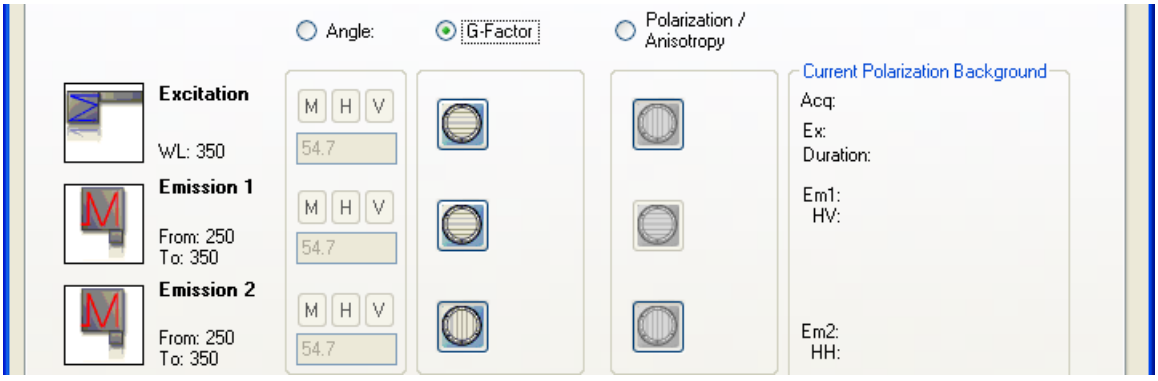
Trigger Mode Low: Starts the scan with TTL OUT high, then the TTL OUT state will change to 0 V when the TTL OUT button is clicked.

Trigger Mode High: Starts the scan with TTL OUT low, then the TTL OUT state will change to +5 V when the TTL OUT button is clicked.

Polarizers Control



This picture shows the Polarizers tab for an emission scan in an L-format QuantaMaster system, using a LUT for the G-Factor, and set to acquire VV and VH data for polarization and anisotropy.



This partial picture shows the Polarizers Accessories tab for an emission scan in a T-format QuantaMaster system, set to acquire HV and HH data for the G-Factor.

Use Polarizers: This check box must be checked to acquire data using the polarizers and configure the display of the data in the **Traces** tab. If this check box is unchecked, the

rest of the dialog is hidden, and no polarizer information is acquired and shown on the **Traces** tab.

The usual polarizer orientations are either **V**ertical [0°] and **H**orizontal [90°]. E.g., in a system with the excitation polarizer at 90° and a single emission polarizer at 0°, the system polarization is designated as HV. In a system with two emission polarizers, an excitation polarizer at 0°, the emission 1 polarizer at 0° and the emission 2 polarizer at 90° the system polarization is designated as VVH. Other angles can be set using the Angle section.

FelixGX 4.0.1 - 4.1.0 can only acquire individual Angle traces, G-Factor (HV and HH) traces, or Polarization (VV and VH) traces. It cannot acquire G-factor and Polarization traces in one scan operation.

Calibration: To perform a polarizer calibration, setup a Timebased acquisition with both monochromators set to the same wavelength and, e.g., 10 points/second and 2 seconds duration. Set the Preference 'Reset clock for time-based acquisitions' OFF. Place a cuvette with a weak scatterer solution in the cuvette holder in the sample compartment. In the Polarizers tab, click the **Angle** radio button. This will disable the normal polarizer settings. A good angle to start calibration is 85°. Set the other polarizer to **V**ertical [0°]. The Traces tab should show only one trace. Save the setup and click **Accept**. **Start** to acquire a data trace. Use the Polarizers Control Panel to change the angle setting (e.g., increment the angle by 1 degree to 86), click **Move**, and then **Start** to acquire the trace at the new angle. Repeat the above until sufficient traces have been acquired to show a clear minimum in the trace intensity vs. polarizer angle.

From the graph estimate the angle at which the minimum occurs. Calibration Number = 90 (expected angle of minimum intensity) - (measured angle of minimum intensity). Enter this number for the respective polarizer in the Hardware Configuration. Repeat the calibration procedure to make sure the minimum occurs at 90°, or increment the Calibration Number in the Hardware Configuration, and repeat the calibration procedure.

Current Polarization Background

You can acquire scalar background values for the polarizer orientations shown. The Duration, Acquire and Use operations are the same as described by the Common Acquisition Setting Parameters.

If you need to acquire background or reference traces of the various polarization orientations this must be done by acquiring G-Factor (HV and HH) and Polarization (VV and VH) traces for background, and then acquiring similar traces for your sample.

Normal polarizer operation

G Factor

Click the G-Factor radio button.

Acquire background values if desired.

If a Timebased scan is used, an average value of the G-Factor will be calculated and saved as a G-Factor Global Value. If an emission scan is to be done, the G-Factor will vary with wavelength. In this case, a derived trace on the Traces tab should be created with **Name** = G-Factor, **Source 1** = HV trace, **Function** = Gfactor,

Source 2 = HH trace. After the scan is done, right-click on the G-Factor trace, **Create Lookup Table**, **Name** = Gfactor, **Type =** Gfactor, **OK**.

Polarization/Anisotropy

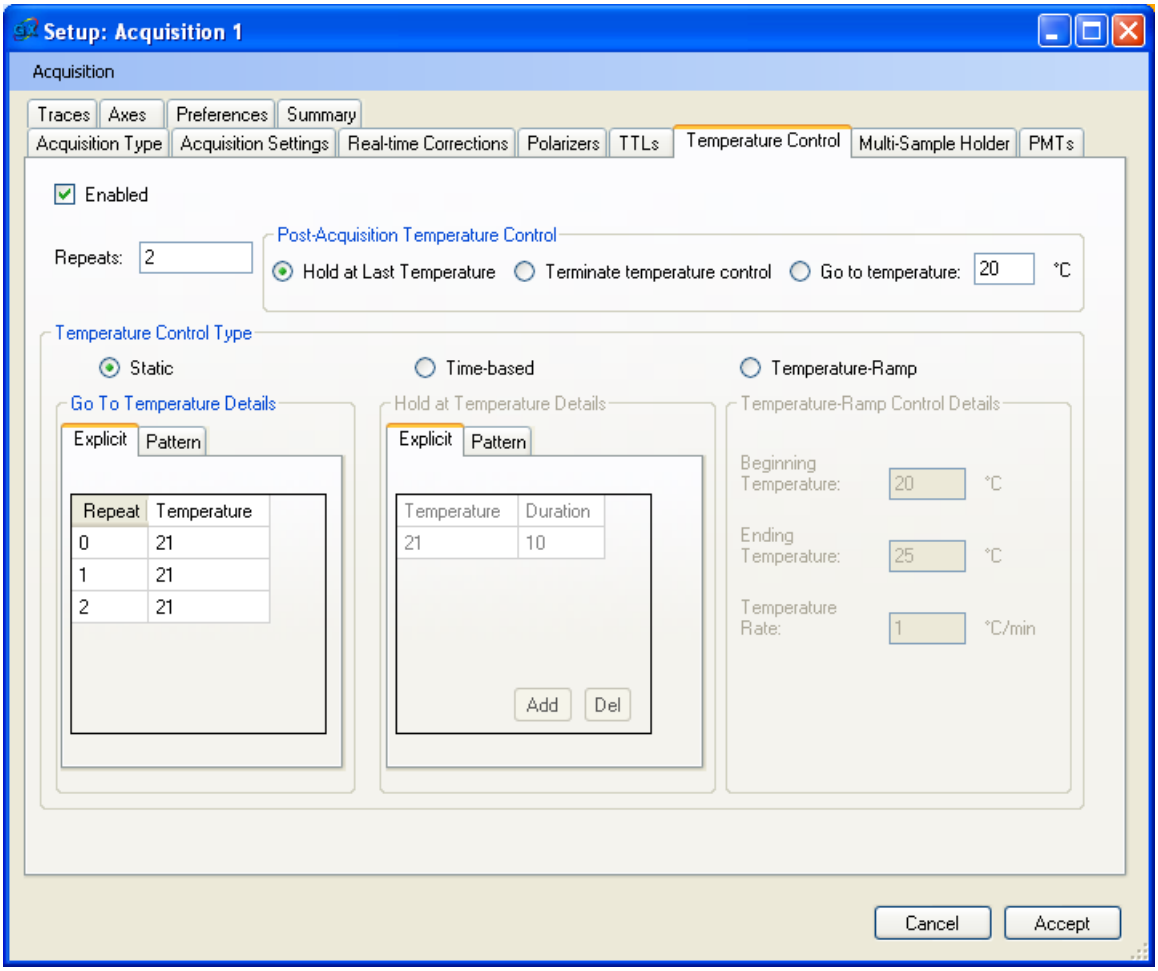
Click the Polarization/Anisotropy radio button.
Acquire background values if desired.
Choose the G-Factor to use by the radio buttons.

Use Last Acquired Global Value: This refers to the most recent G-Factor Global Value (a scalar value) acquired by a timebased scan.
Use Value: Enter a value into the text box.
Use Lookup Table: Use a Lookup Table that is saved as G-Factor values vs. emission wavelength.

Choose: Opens a list of saved G-Factor Lookup Tables. Choose one from the list.
Configure: Opens a Lookup Table editor where you can modify individual X and Y values of the Lookup Table.

On the Traces tab, create a derived trace with **Name** = Polarization or Anisotropy, **Source 1** = VV trace, **Function** = Polarization or Anisotropy, **Source 2** = VH trace.

Temperature Control



The above picture shows the default Temperature Control tab for a timebased scan. Time-based and Temperature-based controls are shown only for Timebased acquisitions. Static control is shown for all acquisition types.

For a system with a single cuvette holder with temperature control, an Enable Stirrer check box is placed above the Post-Acquisition Temperature Control box. Enable/disable this check box to turn the stirrer on or off. To set the stirrer speed check the Enable Stirrer check box, then adjust the Stirrer knob on the front of the QNW Temperature Controller box. This knob is for speed only and is the only way to control the stirrer speed. There, OFF merely means zero speed.

Repeats: This value can be set either in this tab or in the Acquisition Settings tab. Repeats will automatically set the number of temperatures in Static control.

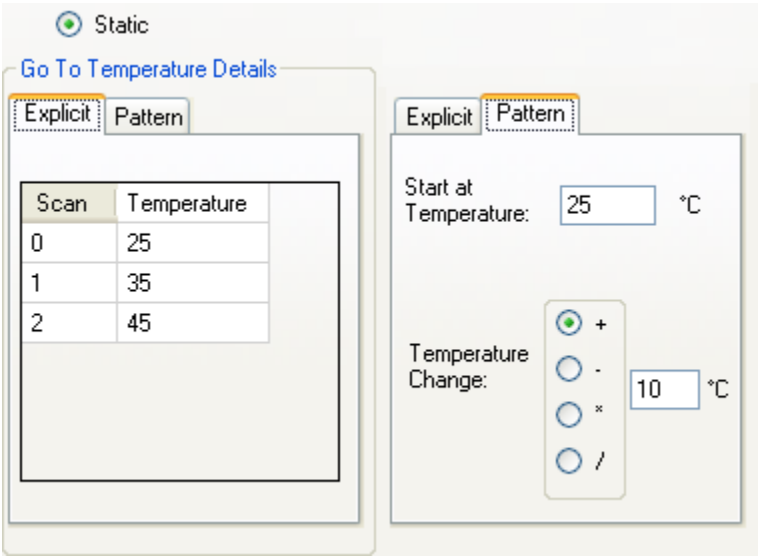
Post-Acquisition Temperature Control:

Click on a radio button to set how temperature will be controlled when the acquisition has completely finished.
Hold at Last Temperature: sends a command to the Temperature Controller to maintain the temperature at the last temperature set value, but the scan is finished and ready to run

a new scan.
Terminate Temperature Control: allows the temperature to change freely, thus going to ambient temperature. The scan is finished and ready to run a new scan.
Go to Temperature: data acquisition is finished, but acquisition control is not released until the Go to temperature is reached.

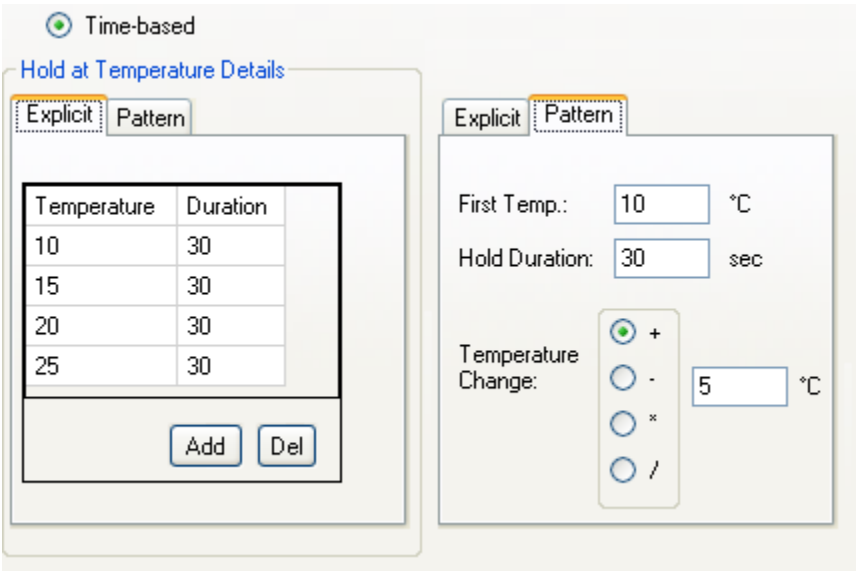
Temperature Control Type

Static – Go to Temperature



A different temperature can be set for each repeat scan. You can either enter temperatures directly into the table, or click on the Pattern tab to increment the table.

Time-based – Hold at Temperature

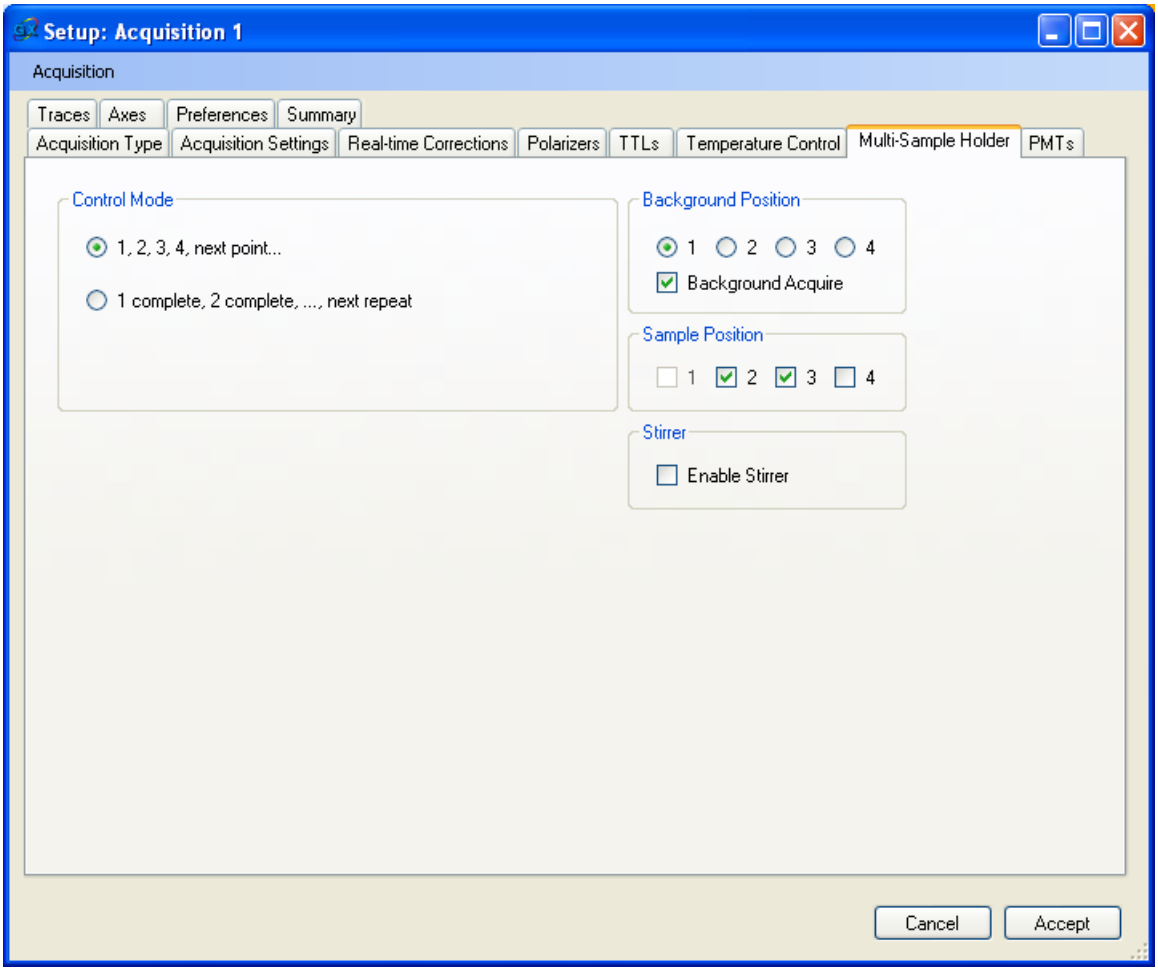


The entire pattern will be repeated for each scan. You can either enter temperatures and durations directly into the table, or click on the Pattern tab to increment the table. Note that different Hold Durations can only be explicitly set in the table.

Temperature-based

Enter the beginning and end temperatures and the temperature rate. The temperature will change from the beginning to the end temperature and the scan will last however long that takes. If Repeats > 0, when the end temperature has been reached, the temperature will go to the beginning temperature before the next scan is allowed to begin. When the last end temperature has been reached the temperature will be directed by the Post-Acquisition Temperature Control.

Accessories - Multi-Sample Holder



The above picture shows the Multi-Sample Holder Accessories tab.

Control Mode

1, 2, 3, 4, next point...: Allows you to acquire data from multiple samples concurrently. One data point is acquired for Sample 1 then one data point is acquired for Sample 2, etc.… This method is commonly used to run temperature ramps with multiple samples – a data point is acquired at each sample at one temperature, then the temperature is increased and a new data point is acquired for each sample, etc.

1 complete, 2 complete, …., next repeat: Allows you to acquire data from multiple samples sequentially. A single scan is acquired for Sample 1, then another scan is acquired for Sample 2, etc. If Repeats > 0, then single scans will be acquired for each position and then these scans will be repeated at each position for the number of Repeats.

Note: Some acquisition types will only allow ***1, 2, 3, 4, next point...***, or only ***1 complete, 2 complete, …., next repeat.***

Background Position

Check the Background Acquire check box to enable the background positions to be active. If you do not wish background data to be collected, toggle the **Background**

Acquire check box off. Selecting a background position will automatically deny that position for samples. Click on a radio button to select the position of the turret from which the background measurement will be acquired. For lifetime measurements this position would be used for the IRF (scatterer).

Sample Position

For sample acquisitions, select the appropriate positions for the samples you wish to run.

Enable Stirrer

Turns the stirrer on or off. To set the stirrer speed check the Enable Stirrer check box, then adjust the Stirrer knob on the front of the QNW Temperature Controller box. This knob is for speed only and is the only way to control the stirrer speed. There, OFF merely means zero speed.

Real-time Corrections

Corrections compensate for intensity variations due to either the light source or the rest of the instrument. Separate corrections are done for the excitation portion of the instrument (EXCORR) and each emission channel (EMCORR), and each of these can be independently enabled or disabled by using the respective checkbox. If both corrections are enabled, then they are both done in real-time, creating one correction trace per emission channel.

Excitation Correction (EXCORR)

The RCQC photodiode measures a portion of the light exiting from the monochromator before it impinges on the sample. The RCQC signal is multiplied by the excorr LUT, which is a calibration of the spectral response of the RCQC. This product is divided into the signal from the PMT detector to compensate for spectral and temporal variations in the light impinging on the sample.

$$Excitation\ correction = \frac{D1}{(RCQC * excorr)}$$

where D1 (or D2 or A1, ..., A4) and RCQC are the real-time raw data from the PMT detector (either digital or analog) and the RCQC photodiode, respectively, and excorr is the excitation correction spectral Lookup Table (LUT) (i.e., a calibration of the RCQC device vs. wavelength). The arithmetic is done point by point in real-time, or using one point in the excorr LUT if using a fixed excitation wavelength (e.g., timebased or emission scan). If necessary, the corrected values are calculated by interpolating between values on the lookup table. If the acquisition is outside the wavelength range of the LUT, then the correction will use the nearest end point of the LUT. The correction is done during sample acquisition, and both the raw and corrected data are available to the user.

To use excitation correction, you must have:

1. The RCQC device enabled in the Hardware Configuration, and
2. Excitation Correction enabled and a Lookup Table chosen in the **Acquisition Setup, Real-time Corrections** tab. Different excitation monochromators, or gratings in the excitation monochromator will have different excitation correction LUTs. The Default Correction LUTs are flat lines with Y = 1 for the wavelength range 0 to 990 nm.

Note: if the RCQC device is enabled in the Hardware Configuration, but not in the Acquisition Setup, a raw data ExCorr trace will be acquired with visibility off in the graph and legend. This trace is not used for correction and can be disregarded.

Note. The EXCORR curve provided by PTI is fully valid at the 5-nm slit at which it was measured. If narrower slit widths are used, the EXCORR curve may need to be measured again at the slit width required for your measurement. Please call PTI for details.

Emission Correction (EMCORR)

The emission correction is much simpler than excitation correction. This correction is intended to compensate for wavelength-dependent variations in the emission channel(s). Each emission channel grating has its own emcorr file. The emcorr file is generated by a comparison of the emission channel response to the spectrum of a NIST traceable tungsten lamp.

$$Emission\ correction = D1 * emcorri$$

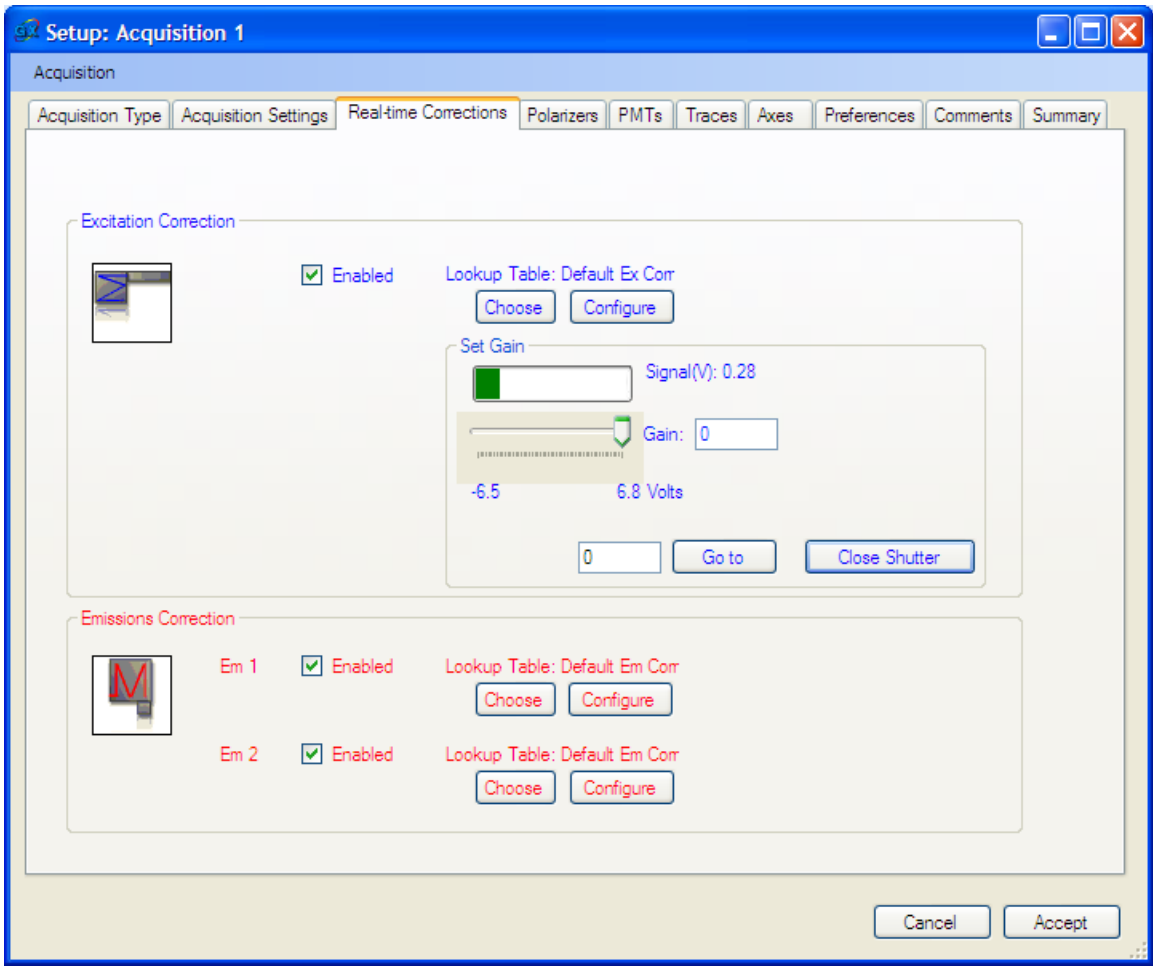
where D1 (or D2 or A1, ..., A4) is the real-time raw data from the PMT detector (either digital or analog) and emcorri is the emission correction spectral LUT (i.e., a calibration of the emission channel vs. wavelength). The arithmetic is done point by point in real-time, or using one point in emcorri if using a fixed emission wavelength (e.g., timebased or excitation scan). If necessary, the corrected values are calculated by interpolating between values on the lookup table. If the acquisition is outside the wavelength range of the LUT, then the correction will use the nearest end point of the LUT.

To use emission correction, you must have Emission Correction enabled and a Lookup Table chosen in the **Acquisition Setup, Real-time Corrections** tab. The Default Correction LUTs are flat lines with Y = 1 for the wavelength range 0 to 990 nm.

Note. If the emission monochromator, grating, PMT, or PMT voltage is changed, the Emission Correction should be re-calibrated for best results. The emission channels will also undergo changes as they age, and the Emission Correction should also be re-evaluated periodically. Please contact PTI for details.

If both excitation and emission correction are enabled, then the corrected trace will be

$$Correction = \frac{D1 * emcorri}{(RCQC * excorr)}$$



The above picture shows the Real Time Corrections tab for a QuantaMaster T-format system, with excitation correction enabled, and emission correction enabled for both emission channels.

You can use the **Create Lookup Table** command in the Trace menu in the legend to create a LUT from a trace, or click on the **Configure** button to open a dialog to create or edit a LUT.

The RCQC signal is acquired in real-time with the detector signal and varies with wavelength and time.

Adjusting the RCQC Gain in a steady state (QM-40) system

Create and save a QM (Steady State) HWC with an RCQC.

1. Click Setup and go to the **Real-time Corrections** tab.
2. Enter a wavelength in the text box beside the **Go to** button and click on the **Go to** button. If your system has a shutter, click on the **Open Shutter** button to open the shutter while setting the gain.
3. Move the **Gain** slider all the way to the right for maximum gain.

4. If the RCQC Signal is too large, then move the **Gain** slider to the left a bit at a time (or press the left arrow key) until a satisfactory signal is observed. The Signal is only updated when control of the **Gain** slider is released.
5. To obtain optimal correction, set the wavelength to the position where the maximum RCQC signal would be obtained for the wavelength range to be acquired, and then adjust the gain to obtain a desired RCQC signal (for a standard monochromator with a 1200 groove/mm grating the maximum RCQC signal occurs about 470 nm). You may want to obtain a quick excitation scan with your experimental parameters to see where the maximum RCQC signal occurs.

Adjusting the RCQC Gain in a pulsed xenon lamp (QM-30) system

Note: Real-time correction is not normally done for decay scans.

Because the lamp is pulsed only while an acquisition is running, the procedure for adjusting the RCQC gain is different.

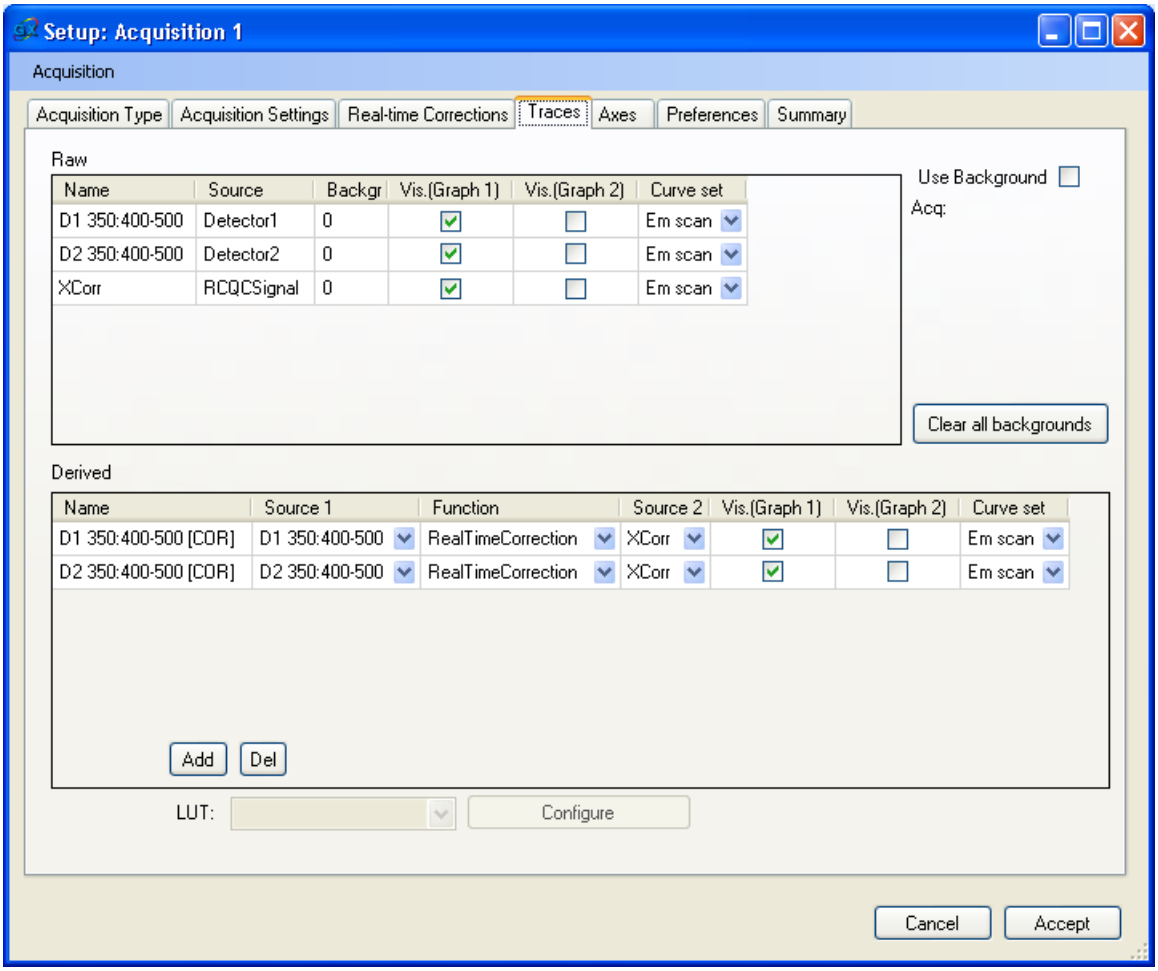
1. Create and save a QM (Phosphorescence) HWC with an RCQC.
2. Click **Setup, Phosphorescence Timebased**, and go to the **Settings** tab.
    a. Set the **Excitation** wavelength to a desired value.
    b. Use the default **XCorr Start** value = 100 μs.
    c. Use the default **XCorr End** value = 140 μs.
    d. Set the duration to e.g., 200 seconds.
    e. On the **Traces** tab, uncheck visibility for A1 and A1 [COR]. I.e., you only need the ExCorr trace to be visible in the following.
    f. **Accept**.
3. Close the excitation slider.
4. Click on the ExCorr Gain control panel to make it visible.
5. **Start**.
6. Open the excitation slider after some data has been acquired. Check that the ExCorr signal is significantly larger with the excitation slider open than when it is closed.
7. **Pause**. The ExCorr Gain control panel is inactive.
8. Click on any other control panel, and then back on the ExCorr Gain control panel to make it active.
9. Click on the slider and press the left or right arrow key to change the gain by 0.01 V increments, or click and drag the ExCorr Gain slider (changes by a minimum of 0.1 V).
10. **Continue**. The ExCorr Signal has changed.
11. Repeat steps 5 to 10 until satisfied with the **ExCorr Signal** value.
12. If you will be running a Time Resolved Excitation Scan, you may want to obtain a quick scan with your experimental parameters to see where the maximum RCQC signal occurs. Then repeat steps 2 to 11 as necessary.
13. When a satisfactory ExCorr Gain Signal has been determined, you must set the respective ExCorr Gain value on the **Real-time Corrections** tab for the type of scan you want to do.

Background Subtraction

Both the PMT detector(s) and the RCQC signals should be background subtracted before applying excitation correction. To acquire background values, close the slider between the arc lamp housing and the excitation monochromator, click **Setup**, **Acquisition Settings** tab, **Background: Acquire**. A message will appear saying "Background acquisition complete. Please refer to the Traces tab for results. OK". Click **OK**. Click on the **Use** checkbox. Click on the **Traces** tab to check if the background values are acceptable, including the RCQC background value.

Once you have acquired the background, open the slider and acquire the rest of your experimental scans. You need to re-acquire this background whenever you restart FelixGX, change any of the hardware in the light path from the excitation monochromator through to the PMT detector(s), adjust the RCQC gain, the PMT detector(s) gain or switch from one acquisition setup to another.

Traces



The above picture shows the Traces tab for a QM-40 T-format system with real-time correction. Derived corrected traces are automatically created. Excitation correction uses two source traces: the PMT Detector signal (in this case digital signal D1) and the RCQC photodiode signal XCorr. Emission correction by itself also creates a corrected trace but does not need the RCQC trace and so shows a blank Source 2.

Raw: data that is collected during acquisition from detectors or other input devices.

Name: The name the trace will show in the legend and exported data files. The trace name can be changed by clicking in the Name text box and entering new text. The default trace names use the following format for PMT detector signals (depending on the detector type and options):

B# or S# A# or D# excitation wavelength or range(excitation polarizer angle): emission wavelength or range(emission polarizer angle)

B# = 4 position turret background position

S# = 4 position turret position

A# = Analog PMT detector

D# = Digital PMT detector

Polarizer angles are shown in parantheses as V for 0°, H for 90°, or the angle if other than V or H.

For example,

D1 350:400 = Digital PMT detector 1, excitation at 350 nm, emission at 400 nm

S2 A2 350-450(V):500(H) = Sample position 2, Analog PMT detector 2, excitation range 350-450 nm, excitation polarizer at V (0°), emission at 500 nm, emission polarizer at H (90°)

Other default trace names are:
ExCorr = RCQC signal
Temperature = Temperature vs. time trace
BB# = Black Box input #

Source: The detector or input device in the hardware configuration.

Backgr: A single background value that is subtracted from each data point in the trace. Background values are acquired by clicking **Background: Acquire**, and then **Use** on the **Acquisition Settings** tab or on the **Polarizers** tab. Backgrounds traces acquired using the Multi-Sample Holder are shown as separate raw traces on the Traces tab.

Vis.(Graph #): Checking this check box will show the trace in Graph 1 or 2, respectively.

Curve set means the same as Group. Enter a name for the Curve set. Once this name is entered for the first trace, it will show up in the choice list for Curve sets for other traces. If a Curve set text box is left blank, then that trace will be placed into its own group in the legend as "Group #". If all Curve set boxes are blank then each trace will be placed into separate groups as "Group #" with different numbers.

Clear all backgrounds: Resets all background values to 0 in this tab and for Current Polarization Backgrounds on the **Polarizers** tab.

Derived: traces that are mathematically generated from the raw traces or other derived traces during acquisition. They may be automatically created by FelixGX (e.g., Real-time correction), or explicitly created by the user by clicking the **Add** button and then selecting one or two source traces and a function. A derived trace can be removed by clicking in any field for that trace and clicking the **Del** button.

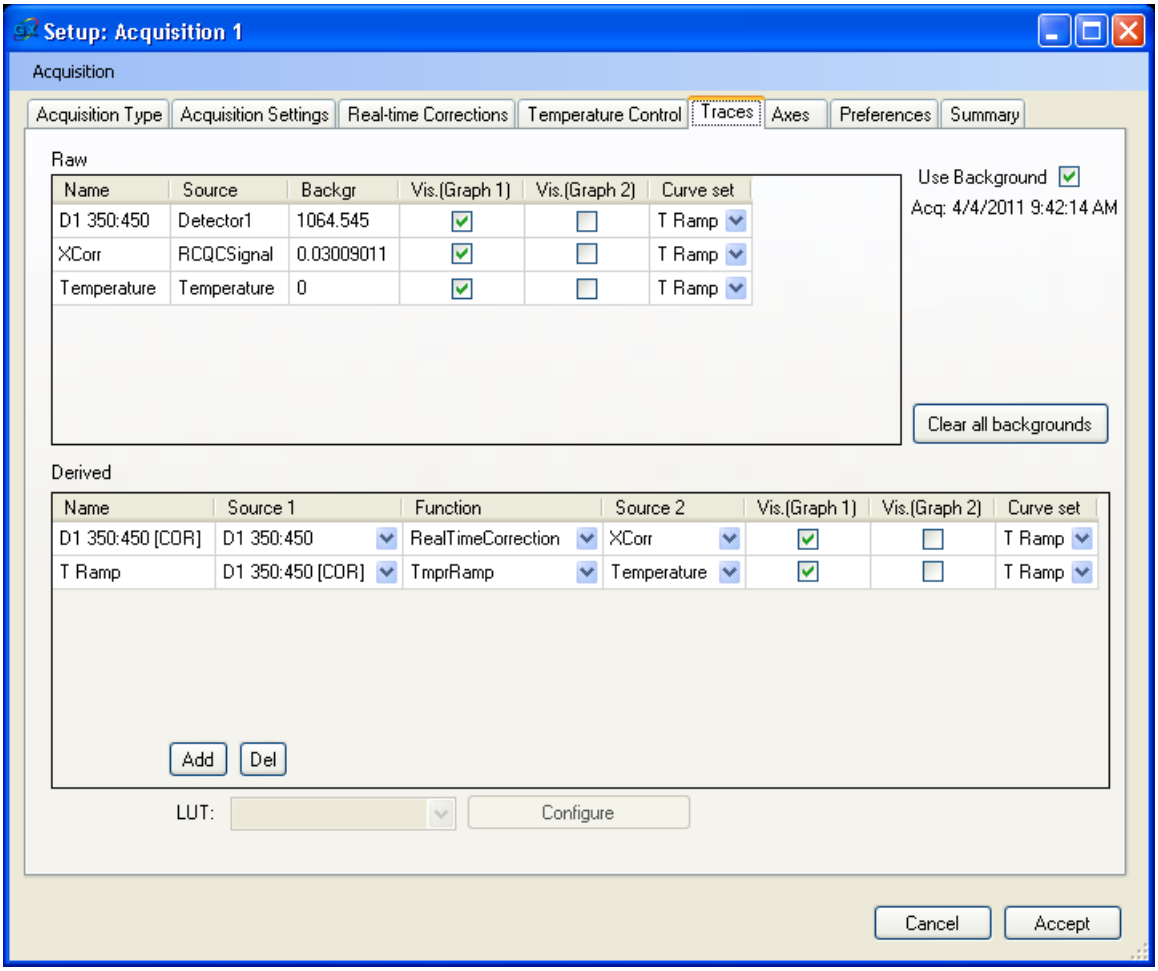
Source 1 and **Source 2**: Select the trace names (raw or derived) from the choice list to be used as operands to create derived traces. Source 1 and Source 2 must be listed for binary operations. Only Source 1 need be listed for unary operations (IntensityToConcLUT, RatioToConcLUT, RatioToPhLUT).

Function: Select the operation from the choice list. Available binary functions are: Add, Subtract, Multiply, Divide, Polarization, Anisotropy, G-Factor, and TmprRamp. Available unary functions are: ConcentrationEquation, IntensityToConcLUT, RatioToConcLUT, RatioToPhLUT. **Note**: Polarization, Anisotropy, G-Factor, and TmprRamp are only visible when the relevant devices are enabled in the Hardware Configuration.

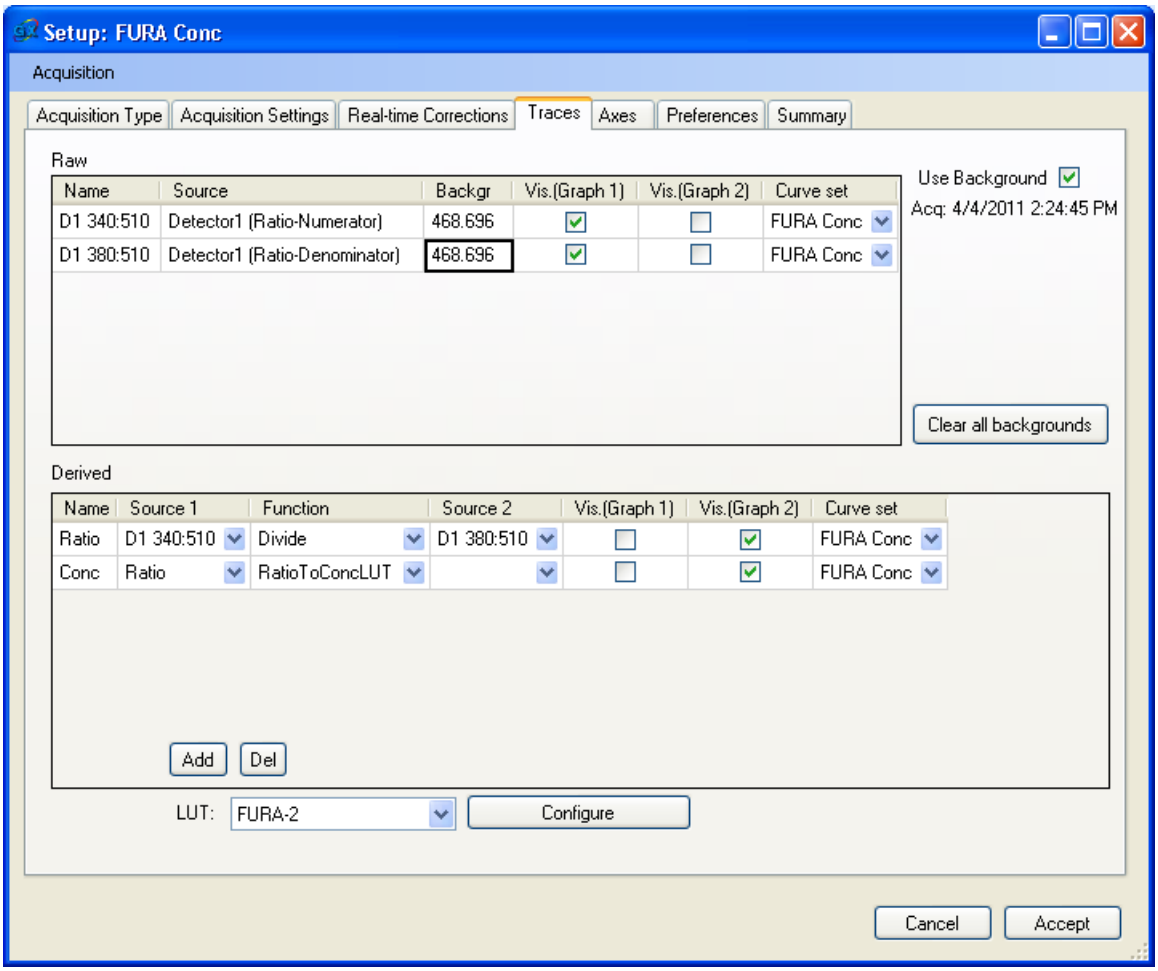
LUT: If a function is chosen that uses a Lookup Table, select the Lookup Table from the choice list.

Configure: Opens the Concentration Map dialog to create or modify a Lookup Table.

Note: Any change on any other tab that affects whether any trace is acquired or not results in the Traces tab being reset to a default setup, and the desired changes to the traces tab must be redone. E.g., changing acquisition type, enabling or disabling Real-time Corrections, Polarization, turret positions, or Temperature Ramps.



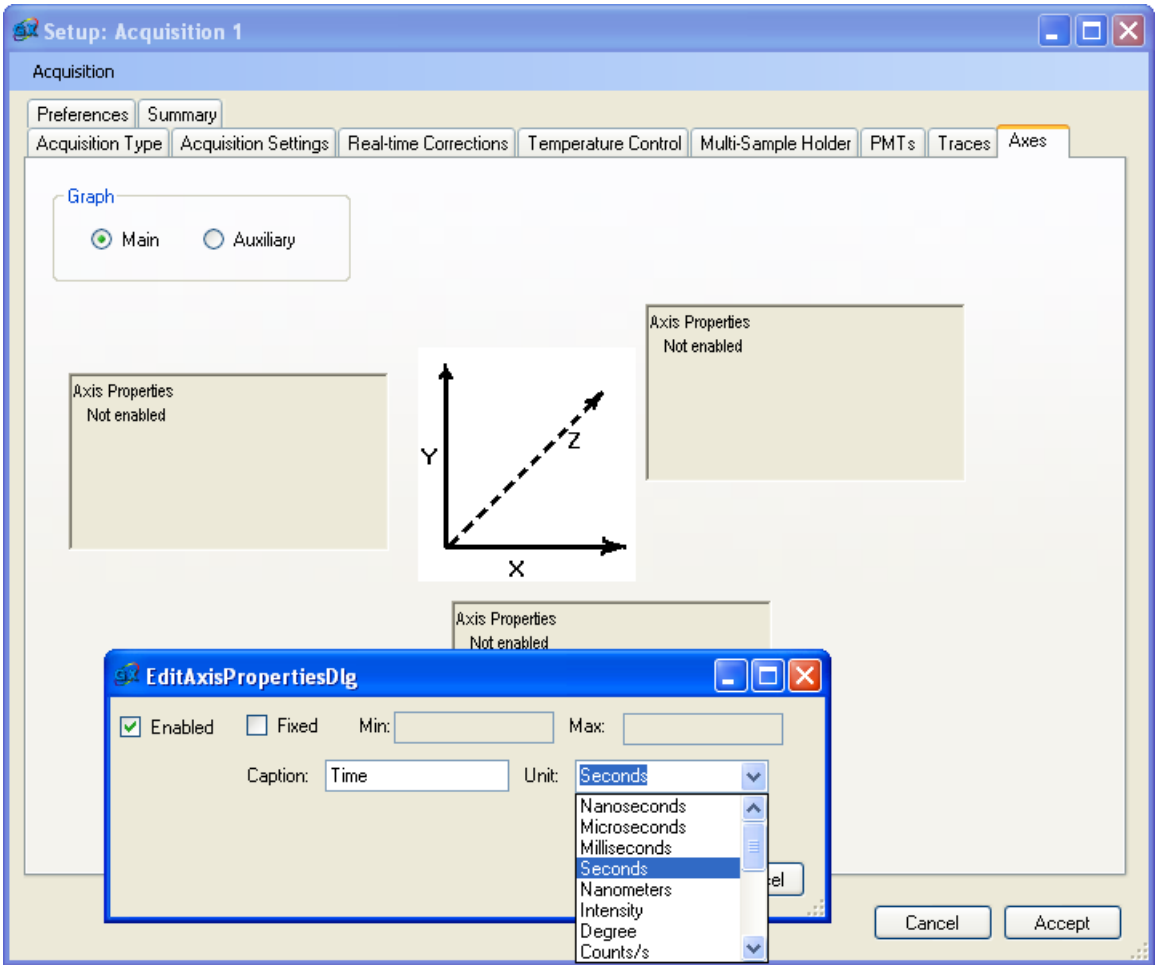
The above picture shows the Acquisition Control Traces tab for a QM-40 system with a temperature controlled Peltier cuvette holder. A derived trace 'D1 350:450 [COR] has been added to show the real-time corrected D1 data. A second derived trace "T Ramp" has been added to show corrected D1 intensity vs. Temperature by choosing the TmprRamp Function.



The above picture shows the Traces tab for an Excitation Ratio acquisition setup type. This setup acquires two raw data traces in real-time and calculates the ratio. The Ratio trace is then used as the source for the RatioToConcLut function. The particular LUT is selected from the LUT choice list.

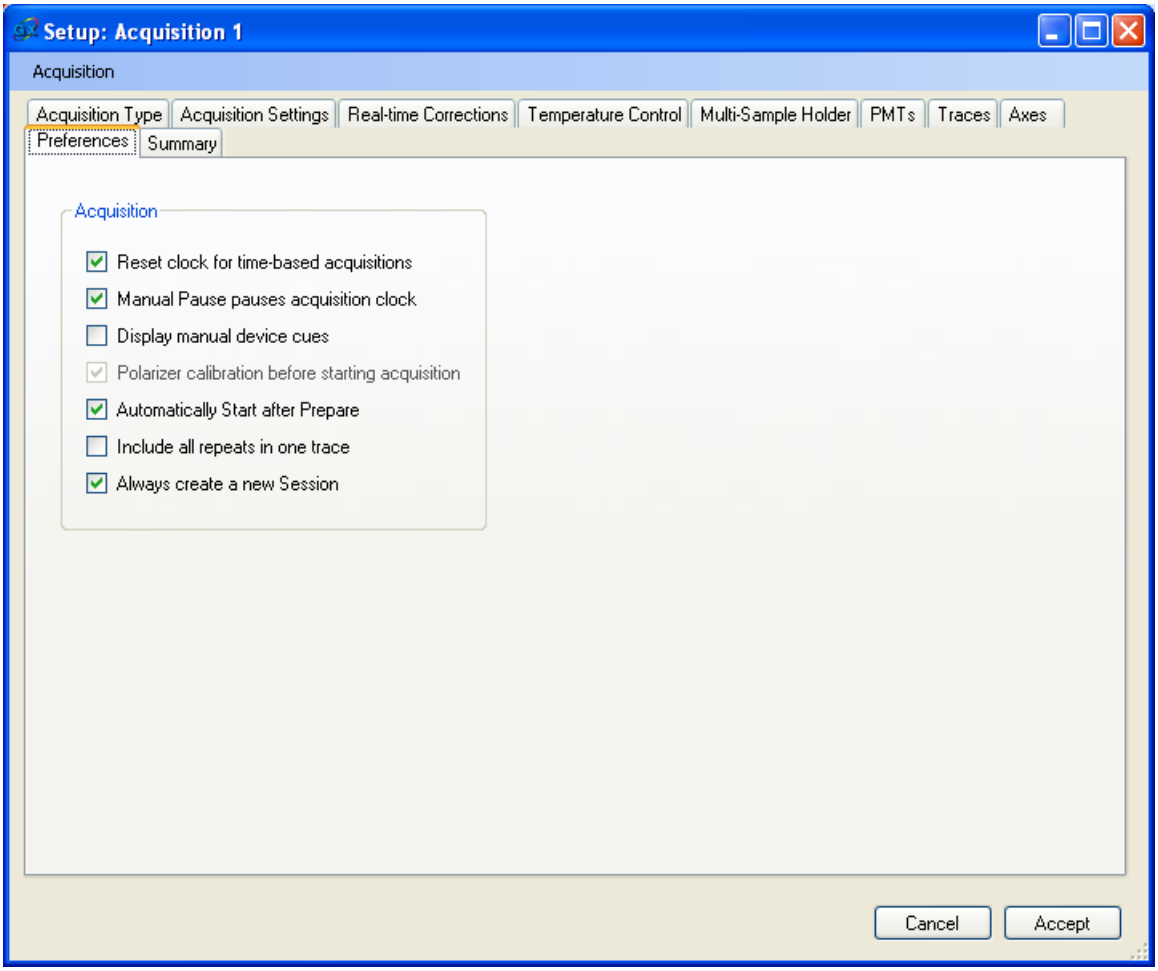
The Ratio and Conc traces are plotted it in the second graph window since they will have values much smaller than the raw data.

Axes



The above picture shows the Axes tab. Click on the Main or Auxiliary radio button to set the axes properties for that graph. Click in an Axis Properties box to show the EditAxisPropertiesDlg for that axis. Fixed Minimum and Maximum limits for an axis apply after the data is acquired. Enter an axis title into the Caption text box, and use the Unit choice list to choose the units for the axis.

Preferences



This dialog adjusts some aspects of how this acquisition setup looks and behaves.

Reset the clock for time-based acquisitions: If this option is ON, the clock and timebased X-axis will reset to zero at the beginning of each acquisition. If this option is OFF, the clock and timebased X-axis will continue increasing as long as this acquisition is repeated.

Manual Pause pauses acquisition clock: If this option is ON, clicking the **Pause** button will pause the clock and data display on a timebased X-axis. The clock and data display will continue when the **Continue** button is clicked. If this button is OFF, clicking the **Pause** button will not pause the clock. When the **Continue** button is clicked the data display will show a jump in the data display that is equal to the duration of the pause.

Display manual device cues: When ON FelixGX will show popups to prompt the user to adjust manual devices such as manual slits. Turn this preference OFF if you will not change these devices and do not wish to see the prompts.

Polarizer calibration before starting acquisition: Inactive at this time.

Automatically Start after Prepare: If this preference is ON, clicking the **Start** button on the Acquisition Control Panel will move the motorized devices to their start positions and start acquiring data automatically. If OFF, clicking the **Start** button will move the motorized devices to their start positions and the status bar will show 'Preparing'. When this step is complete, the **Start** button will re-activate and the status will change to 'Prepared'. When the **Start** button is hit again, then data will start being acquired and the status bar will show 'Running'.

Include all repeats in one trace: Use this in conjunction with Repeats and Pause during timebased scan types. If this preference is ON, then subsequent repeats will be appended to the current trace after the pause time. If OFF, then subsequent repeats will show in the legend and graph as separate traces, all starting at time = 0.

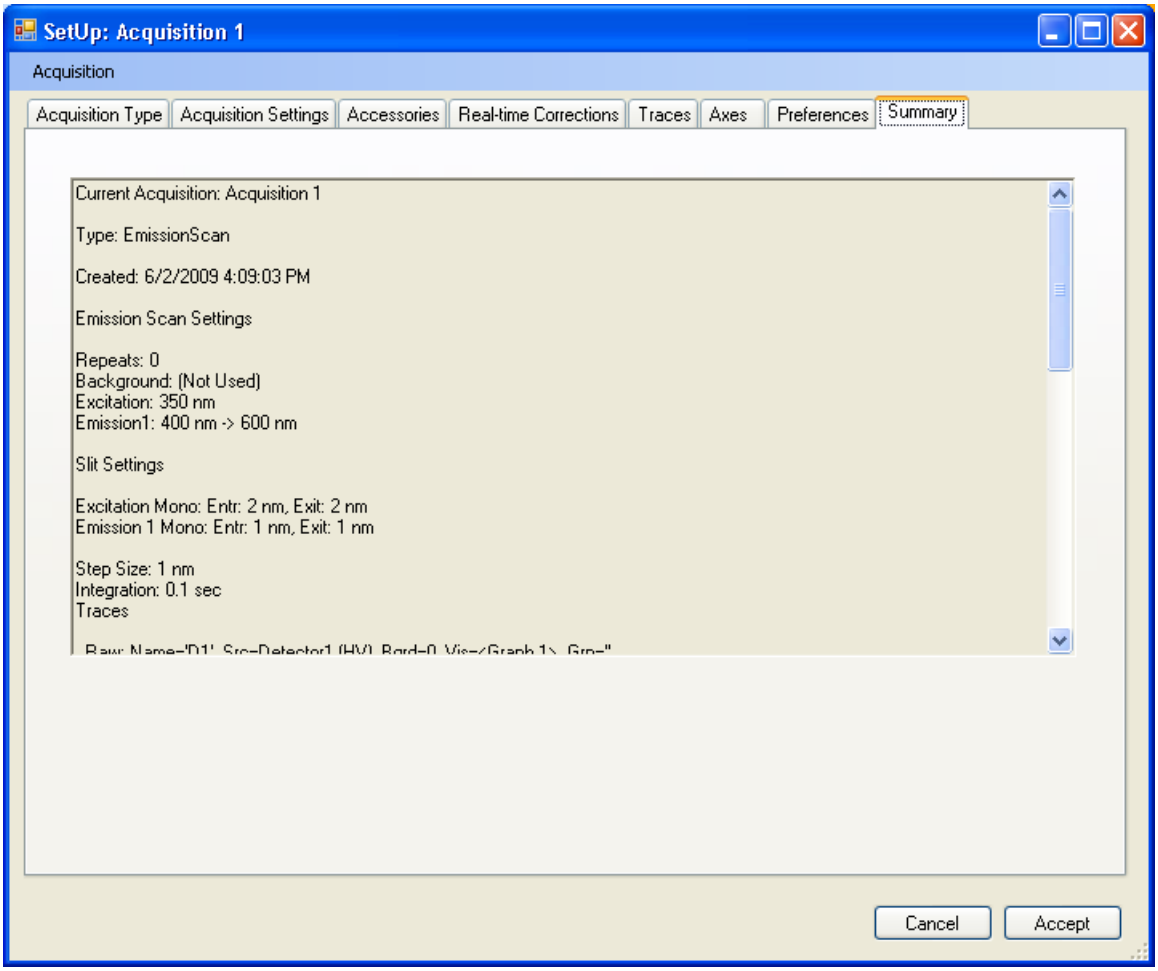
Always create a new Session: If this preference is ON, then every new scan will be placed in a new session. If this preference is OFF, then new traces will be added to the same groups as in the previous scan.

When a new session is started, it is automatically named with the acquisition name plus the time stamp YYYY-MM-DD HH:MM:SS AM/PM.

Comments

Enter any comments into the box provided.

Summary



The above picture shows the Summary tab. The information shown here is the same shown in the Acquisition Description below the legend and appended to Trace Properties. The first line shows the acquisition name if it has already been saved. The second line shows the hardware type, underscore character, and the acquisition type.

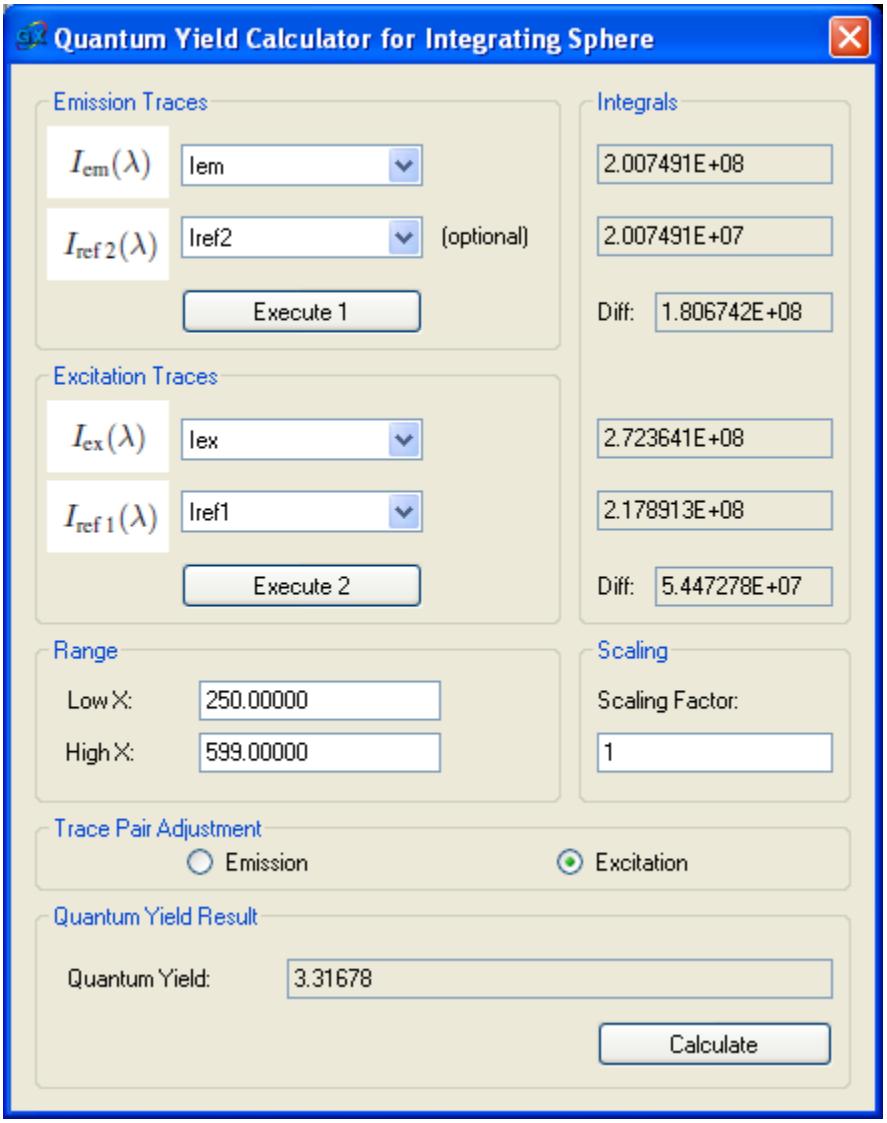
Optional Features

Quantum Yield Calculator

Acquire the data:

1. Sample emission spectrum, $I_{em}(\lambda)$ with the excitation wavelength at λ_{ex}. The spectrum should include the entire emission range. The spectrum should be corrected for the spectral sensitivity of the emission channel and expressed in quanta.
2. Sample scattered spectrum $I_{ex}(\lambda)$ with the excitation wavelength at λ_{ex}. This emission scan should be acquired starting before λ_{ex} and include the excitation wavelength range. The spectrum should be corrected for the spectral sensitivity of the emission channel and expressed in quanta. *(Note: $I_{em}(\lambda)$ and $I_{ex}(\lambda)$ can be represented by a single spectrum measured from before λ_{ex} until the end of the emission spectrum if the intensities of the scattered peak and the emission band can be measured at the same slit settings and none of the peaks requires attenuation).*
3. Reference scattered spectrum $I_{ref1}(\lambda)$ that does not include the fluorophore (e.g. solvent alone) measured under identical conditions as $I_{ex}(\lambda)$, emission-corrected and expressed in quanta.
4. If the sample scattered spectrum $I_{ex}(\lambda)$ and the reference scattered spectrum $I_{ref1}(\lambda)$ were acquired with attenuation, then this attenuation must be measured. Repeat step 3 without attenuation to give reference scattered spectrum without attenuation $I_{ref1\text{-}att}(\lambda)$.
5. Optional: Reference emission spectrum $I_{ref2}(\lambda)$ that does not include the fluorophore (e.g. solvent alone) measured under identical conditions as $I_{em}(\lambda)$, emission-corrected and expressed in quanta. *(Note: $I_{ref1}(\lambda)$ and $I_{ref2}(\lambda)$ can be represented by a single spectrum measured from before λ_{ex} until the end of the emission spectrum if items (a) and (b) were also represented by a single spectrum.*

Analyze the data



Emission Traces

6. **$I_{em}(\lambda)$**: Choose the sample emission spectrum from the choice list.
7. **$I_{ref2}(\lambda)$**: Choose the reference emission spectrum from the choice list, or <*none*>.
8. Select the integration range for the emission spectra by clicking on $I_{em}(\lambda)$ or $I_{ref2}(\lambda)$ in the legend and dragging left and right range bars.
9. Click **Execute** to calculate the areas (Integrals) under $I_{em}(\lambda)$ (= A1) and $I_{ref2}(\lambda)$ (= A2) within the selected integration range and to calculate the difference (A3 = A1 - A2). If <*none*> was selected in step 3, then A2 = 0 for any integration range.

Excitation Traces

10. **$I_{ex}(\lambda)$**: Choose the sample scattered spectrum from the choice list.
11. **$I_{ref1}(\lambda)$**: Choose the reference scattered spectrum from the choice list.

12. Select integration range for the excitation peak by dragging the mouse from integration start to integration end.
13. Select the integration range for the excitation peak by clicking on $I_{ex}(\lambda)$ or $I_{ref1}(\lambda)$ in the legend and dragging left and right range bars.
14. Click **Execute** to calculate the areas (Integrals) under $I_{ex}(\lambda)$ (= A4) and $I_{ref1}(\lambda)$ (= A5) within the selected integration range and to calculate the difference (A6 = A4 – A5).
15. If the sample scattered spectrum $I_{ex}(\lambda)$ and the reference scattered spectrum $I_{ref1}(\lambda)$ were acquired without attenuation, then the scaling factor R = 1. If reference scattered spectrum without attenuation $I_{ref1\text{-}att}(\lambda)$ was acquired, then use **Trace Math, Combine** to divide $I_{ref1}(\lambda)$ by $I_{ref1\text{-}att}(\lambda)$ to produce the trace Attenuation(λ). Near λ_{ex}, Attenuation(λ) may drop if the peak was saturated. Find the region away from λ_{ex}, that is reasonably level and use **Trace Math, Average** to find the average value of this region. Enter this value as the scaling factor R in the **Scaling Factor** box. The scaling factor accounts for potential attenuation of the scattered excitation peak (i.e. different slits, ND filter etc…) compared to the emission peak.
16. Click **Calculate** to calculate the Quantum Yield = A3*R/A6.

Trace Pair Adjustment: These radio buttons automatically shift between **Emission** and **Excitation** whenever selecting the respective traces in the calculator. Also, the user can click on one of these radio buttons and then drag the integration range bars in the graph, and the respective emission or excitation integrals and difference value and the quantum yield value will change immediately.

Color Coordinate Calculator

Introduction

The purpose of a color coordinate system is to quantify visual (human eye) perception of a color. In the retina of human eye there are photoreceptor cells (rods and cones) that react to some range of light colors and intensities and transmit the light signal to the brain. There are 3 types of the photoreceptors, each sensitive to a different spectral range within the total human eye sensitivity range of 380-700 nm. These are 'blue', 'green' and 'red' cones with sensitivity peaking at about 450, 540 and 600 nm, respectively. Perception of any color or hue can be considered a linear combination of responses from these different photoreceptor types.

Loosely based on the above photo physiology, in 1931 the International Commission on Illumination (Commission Internationale de l'Eclairage), CIE, defined the color system called CIE 1931 color space. They defined 3 'color matching functions' (akin to the human eye sensitivity functions) which represent the chromatic response of an observer. One can envision this as having 3 hypothetical photomultipliers whose respective sensitivities are described by the 3 color matching functions and each PMT produces some kind of average response for its sensitivity range (so-called tristimulus values).

In 1976 CIE introduced a modified color coordinate standard.

Calculations

1. CIE 1931 chromaticity coordinates

We measure the intensity distribution as a function of wavelength, I(λ). For fluorescence data, this could be an excitation or an emission spectrum. The color matching functions, as designated by CIE 1931, are x'(λ), y'(λ) and z'(λ).

The tristimulus values are defined as:

$$X = \int_0^\infty \mathrm{I}(\lambda)\mathrm{x}'(\lambda)\, d\lambda \tag{1}$$

$$Y = \int_0^\infty \mathrm{I}(\lambda)\mathrm{y}'(\lambda)\, d\lambda \tag{2}$$

$$Z = \int_0^\infty \mathrm{I}(\lambda)\mathrm{z}'(\lambda)\, d\lambda \tag{3}$$

These values are then normalized yielding the chromaticity values x, y and z:

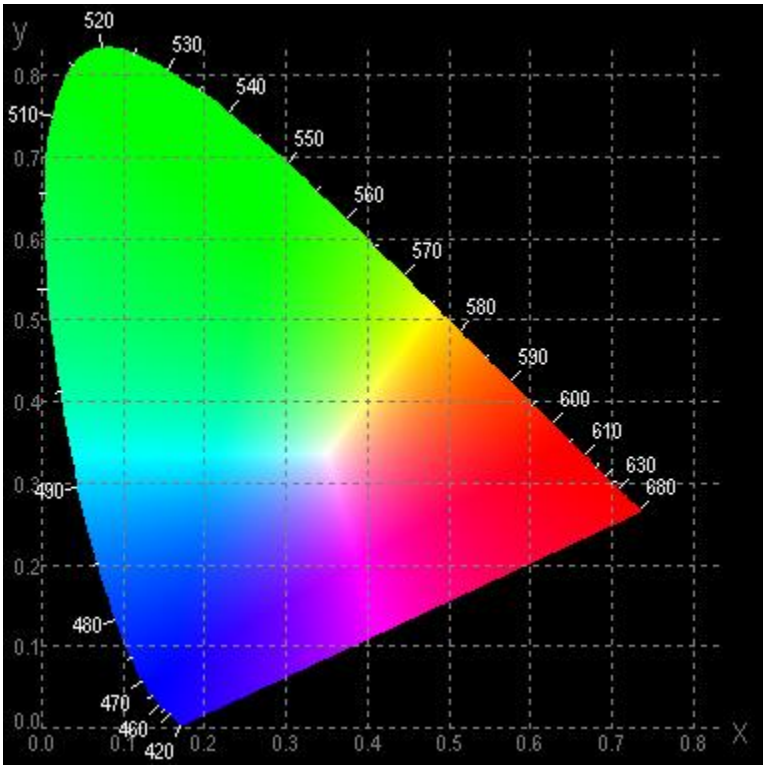
$$x = \frac{X}{X+Y+Z} \tag{4}$$

$$y = \frac{Y}{X+Y+Z} \tag{5}$$

$$z = \frac{Z}{X + Y + Z} \tag{6}$$

Since
$$x + y + z = 1 \tag{7}$$

only 2 chromaticity values are independent, i.e. only x and y need to be reported.



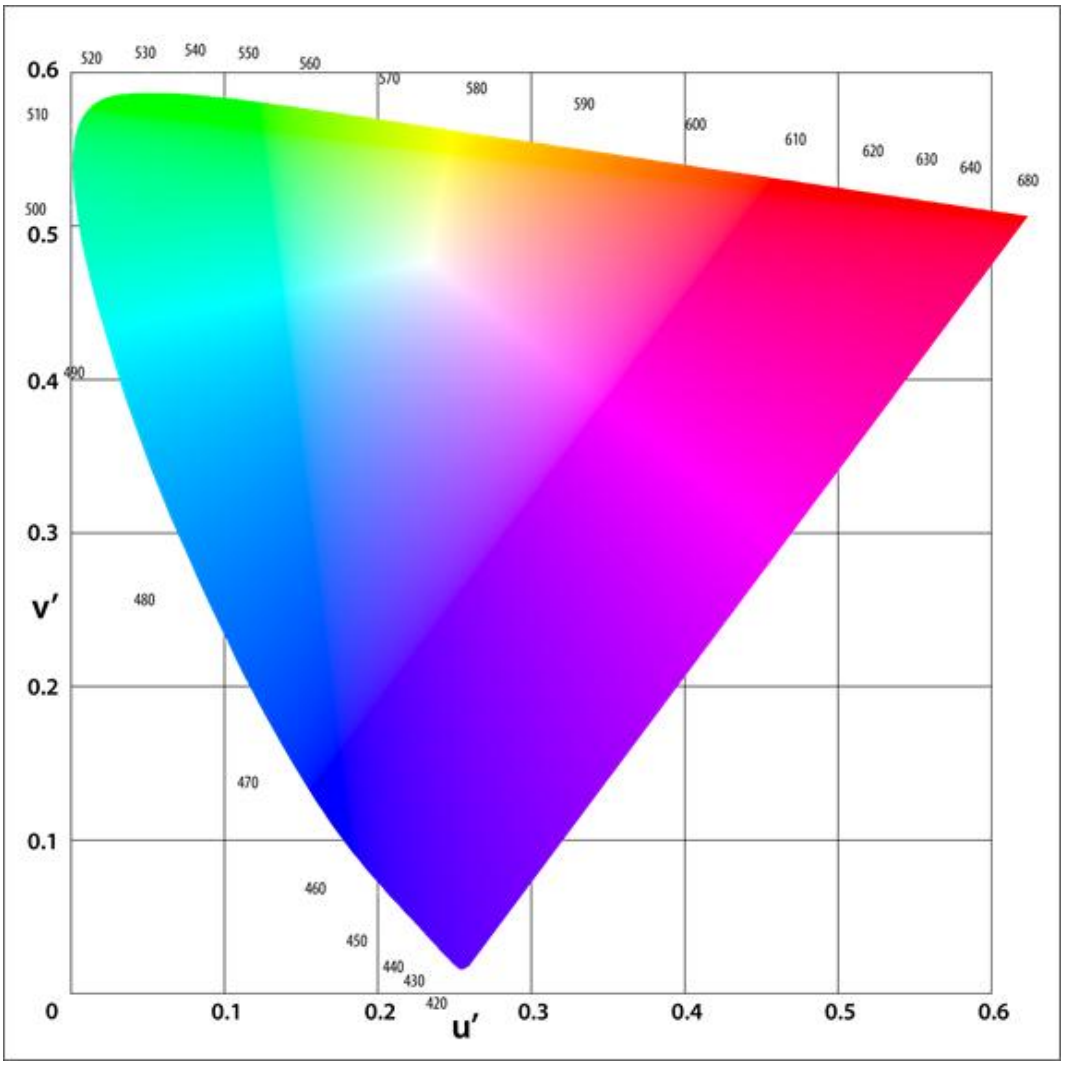
The x,y chromaticity coordinates define the CIE 1931 chromaticity diagram above. Each x,y pair corresponds to a unique color within the colored shape. The outline (boundary) of the shape corresponds to monochromatic colors within the visible spectrum.

2. CIE 1976 chromaticity coordinates

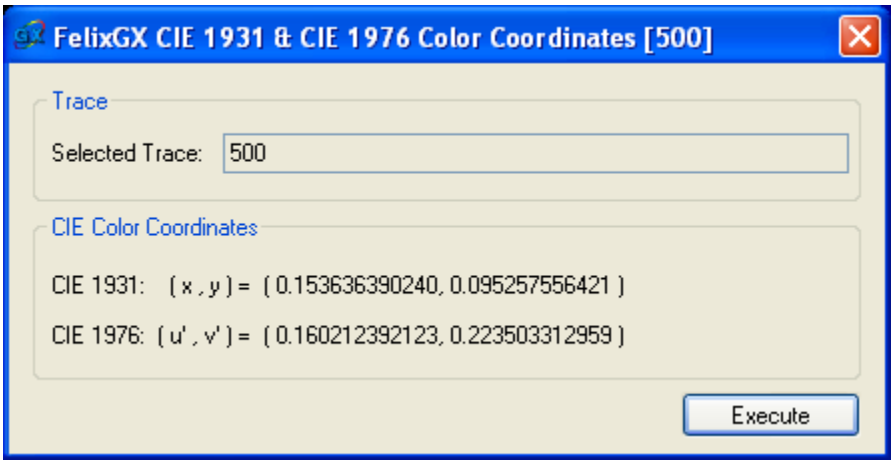
There was a number of other chromaticity coordinates defined after 1931, among them the CIE 1976 color space, which present 'more uniform perceptual chromaticity'. The CIE 1976 color space is defined by u', v' coordinates, which can be calculated via nonlinear transformations of x, y (CIE 1931) coordinates according to the following equations:

$$u' = \frac{4x}{-2x + 12y + 3} \tag{8}$$

$$v' = \frac{9y}{-2x + 12y + 3} \tag{9}$$



Math, Trace Math, CIE 1931 and CIE 1976 Color Coordinates to show the dialog.



In the legend, click on any spectral trace and then click on **Execute** to show the CIE 1931 and CIE 1976 Color Coordinates.

Chapter 14

FelixGX at Work

Perhaps the best way to understand how all of the features of FelixGX and your instrument go together is to walk through some examples of fluorescence analyses on samples that are easily reproduced.

As one exercise, we will measure the Raman scatter of water, which can be used to determine the sensitivity of your instrument. The second exercise is a titration of the calcium indicator Fura-2 with calcium.

Raman Scatter of Water

The Raman scatter of water can be used as a quick check of an instrument's overall functional integrity, and also to measure its sensitivity. The peak in the spectrum of water is not due to fluorescence; it is Raman scattering that gives rise to the fluorescence-like response of water. It simulates fluorescence nicely in that the scattered light is observed at a longer wavelength than excitation. The signal is of low intensity, making it an appropriate test for the sensitivity of a fluorescence spectrometer.

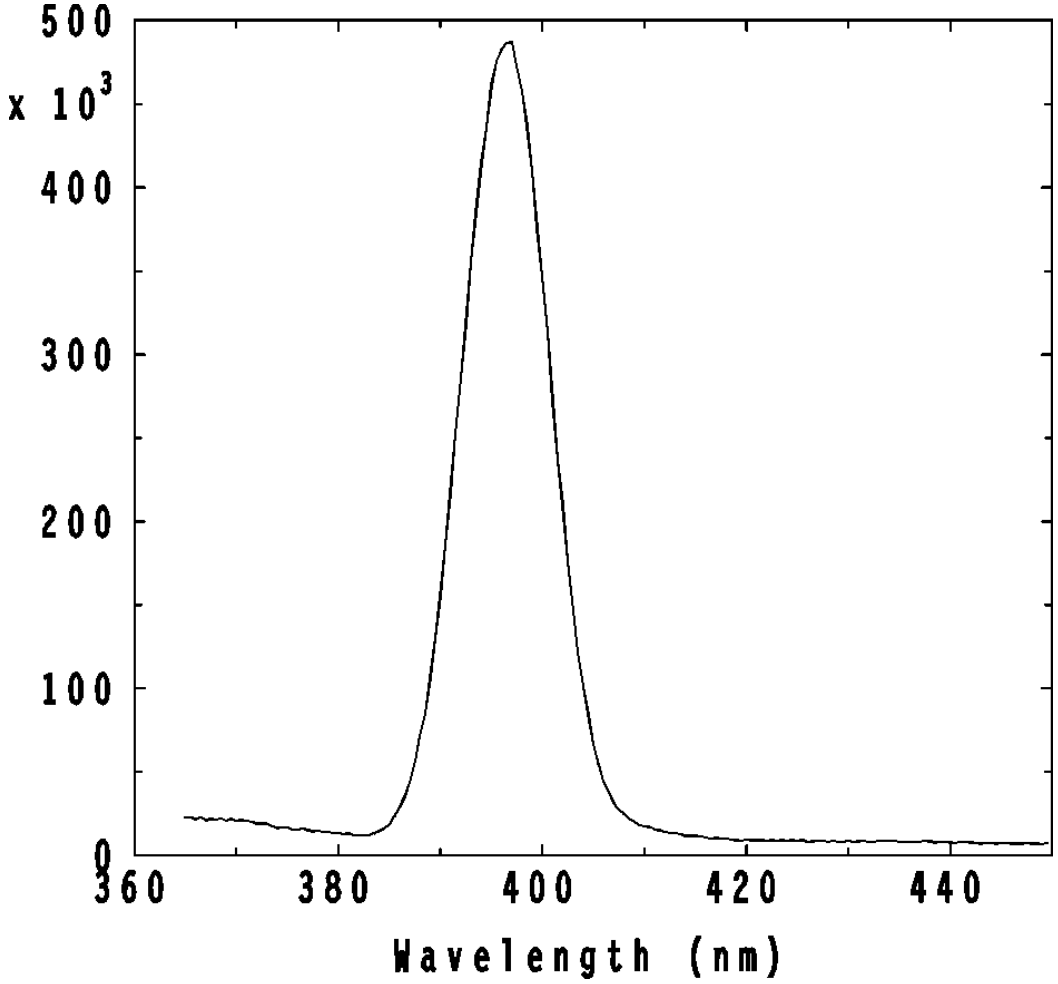
The wavelength maximum of the Raman band of water is dependent on the excitation wavelength. The scatter peak is always red-shifted (toward longer wavelengths) 3382 cm^{-1} from the excitation wavelength. If your excitation monochromator is set at 360 nm, for example, the peak will be at 410 nm.

Choose New Acquisition/Emission Spectra and set it up as follows:

Acquire:	Emission Scan
Excitation:	350 nm
Start:	365 nm
Stop:	450 nm
Length:	85 nm
Step Size:	0.5 nm
Integration:	1 second
Bandpass:	5 nm*

*for the entrance and exits sides of both the excitation and emission monochromators (2.5 turns of the slit micrometers for a model 101M monochromator with a 1200 line/mm grating and the DeltaRAM X).

Fill a clean, 1 cm, quartz cuvette with distilled water, tap it to displace any bubbles adhering to the walls, and place it in the sample compartment. Click **START**. The Raman band should appear as shown with the peak at 397 nm. The intensity at the peak should be between 300,000 and 800,000 counts per second (cps) and the data on the baseline should be relatively noise-free. The number of counts apply for a model 101M monochromator with standard gratings, other systems may give differing results.



Signal-to-Noise Ratio

For details on the measurement of the signal-to-noise ratio of the Raman band of water, ask for the PTI Technical Note, "The Measurement of Sensitivity in Fluorescence Spectroscopy," see American Laboratory, September 1994, page 32G, or visit our website at www.pti-nj.com.

Titration of Fura-2 with Calcium

This section outlines a procedure for calibration of Fura-2 experiments for the measurement of calcium. A Fura-2 titration is carried out with known concentrations of free Ca^{++} that are controlled by Ca^{++}/EGTA buffers. The resulting data are used to determine the dissociation constant, Kd, of the Ca^{++}/Fura-2 complex. Essentially, this procedure reproduces the results in figure 3 of the original work by G. Grynkiewicz, M. Poenie, and R.Y. Tsien, "A New Generation of Ca^{++} Indicators with Greatly Improved Fluorescence Properties", Journal of Biological Chemistry, **260**, 3340 (1985).

This is approximately a three-hour exercise that is meant to acquaint you with the operation of FelixGX and your instrument. Although a more rigorous calibration procedure may be needed in some cases, this exercise will provide valuable experience with steady state ratio fluorescence measurements and result in data that will unequivocally indicate your mastery of the technique and the thorough understanding of FelixGX.

Obtaining a satisfactory set of titration traces, which yield a dissociation constant comparable to the literature value, will also confirm the performance of the instrument and the condition of the reagents.

In the following, subscripts 1 will refer to 340 nm and 2 will refer to 380 nm. These wavelengths are appropriate for Fura-2. It should be understood that other wavelengths may be chosen and that different indicators will have different wavelength pairs that should be selected for the calibration equation.

The calibration equation is:

$$\left[Ca^{++}\right] = K_d \bullet \left(\frac{R - v \bullet R_{\min}}{v \bullet R_{\max} - R}\right) \bullet \left(\frac{Sf_2}{Sb_2}\right)$$

$R = F_1/F_2$, the ratio of fluorescence intensities obtained with excitation at $\lambda_1 = 340$ nm and $\lambda_2 = 380$ nm.

R_{min}, $R_{max} = F_1/F_2$ ratios of the calcium-free and calcium-saturated Fura-2 sample, respectively.

$Sf_2 = F_{2,min}$ of the calcium-free Fura-2 sample (i.e., the fluorescence intensity at 380 nm of the calcium free sample).

$Sb_2 = F_{2,max}$ of the calcium-bound (saturated) Fura-2 sample (i.e., the fluorescence intensity at 380 nm of the calcium-bound (saturated) sample).

K_d is the effective dissociation constant for the Ca^{++}/Fura-2 complex.

v is the intracellular viscosity. For water this value is close to 1.0.

The calibration equation becomes

$$\left[Ca^{++}\right] = K_d \bullet \left(\frac{R - R_{\min}}{R_{\max} - R}\right) \bullet \left(\frac{F_{2,\min}}{F_{2,\max}}\right)$$

Preparation for Measurement

Prepare two solutions of pH 7.0, 10 mM EGTA buffers containing 100 mM KCl and 10 mM K-MOPS. One of the buffers will contain 10 mM Ca^{++} (use a 1 M $CaCl_2$ stock solution); the other will contain no Ca^{++}. They will be called CaEGTA and EGTA buffers, respectively.

Fura-2 will be added to both buffers during the exercise. If 1 mM Fura-2 stock solutions are used, a thousand-fold dilution of the stock would yield 1 μM final Fura-2. We have found it convenient to store Fura-2 frozen in 50-μl quantities.

During the exercise, you will prepare a range of free calcium concentrations by removing specific volumes of EGTA buffer and replacing them with CaEGTA buffer.

To a washed and dried test tube, add 8.991 ml of CaEGTA buffer and 9 μl of the 1 mM Fura-2 stock solution to obtain a final Fura-2 concentration of 1 μM. Mix the contents of the test tube thoroughly.

Excitation Scan Measurements

This section describes the preparation of a range of calcium-Fura-2 solutions by serial exchange of reagents. An excitation scan is performed for each solution. The excitation scans will be used to calculate the dissociation constant.

In FelixGX, select *Excitation Scan* from the **New Acquisition** menu. Enter the following parameters:

Start:	300 nm
Stop:	450 nm
Emission:	510 nm
Step Size:	0.5 nm
Integration Time:	0.25 sec.

Set all slits to 3 nm bandpass (1.5 turns of the slit micrometers for a model 101M monochromator and DeltaRAM X with standard gratings and 3 turns for a model 201M monochromator).

You will be making several volume exchanges in the cuvette and measuring the excitation spectrum of each.

Add 2.997 ml of EGTA buffer to a clean, dry cuvette (use a 1 ml digital pipette set to 0.999 ml) that is optically transparent above 300 nm. A quartz cuvette is the best choice. Use caution with plastic cuvettes. A plastic cuvette may be opaque to 340 nm light.

Click **Start** to verify that you have a relatively flat baseline with no fluorescence due to contaminants. Your instrument is very sensitive, so you may observe a peak at about 435 nm from the Raman band of water. This will not affect the measurement since it is beyond the wavelength of interest. Keep this trace. If it is of comparable intensity to the

subsequent traces with Fura-2 in the solution, then you may want to use it to subtract it from all of the subsequent traces before analyzing the results.

Introduce 3 µl of Fura-2 into the 2.997 ml EGTA buffer directly into the cuvette using a 10 µl adjustable pipette. This results in a 1 µM final concentration of Fura-2. Using a 1 ml digital pipette set to 1 ml, carefully siphon and subsequently release the sample in the cuvette to ensure thorough mixing. 4-5 such cycles should suffice. (If 3 µl cannot be delivered with precision to the cuvette, add 9 µl of Fura-2 to 2.991 ml of the EGTA buffer in the cuvette and remove 2 ml after mixing. With a fresh pipette tip, dilute the remaining 1 ml in the sample cuvette with 2.0 ml of EGTA buffer. Repeat 4-5 cycles of mixing.)

Take the fluorescence excitation spectrum of the sample containing 1 µM Fura-2 by scanning from 300 to 450 nm. Make sure that a peak is observed at about 370 nm. If the maximum wavelength is much shorter, calcium may have been introduced at some point or the cuvette was not calcium-free when you added Fura-2. In that case, the procedure must be repeated from the beginning.

You may save the spectrum of the sample with no calcium by using the File/Save As command. Remove 300 µl of the sample with the digital pipette, discard and replace with 300 µl of the CaEGTA buffer preparation containing Fura-2. Take the excitation spectrum again and save it with File/Save.

Remove 333 µl of the sample from the cuvette and replace it with 333 µl of CaEGTA buffer containing Fura-2. Measure the excitation spectrum and save it.

See the Table on the following page. Continue to exchange the volumes in the first column and measure the excitation scan. These serial exchanges take you through a series of measurements of a solution containing 9 mM EGTA and 1 mM CaEGTA, 8 mM EGTA and 2 mM CaEGTA, etc… The CaEGTA concentration is increased by 1 mM and the EGTA concentration is concurrently decreased by 1 mM at each subsequent step by replacing a volume of 3/(11–n) ml, where n is the number of iterations.

The Table also tabulates the respective free Ca^{++} concentrations that are controlled by the two buffers, assuming an apparent dissociation constant for the Ca^{++} EGTA complex of 380 nm at pH 7.0 in 100 mM KCl at 20°C. Note that the temperature dependence of this dissociation constant may mean that this value is only appropriate for data gathered at 20°C and conversely, that K_d values measured at 20°C may not be correct for analyzing experimental data gathered at other temperatures. Thus:

$$Kd = [Ca^{++}][EGTA]/[CaEGTA]$$

$$[Ca^{++}] = K[CaEGTA]/[EGTA]$$

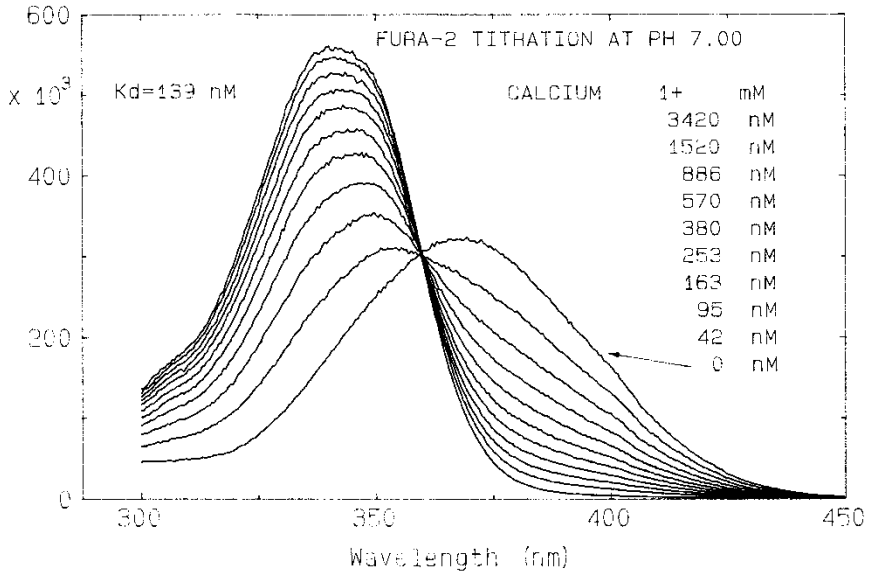
$$= 380 \times 1/9,\ 380 \times 2/8,\ 380 \times 3/7,$$
$$380 \times 4/6,\ 380 \times 5/5,\ ...380 \times 9/1.$$

CALCULATION WORKSHEET

Volume Exchange, ml	$[Ca^{++}]$ nm	R	$R - R_{min}$	$\frac{R - R_{min}}{[Ca^{++}]}$
0	0	.61776	—	—
0.3	42.2	1.11306	0.4953	0.01174
0.333	95	1.73439	1.11663	0.01175
0.375	162.85	2.44889	1.83113	0.01124
0.429	253.33	3.31391	2.69615	0.01064
0.5	380	4.53287	3.91511	0.01030
0.6	570	5.94783	5.33007	0.00935
0.75	880	7.81845	7.20069	0.00813
1.0	1520	10.60804	9.99028	0.00657
1.5	3420	15.20347	14.58571	0.00426
2.990	>0.1-mM	22.15208	—	—

Note that the first value for R, 0.61776, becomes R_{min}. Following the last measurement, 30 µl of 100 mM $CaCl_2$ solution are added to bring the free calcium concentration above 1 mM and saturate Fura-2, and another spectrum is taken. That value becomes R_{max}.

Isosbestic Point



The superimposed excitation spectra all intersect at a single point, the isosbestic point, indicating that the spectra are linear combinations of two components and reflect an equilibrium between these two components. It is very critical to perform the above dilution series as carefully as possible. Otherwise, the isosbestic point will not be well defined, and the ratios obtained from the spectra may not reflect the true concentrations. (The isosbestic point does not depend on the tabulated values of dilutions; any haphazard value of exchange volume will result in the traces intersecting at the isosbestic point as long as the exchanged volumes are precisely identical, e.g., 1.23456 ml exchanged for 1.23456 ml. However, the calcium concentration in the table will obviously change accordingly.)

Ratio Determination

Using the Data Cursor, measure and note the intensity values at 340 and 380 nm for each excitation spectrum. Calculate the ratio $R = F_{340}/F_{380}$ and enter it into a separate column next to the corresponding calcium concentration. Calculate the difference $R - R_{min}$ and the ratio $(R - R_{min})/[Ca^{++}]$ and enter these values into a separate column as well.

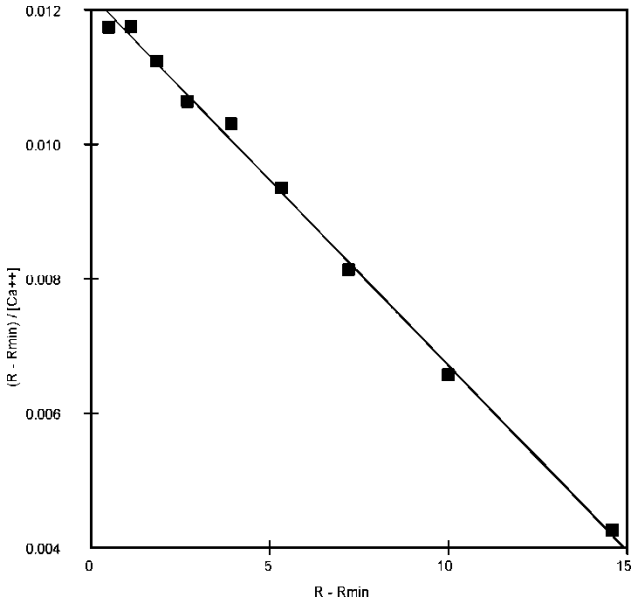
K_d Determination

Plot the values $(R - R_{min})/[Ca^{++}]$ as a function of $R - R_{min}$ and obtain a linear fit. Note that the final measurement of R is not used because of uncertainty in the calcium concentration. The slope of the fitted line is m and thus $K_d = -1/(m \times Sf_2/Sb_2)$ where Sf_2 is the fluorescence intensity at 380-nm of the Ca-free sample and Sb_2 is the fluorescence intensity at 380 nm of the Ca-bound sample. Linearization of the calibration equation is given at the end of this section. Data from the family of excitation scans yield $Sf_2/Sb_2 = 12.917$.

Linear Fit of Data from Worksheet

Calculating: $K_d = -(1/(m \times (Sf_2/Sb_2)))$

Yields: $K_d = 139$ nm



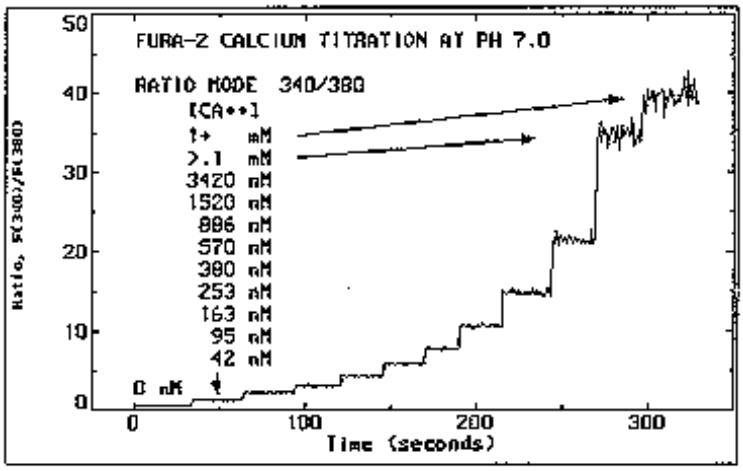
Excitation Ratio Mode Measurement

PTI RatioMaster systems are capable of direct measurement of R-values. Instead of measuring the excitation spectra of Fura-2 at various calcium concentrations and subsequently determining the F_{340}/F_{380} ratios from the spectra, the ratios can be directly measured in Excitation Ratio mode.

Select **Setup**, **Excitation Ratio**. Enter the following parameters in the **Acquisition Settings** tab:

Excitation 1:	340 nm
Excitation 2:	380 nm
Emission:	510 nm
Points/sec:	15 (DeltaRAM
Integration:	0.1 sec (monochromator-based systems
Duration:	650 sec
Repeats:	1
Pause:	Not applicable
View Window:	650 sec

Go to the **Traces** tab. Add a new **Derived Data** trace, select the 340 nm trace for **Source 1** and the 380 nm trace for **Source 2** on the choice lists to and select **Divide** from the function box to calculate and display the ratio. We recommend that the ratio and intensity be displayed in separate groups.

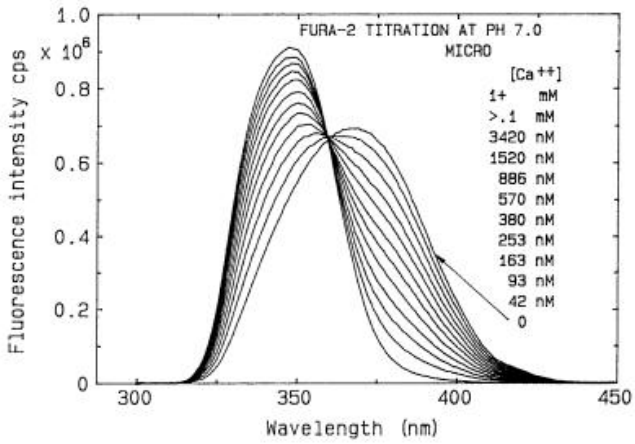


The titration proceeds exactly as before. Move the **Acquisition Control Pane**l up to see a clock. The excitation ratio is paused between sample dilutions: click **Pause** on the **Acquisition Control Panel** after 50 seconds of data acquisition to pause for sample manipulation, then click on **Start** to resume data acquisition. The ratio values are determined from the stepwise titration trace by taking the average value of each step segment. Subsequent calculations are the same as above.

Fura-2 Titration with a Microscope-Based System

When the titration is done in a tissue chamber on a microscope stage, the fluorescence excitation spectra of Fura-2 will differ from those obtained in a cuvette-based system.

The light transmission properties of the optical path of the microscope are responsible for a significant reduction in UV intensity. Therefore, the excitation peak of the Ca-saturated form of the dye will be less than twice as intense as the Ca-free form. Also, the excitation peak position will tend to be shifted towards the longer wavelengths. The extent of this observed shift is strongly dependent on the quality of the UV optics in the microscope objective that is employed. An example of this effect is shown in the figure below. The background was subtracted from all traces prior to display.



The calculations presented in the preceding section will still be valid and correct K_d values may be obtained from this family of traces as well. It is understood, however, that the experimental values to be tabulated for the calculations will be different.

Chapter 14

Troubleshooting

Loss of communication with the ASOC-10 MD-4000, or QNW Controller

If this happens, you can leave FelixGX open, and leave the ASOC-10, MD-4000(s), and QNW Controller powered on. Simply disconnect the USB cables from these control boxes to the computer and plug them back in again. Then retry the command that led to the loss of communication message.

If this does not work, check that InstaCal recognizes the ASOC-10. Here, you must close FelixGX, then run InstaCal to see if it recognizes the ASOC-10 and assigns a board number to it.

If the above fails contact PTI Service.

Service Calls to PTI

Before contacting PTI 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 contacting PTI.

- Your system serial number, or as many other component serial numbers as possible
- The name of the purchaser or principal investigator, and the company or institution where the instrument is located.
- Your instrument type and hardware configuration
- Computer details, especially if the computer was not purchased from PTI:
  - Operating System and Operating System Service Packs installed (e.g., Windows XP Professional, year, Service Pack 3)
  - CPU – Intel or AMD
  - Hard drive size and amount of free space on the hard drive
  - RAM memory size
  - Video RAM size
  - Video card manufacturer and model number (if not on the computer motherboard)
  - An Ethernet port other than on the computer motherboard – manufacturer and model number
  - Any other peripherals attached to the computer
- The software name and version (in the program window, click on **Help, About** to find the software name and version information).
- The date on which your instrument was installed
- 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.
- If possible, be prepared to send sample data and hardware and acquisition setup files as e-mail attachments to PTI service personnel.

Contact PTI Service at
Toll Free: 877-784-4349 US/Canada
Phone: 609-894-4420 Ext 115
Fax: 609-894-1579
E-mail: PTIService@pti-nj.com