FLSP920 Series User Guide

FS920 - FL920 - FP920 - FSP920 - FLP920 - FLS920 - FLSP920

Revision 4

Copyrights

Copyright © 2012 Edinburgh Instruments Ltd. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or any means electronic or mechanical, including photocopying and recording for any purpose other than the purchaser's personal use without written permission.

Information in this document is subject to change without notice. While every effort has been taken to make sure that the information given is correct, Edinburgh Instruments Limited cannot accept any liability for errors and omissions or their consequences. The software described in this document is furnished under a license agreement or nondisclosure agreement. The software may be used or copied only in accordance with the terms of those agreements.

Edinburgh Instruments Ltd.

2 Bain Square

Kirkton Campus

Livingston

EH54 7DQ

UK

www.edinst.com

Contents

OVERVIEW **3**

1. INTRODUCTION TO THE FLSP920 SERIES OF FLUORESCENCE SPECTROMETERS **4**

1.1. FLSP920 SERIES MODELS **5**
1.2. SPECTROMETER UPGRADES **10**

2. SPECTROMETER COMPONENTS **11**

2.1. LIGHT SOURCES **11**
2.1.1. Xe900 Continuous Xenon Lamp *11*
2.1.2. µF920H Xenon Flashlamp *13*
2.1.3. nF920 Nanosecond Flashlamp *15*
2.1.4. Other Light Sources *17*
2.2. MONOCHROMATORS **17**
2.2.1. Excitation Monochromator *18*
Emission Monochromator *19*
2.2.2. Monochromator Gratings *20*
2.3. SAMPLE CHAMBER **21**
2.3.1. Standard Lens Optics *22*
2.3.2. Mirror Optics and Other Beam Steering Options *23*
2.4. SAMPLE HOLDERS **23**
2.4.1. Standard Single Cuvette Holder *23*
2.4.2. Other Sample Holders and Sample Positioning Options *24*
2.5. DETECTORS **25**
2.5.1. Red Sensitive Photomultiplier in Cooled Housing *25*
2.5.2. Other Detectors *26*

3. INTRODUCTION TO F900 SOFTWARE **27**

3.1. STARTING F900 .... **27**
3.2. GENERAL FEATURES OF THE F900 MAIN WINDOW **27**
3.3. SIGNAL RATE WINDOW **29**
3.4. SETUP MENUS **30**
3.4.1. nF Lamp Setup *30*
3.4.2. µF Lamp Setup *31*
3.5. SCAN TYPES AND SCAN PROPERTIES **32**
3.5.1. Spectral Data *32*
3.5.2. Time-Resolved Data *36*
3.6. GRAPHICAL PRESENTATIONS AND PLOT OPTIONS **37**
3.6.1. 2D Data Presentation *37*
3.6.2. 3D Data Presentation *39*
3.6.3. Contour Plots *40*
3.6.4. View Numerical Data *41*
3.7. DATA OPERATION AND ANALYSIS WITH F900 .... **42**
3.8. SOFTWARE AND HARDWARE OPTIONS **43**

4. MAKING SPECTRAL MEASUREMENTS **45**

4.1. EMISSION SCANS **46**
4.2. EXCITATION SCANS **47**
4.3. SYNCHRONOUS SCANS **48**
4.4. CORRECTION SCANS **49**
4.4.1. Excitation Correction Scans *50*
4.4.2. Emission Correction Scans *51*
4.5. SPECTRAL ANISOTROPY SCANS **53**
4.5.1. Excitation Anisotropy Scans *54*
4.5.2. Emission Anisotropy Scans *55*

4.6. EMISSION MAPS 57
4.7. SYNCHRONOUS MAPS 59
4.8. TIPS FOR MAKING GOOD SPECTRAL MEASUREMENTS 60

5. MAKING TIME RESOLVED MEASUREMENTS 65

5.1. KINETIC MEASUREMENTS 67
5.2. MANUAL TIME RESOLVED MEASUREMENTS – MCS 68
5.2.1. Operation of the μF900H 68
5.2.2. Measurement of a Phosphorescence Decay 69
5.2.3. Measurement of the Instrument Response Function 70
5.3. MANUAL TIME RESOLVED MEASUREMENTS – TCSPC 71
5.3.1. Operation of the nF920 71
5.3.2. Measurement of a Fluorescence Decay 72
5.3.3. Measurement of the Instrument Response Function 74
5.4. MULTIPLE TIME RESOLVED MEASUREMENTS 74
5.5. TRES-MAP MEASUREMENTS 75
5.5.1. Excitation TRES-Map Measurements 75
5.5.2. Emission TRES-Map Measurements 76
5.6. TIME RESOLVED ANISOTROPY MEASUREMENTS 77
5.7. TIPS FOR MAKING GOOD LIFETIME MEASUREMENTS 80

6. DATA OPERATIONS ON SPECTRAL DATA 85

6.1. ADD, SUBTRACT, MULTIPLY, DIVIDE 85
6.2. APPEND 86
6.3. SCALE, NORMALISE, SUBTRACT BASELINE, CROP RANGE 86
6.4. SMOOTH 88
6.5. DIFFERENTIATE AND INTEGRATE 89
6.6. ANISOTROPY 90
6.7. SPECTRAL CORRECTION 91

7. DATA OPERATIONS AND ANALYSIS ON TIME RESOLVED DATA 93

7.1. ADD, SUBTRACT, MULTIPLY, DIVIDE 93
7.2. SCALE, NORMALISE, SUBTRACT BASELINE, CROP RANGE 93
7.3. REVERSE 93
7.4. ANISOTROPY 94
7.5. TAIL FIT ANALYSIS 95
7.6. RECONVOLUTION FIT ANALYSIS 98
7.7. AUTOCORRELATION 102
7.8. TRES DATA SLICING 102

FORMULAS AND DEFINITIONS 103

GLOSSARY 105

INDEX 106

Overview

The FLSP920 Series of Fluorescence Spectrometers enable steady-state, fluorescence lifetime and phosphorescence lifetime measurements.

Read this document if you plan to use the spectrometer to make measurements or use the software to analyse your measurement results. It describes the spectrometer components, the software you use to control the spectrometer and how to make spectral and lifetime measurements. It also describes how to use the software to analyse the results.

This User Guide coveres the operation of the standard components, with two exceptions: Double monochromators and anisotropy measurements. Double monochromators are a popular alternative to the standard single monochromators, they are mentioned in chapters 1 and 2. Anisotropy measurements require the optional polariser upgrade. The acquisiton of anisotropy measurements is covered in chapters 4 and 5 and the data operation and analysis procedures are explained in chapters 6 and 7.

More Information

For details on how to install and setup the spectrometer, refer to the ***FLSP920 Series Installation Guide***.

For more detail on all the spectrometer components (standard and optional components), see the ***FLSP920 Series Reference Guide***. This contains instructions on how to maintain, clean, and replace component parts as well as specification sheets and software references.

1. Introduction to the FLSP920 Series of Fluorescence Spectrometers

The FLSP920 Series is a series of modular software-controlled spectrometers for the acquisition of steady state and time resolved photoluminescence. They can measure:

- Fluorescence and phosphorescence spectra
- Fluorescence and phosphorescence lifetimes

Data can be obtained in a wide spectral range, from the ultraviolet to near-infrared, with single photon counting sensitivity. The spectrometer series combines extremely high sensitivity with high spectral and temporal resolution.

The FLSP920 Series of Fluorescence Spectrometers is ideal for demanding applications in such areas as:

- Photophysics
- Photochemistry
- Biophysics
- Materials research

An FLSP920 Series spectrometer has the following main components:

- *Light Source* – an excitation source to generate photoluminescence from the sample
- *Excitation Monochromator* – for the selection of specific monochratic light from the full spectrum of the excitation source
- *Sample Chamber* – for optical components and sample holders
- *Emission Monochromator* – for the selection of specific monochromatic light from the full spectrum of the sample emission
- *Detector* – detects the photon flux of the sample emission at the selected wavelength
- *dedicated PC – houses data acquisition cards and is loaded with spectrometer operating software F900.*

Depending on the type of measurments you want to make, different light sources are used together with different data acquisition techniques:

Measurement Type	Standard Light Source	Data Acquisition Technique
Spectral masurements	Continuous xenon lamp (Xe900)	Single Photon Counting
Time resolved measurements in the microsecond to second range	Microsecond flashlamp (μF920H)	Multi-channel scaling (MCS) for time resolved photon counting
Time resolved measurements in the pico- to nano-second range	Nanosecond flashlamp (nF920)	Time Correlated Single Photon Counting (TCSPC)

The following section (chapter 1.1) introduces each of the seven models in the FLSP920 Series of fluorescence spectrometers. Your own spectrometer may differ from those shown in respect to light sources, detectors and optional accessories. Upgrade options are listed in chapter 1.2 on page 10. For a detailed description on those options refer to the ***FLSP920 Series Reference Guide.***

1.1. FLSP920 Series Models

The seven FLSP920 Series models are listed below.

FS920

This is a spectrometer for the acquisition of fluorescence spectra and fluorescence kinetic measurements. The FS920 is the world's most sensitive steady-state fluorescence spectrometer, with a specified signal-to-noise ratio of the Water Raman signal of 6000:1.

Figure 1-1a shows the layout of the standard version of the FS920 with single grating monochromators; Figure 1-1b shows the FS920 with double grating monochromators.

Component	Standard
Light Source	Xe900 continuous xenon lamp
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900
Acquisition Technique	Single Photon Counting (spectral scanning, kinetic measurements)

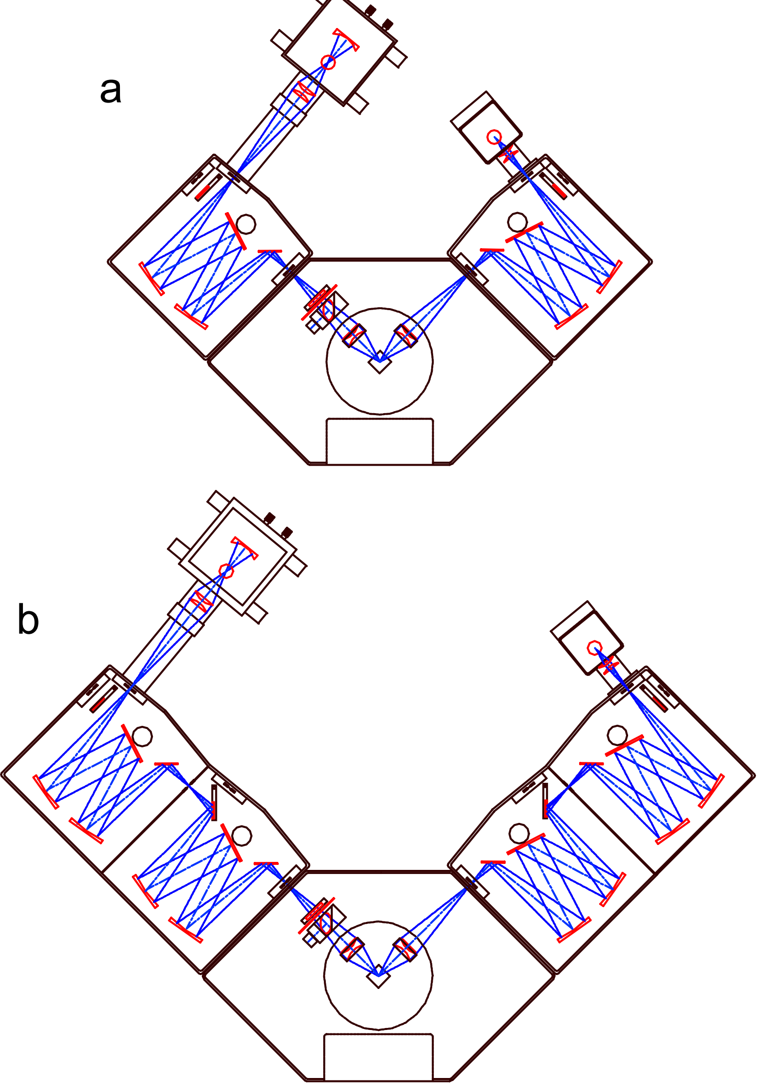


Figure 1-1: General layout of the FS920

FL920

The FL920 is a dedicated spectrometer for the acquisition of fluorescence decay kinetics the time range from picoseconds to microseconds, based on the technique of Time Correlated Single Photon Counting (TCSPC).

Component	Standard
Light Source	nF920 nanosecond flashlamp, EPLs and EPLEDs as options
Detector	Single Photon Counting PMT
Data Acquisition Card	TCC900
Acquisition Technique	TCSPC (fluorescence decay acquisitions)

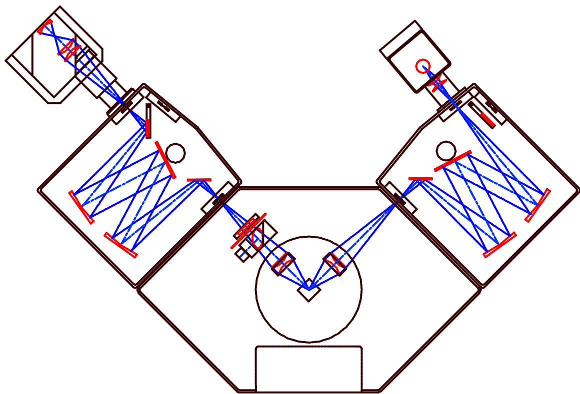


Figure 1-2: General layout of the FL920

FP920

The FP920 is a dedicated spectrometer for the acquisition of phosphorescence decay kinetics in the time range from microseconds to seconds, based on the technique of multi-channel scaling (MCS).

Component	Standard
Light Source	µF920H microsecond flashlamp
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900
Acquisition Technique	MCS (phosphorescence/lanthanide decay acquisitions)

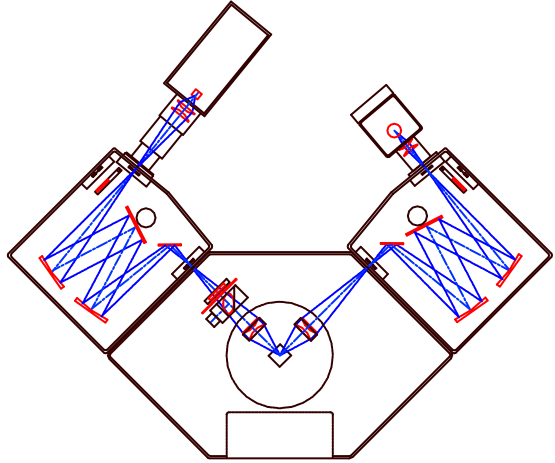


Figure 1-3: General layout of an FP920

FSP920

The FSP920 is a steady state and phosphorescence lifetime spectrometer. It can be used to measure fluorescence and phosphorescence spectra, as well as phosphorescence decay kinetics.

Component	Standard
Light Sources	Xe900 continuous xenon lamp, μF920 microsecond flashlamp
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900
Acquisition Technique	MCS (spectral scanning and phosphorescence/lanthanide decay acquisitions)

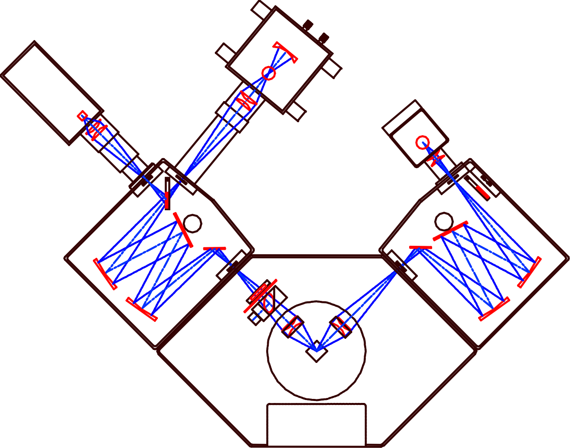


Figure 1-4: General layout of an FSP920

FLP920

The FLP920 is a dedicated instrument for the acquisition of fluorescence and phosphorescence kinetics throughout all time ranges (picoseconds to seconds).

Component	Standard
Light Sources	μF920H microsecond flashlamp, nF920 nanosecond flashlamp, EPLs/EPLEDs
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900, TCC900
Acquisition Technique	MCS and TCSPC (fluorescence and phosphorescence decay acquisitions)

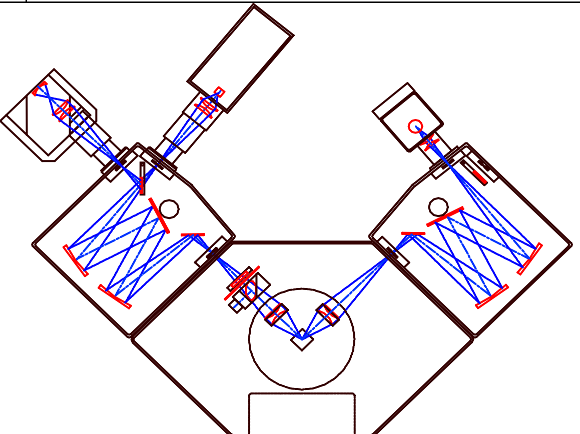


Figure 1-5: General layout of an FLP920

FLS920

The FLS920 is a combined steady state and time resolved fluorescence spectrometer.

Component	Standard
Light Sources	Xe900 continuous xenon lamp, nF920 nanosecond flashlamp, EPLs and EPLEDs as options
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900, TCC900
Acquisition Technique	Single Photon Counting, MCS and TCSPC (spectral scanning, kinetic measurements, fluorescence decay acquisitions)

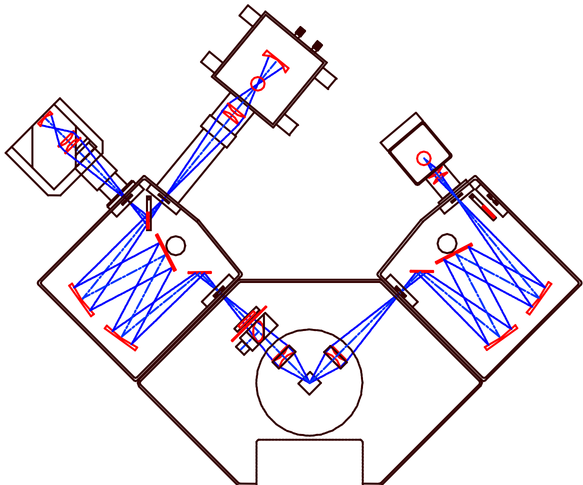


Figure 1-6: General layout of an FLS920

FLSP920

The FLSP920 is a complete fluorescence laboratory in a single instrument. It combines all the features of the FS920, FL920, and FP920.

Figure 1-7a shows the layout of the standard version of the FLSP920 with single grating monochromators, Figure 1-7b shows the FLSP920 with the popular upgrade of double grating monochromators.

Component	Standard
Light Sources	Xe900 continuous xenon lamp, μF920 microsecond flashlamp, nF920 nanosecond flashlamp, EPLs and EPLEDs as options
Detector	Single Photon Counting PMT
Data Acquisition Card	PCS900, TCC900
Acquisition Technique	Single Photon Counting, MCS and TCSPC (spectral scanning, kinetic measurements, fluorescence and phosphorescence decay acquisitions)

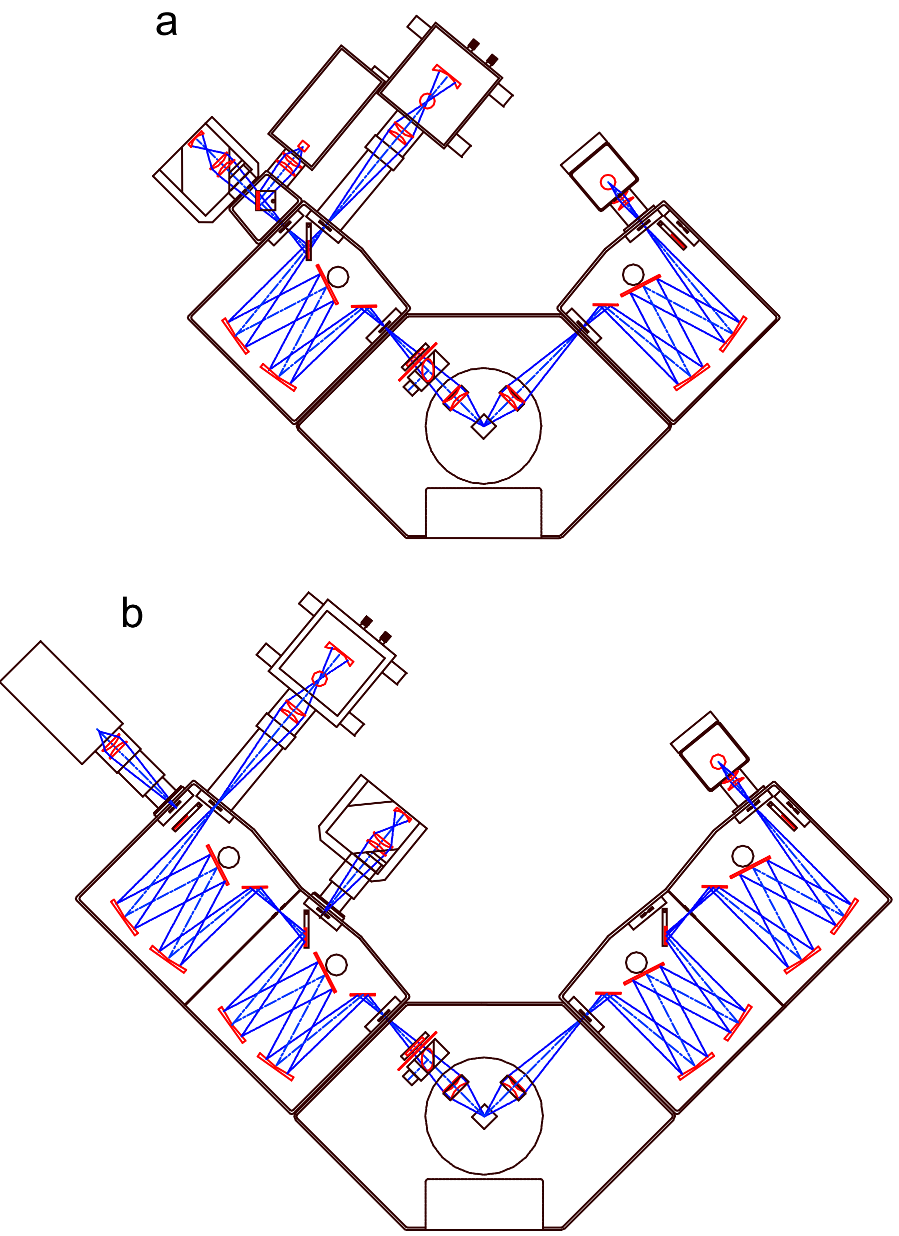


Figure 1-7: General layout of an FLSP920

1.2. Spectrometer Upgrades

The fluorescence spectrometers listed in Section 1.1 are standard configurations. Various upgrades, modifications, and enhancements are also available, giving the FLSP920 Series the flexibility to be used with a wide range of specific applications and sample requirements.

Common upgrades and enhancements include:

- Double monochromators with increased stray light performance for better measurements of highly scattering samples
- Additional emission monochromators to form a "T" geometry, enabling more than two detectors to be permanently installed (or more than three when using double monochromators)
- Polarisers for automatic acquisition of fluorescence anisotropy data
- EPLs (picosecond pulsed diode lasers) to increase temporal resolution for samples with picosecond fluorescence decays
- EPLEDs (picosecond pulsed LEDs) to increase pulse energy and pulse repetition rate for faster fluorescence lifetime measurements
- Alternative data acquisition cards for increased temporal resolution of samples with decay in nanosecond time range
- Gated photomultipliers for better discrimination between (long) phosphorescence decay and strong (short) fluorescence and/or strong sample scattering
- Near Infrared detectors

Additionally, a range of sample holders and sample cooling options are available:

- Cuvette holders with options for water circulation (for cooling or heating) with a temperature probe
- Magnetic stirrers
- Front face sample holders on slides, rotational stages, X-Y stages
- Thermo-electrically temperature-controlled cuvette holder with magnetic stirrer option
- EPR dewar option for cooling at liquid nitrogen temperature
- Cryostat options (Helium or Nitrogen)
- Multi-position sample holders, with water circulation or thermo-electric based temperature control
- Plate Reader attachment
- Fibre attachments
- Fibre couplers to microscopes
- Integration Sphere
- Absorption accessory
- X-ray excitation

For more details on these options, refer to the ***FLSP920 Series Reference Guide***.

2. Spectrometer Components

The following sections describe the FLSP920 Series standard components. For details on other non-standard components, see the *FLSP920 Series Reference Guide*.

2.1. Light Sources

There are three standard light sources in the FLSP920 spectrometer series:

- A 450W continuous xenon arc lamp for continuous sample excitation (FLSP, FLS, FSP, FS spectrometers)
- A low repetition rate pulsed 60W xenon flashlamp for pulsed sample excitation in multichannel scaling experiments (FLSP, FSP, FLP, FP spectrometers),
- A high repetition rate nanosecond pulsed flashlamp for Time Correlated Single Photon Counting experiments (FLSP, FLS, FLP, FL spectrometers).

2.1.1. Xe900 Continuous Xenon Lamp

The standard light source for steady state applications is a 450W xenon arc lamp, as shown in Figure 2-1. It produces “white” light composed of a continuum superimposed with lines. The optimal spectral range extends from below 250nm to more than 1000nm.

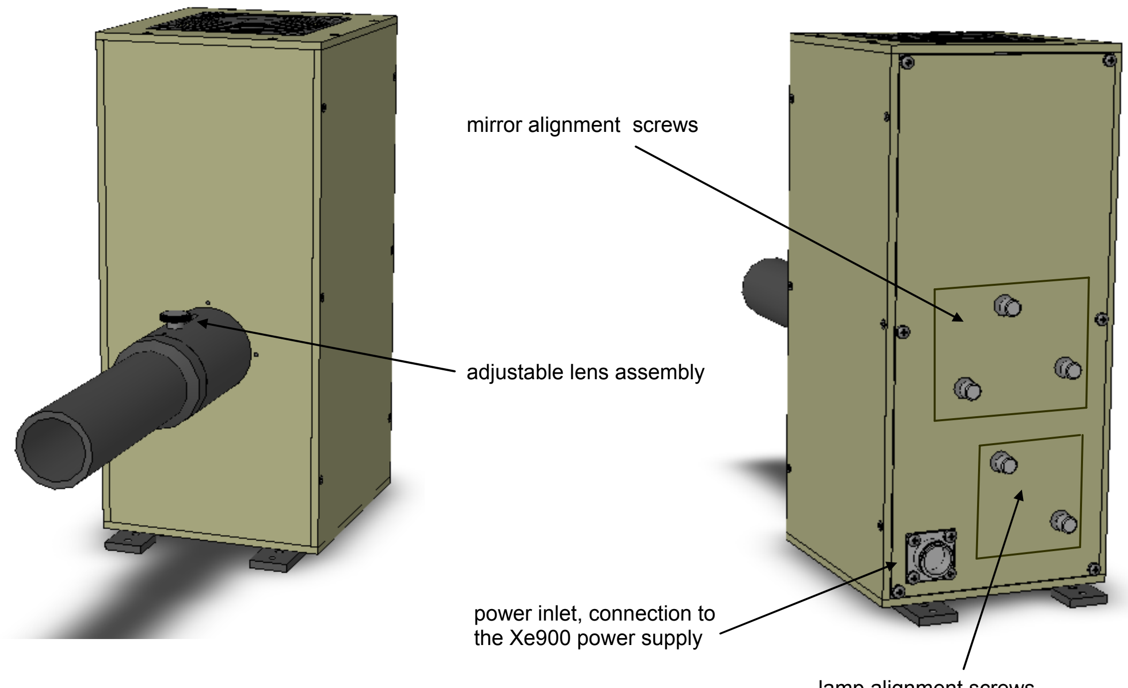


Figure 2-1: Xe900 Xenon Lamp

The Xe900 has a separate power supply connected to the lamp head by a well-shielded power cable. The lamp has an automated start-up procedure, a run-time monitor on the rear of the power supply, failure indicators and a voltage/current display on the front of the power supply unit.

The emission of the arc is focussed onto the slit of the excitation monochromator using an adjustable lens system. The rear of the xenon lamp head has two adjustment screws (horizontal, vertical) for the xenon bulb and three adjustment screws (horizontal, vertical, focus) for a reflector mounted behind the bulb for additional light collection.

Xe900 Output Spectrum

Figure 2-2 shows the spectral output of the 450W xenon arc lamp, in relative power units. This is the spectral output of the xenon lamp only, un-affected by the throughput function of the excitation monochromator.

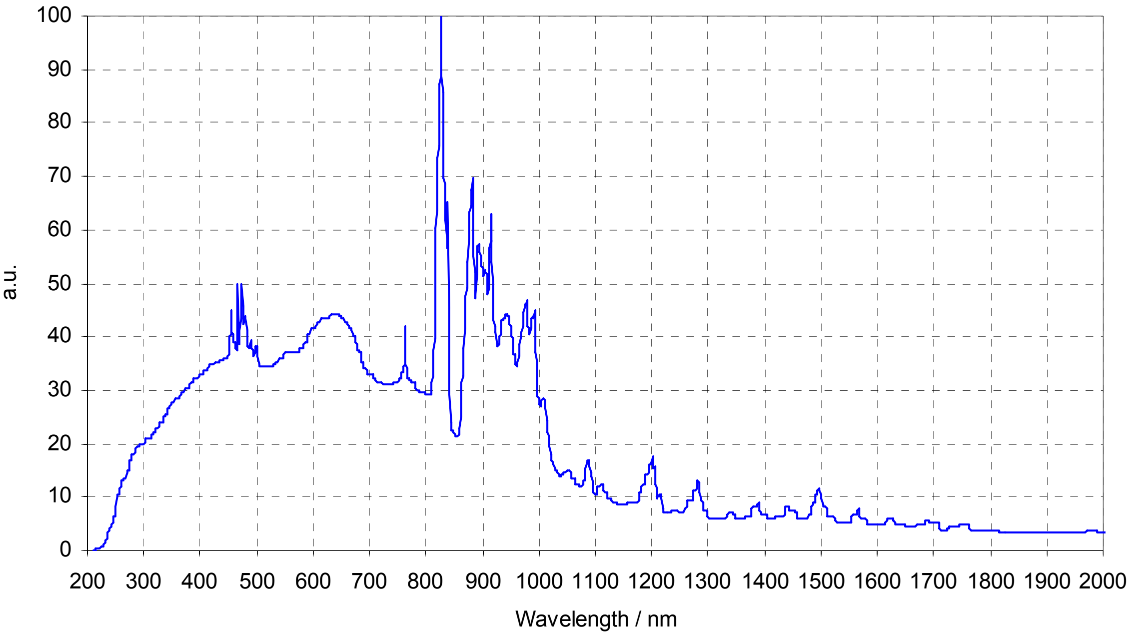


Figure 2-2: Spectral Output of the 450W Xenon Arc Lamp

The xenon lamp can be fitted with an ozone-free bulb or with an ozone generating bulb (optional). When fitted with an ozone generating bulb, the spectral output in the UV spectral range is enhanced and the lower wavelength limit shifts from about 250nm to 230nm. Further enhancement in the UV can be achieved by selecting special UV gratings for the excitation monochromator.

WARNING!

When working with ozone-generating bulbs, an air extraction system **must** be fitted to the lamp head.

Maintenance

The Xe900 is mostly maintenance-free. Optical alignment is made as part of the installation process: see the ***FLSP920 Series Installation Guide*** for details.

The xenon bulb has an average lifetime of 2000 hours (1000 hours guaranteed). For instructions on how to change and align the bulb, refer to the ***FLSP920 Series Installation Guide*** for details.

2.1.2. µF920H Xenon Flashlamp

The µF920H is a compact 60W xenon flashlamp, triggered by the spectrometer controller. The output is focussed by an adjustable lens assembly onto one of the entrance slits in the spectrometer's excitation monochromator.

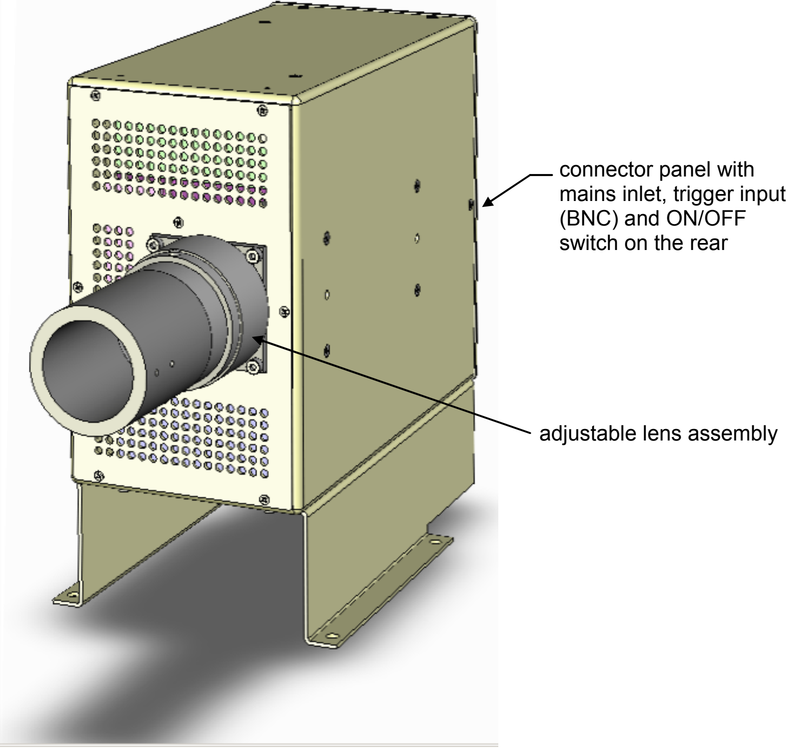


Figure 2-3: µF920H Xenon Flashlamp

The µF920H is optimised for multi-channel scaling (MCS) lifetime measurements, with a narrow optical pulse width and a pulse repetition rate of between 0.1Hz and 100Hz.

For some applications (for example, time-gated spectral scanning), the µF920H lamp can be used as an excitation source for spectral scans.

When using the µF920H, your samples should have intrinsically long lifetimes since the spectrometer uses the highly sensitive single photon counting mode for spectral scanning. With a repetition rate of 100Hz and a typical pulse width of 1µs, the number of instantaneous photons counted per dwell time is limited.

µF920H Output Spectrum

The typical output spectrum of the µF920H is shown in Figure 2-4. Compared to the spectrum of a continuous xenon lamp (see Section 2.1.1 *Xe900* Continuous Xenon Lamp on page 11), the microsecond pulsed xenon flashlamp has an optical output that is more line-structured and has comparatively higher content of UV output.

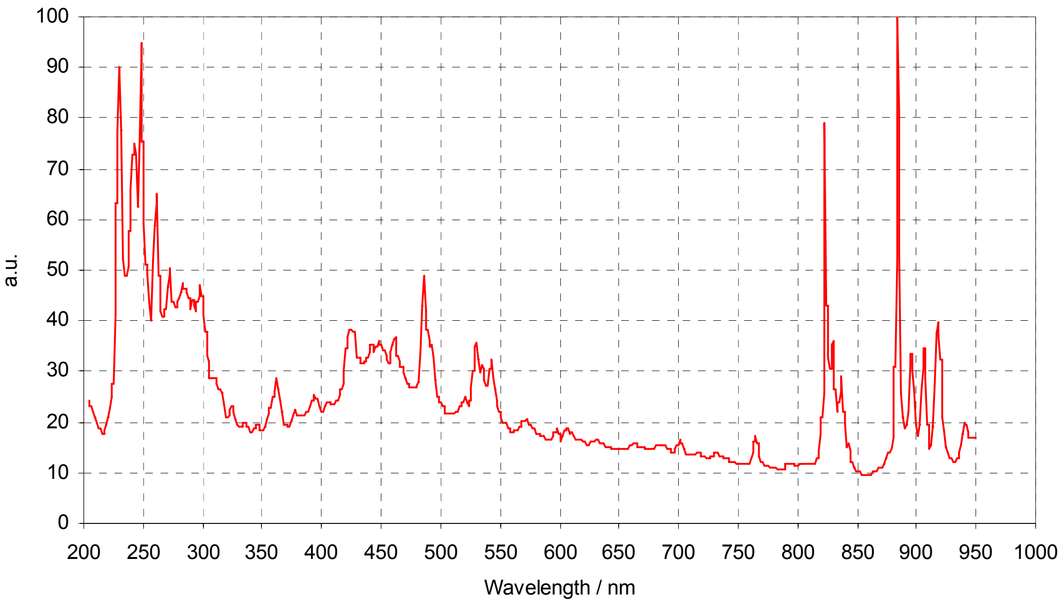


Figure 2-4: Spectral Output of the Xenon Flashlamp

µF920H Pulse Profile

Figure 2-5 shows the temporal output of the µF920H flashlamp, measured at 300nm (blue), 450nm (green) and 600nm (red). The typical pulse width is 2µs. Note that the shape and the tail is somewhat wavelength dependent.

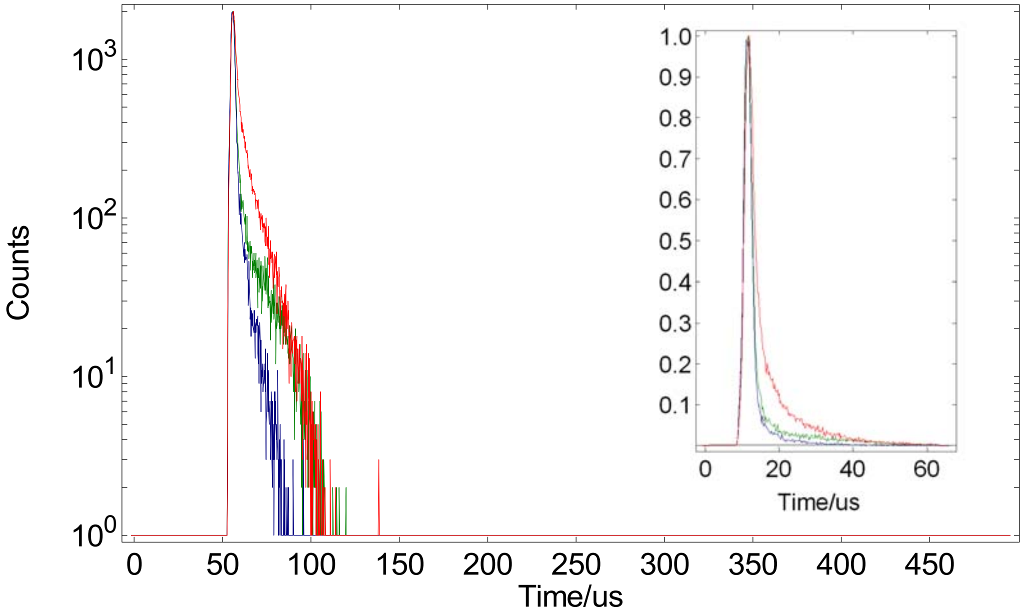


Figure 2-5: Temporal Output of µF920H Flashlamp

2.1.3. nF920 Nanosecond Flashlamp

The nF920 is an all-metal triggered flashlamp with a fast switching thyratron, spark gap and charging resistor. The flashlamp can produce nanosecond and sub-nanosecond optical pulses with repetition rates up to 50kHz. It is the only discharge light source available with narrow pulses of sufficiently high repetition rate to be suitable for TCSPC measurements. However, upgrades picosecond diode lasers and LEDs are available, refer to the ***FLSP920 Series Reference Guide*** for EPLs and EPLEDs, respectively.

Depending on the discharge gas, the nF920 can produce a broad spectral continuum. This makes the nF920 a suitable light source in fluorescence lifetime spectrometers.

The nF920 ensures maximum electrical-to-optical energy conversion during a clean, controlled spark discharge. The compact metal body ensures robustness and effectively shields any RF emission from the spark discharge at source. Stray capacitance is kept to a minimum, ensuring maximum photon yield of the light pulses with minimum pulse width and tail.

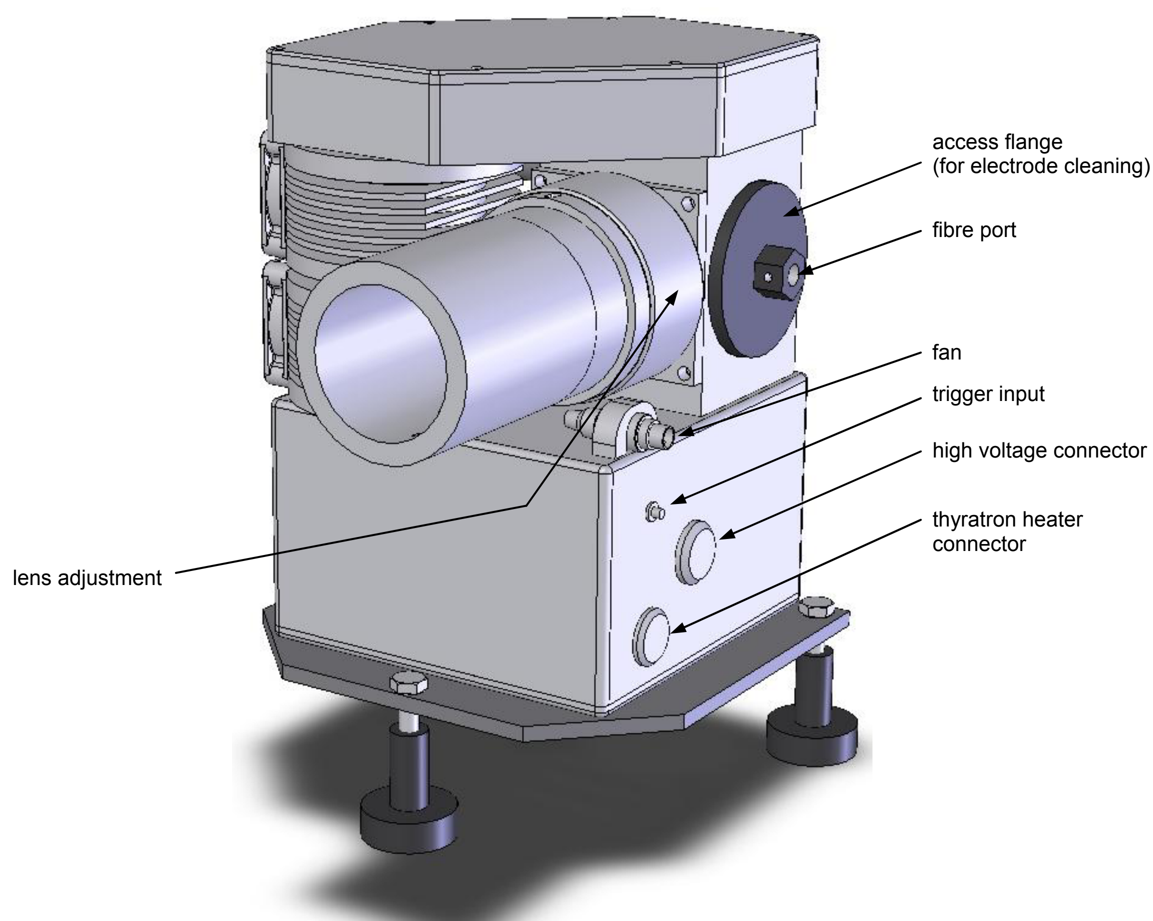


Figure 2-6: nF920 Nanosecond Flashlamp

nF920 Power Control Unit

The nF920 lamp is powered and controlled by a dedicated power supply unit. The nF920 power control unit delivers a high voltage for the gas discharge, heater current for the thyratron cathode, trigger pulses for the thyratron discharge trigger and power for the fans that control the thyratron temperature. The power control unit connects to spectrometer controller so that the high voltage and the trigger frequency can be controlled and monitored by the spectrometer software. The software also monitors the lamp's gas pressure.

START Photomultiplier Box

The synchronisation photomultiplier monitors the optical pulses generated by the flashlamp via optical fibre. The optical pulses are converted into electrical pulses which are delivered to the timing electronics of the lifetime spectrometer where they are used as START pulses in TCSPC measurements.

nF920 Filler Gas

The nF920 can use many filler gases and gas mixtures: typically pure hydrogen and nitrogen are used. The discharge chamber is sealed from other lamp components by Pyrex insulators and O-rings to give excellent vacuum integrity over a wide pressure range.

Standard vacuum couplings are fitted for connection to the gas pump and a fibre optic interface connects the discharge chamber to the synchronisation photomultiplier.

For details on filler gas tolerance ranges and standard settings, refer to the *FLSP920 Series Reference Guide*.

Maintenance

The nF920 must be maintained frequently to ensure stable operation and best performance. This involves cleaning the electrodes, aligning the spark gap and readjusting the rear reflector. Refer to the *FLSP920 Series Reference Guide* for details.

nF920 Output Spectrum

The typical output spectra of the nF920 for hydrogen, deuterium and nitrogen as filler gases are shown in Figure 2-7. This is the spectral output of the flashlamp only, un-affected by the throughput function of the excitation monochromator.

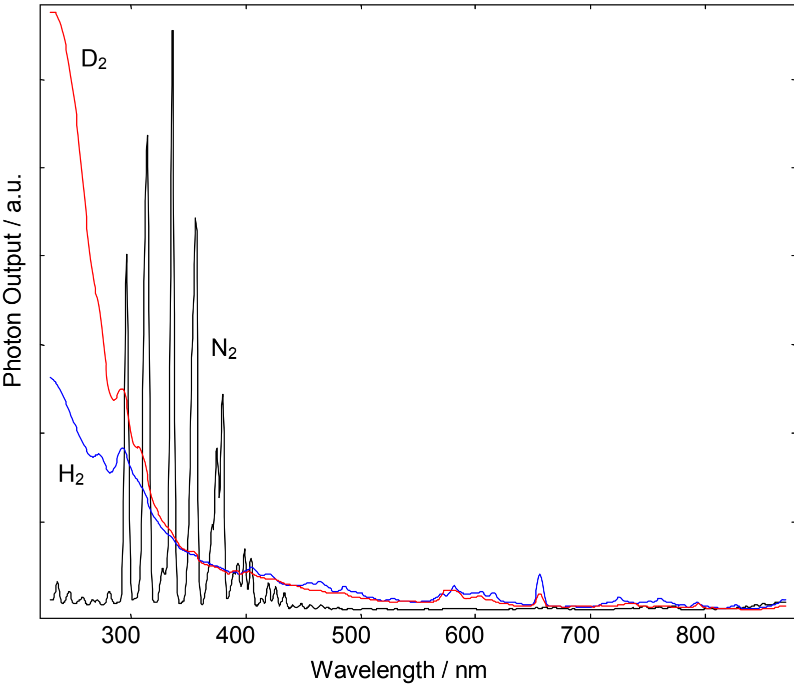


Figure 2-7: Spectral Output of the Nanosecond Flashlamp for H_2, N_2 and D_2

nF920 Pulse Profile

Figure 2-8 shows typical instrumental response functions of the nanosecond flashlamp for different filler gases (measured with TCSPC, in semi-logarithmic plots). Note that these are typical curves – characteristics can vary with operating conditions.

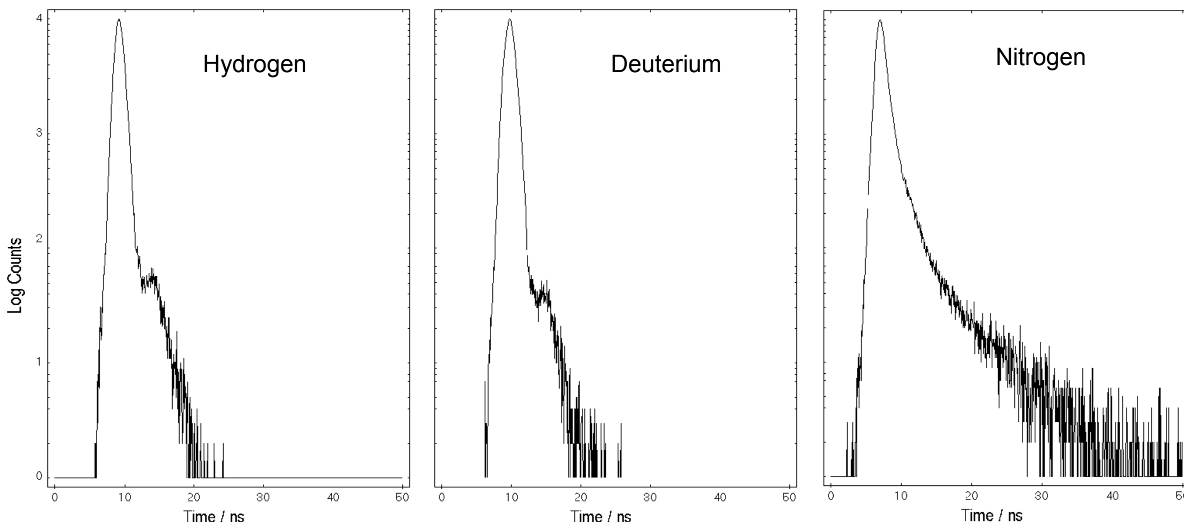


Figure 2-8: Temporal Output (Instrumental Response Functions) of the nF920 for different filler gases

2.1.4. Other Light Sources

For details on other light sources, refer to the ***FLSP920 Series Reference Guide***.

2.2. Monochromators

A *monochromator* transmits a selectable narrow band of wavelengths of light from a wider range of wavelengths available at the input. Every FLSP920 Series of fluorescence spectrometers has at least two monochromators: an *excitation monochromator* and an *emission monochromator*. These can be either single-grating or double-grating monochromators, depending on your configuration. You can also add an additional emission monochromator to form a “T” geometry, enabling more than two detectors permanently installed. See the ***FLSP920 Series Reference Guide*** for details.

FLSP920 Series of fluorescence spectrometers use symmetric Czerny-Turner type monochromators, specifically designed to meet the requirements of the modular concept of the spectrometer. The monochromators have two chambers:

- An upper optical chamber coated with absorbent optical grade paint. It is fitted with baffles and shields to minimise reflections of scattered light on shiny surfaces and is sealed against ambient light for “single photon integrity”
- The lower chamber contains mechanical and electrical components

The focal length of the monochromators are 300mm with an F-number of 4.1. They are fitted with one (single monochromators) or two (double monochromators) grating turrets, each one carrying up to three gratings. The monochromators also have several computer-controlled slits, swing mirrors and shutters.

When you switch on the spectrometer, the monochromators calibrate by finding defined positions for both the grating turrets and slit mechanism.

2.2.1. Excitation Monochromator

The single-grating excitation monochromator has two entrance ports for lamps and one exit port mounted to the spectrometer's sample chamber:

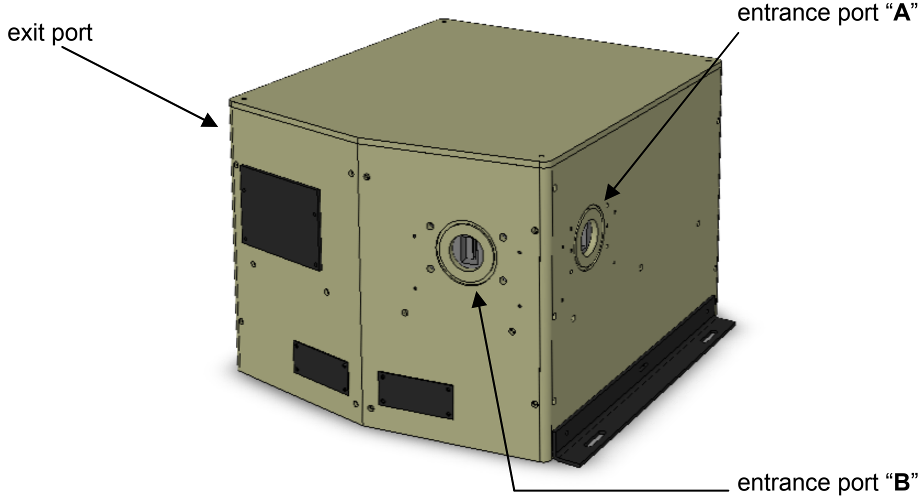


Figure 2-9: TMS300-X Single-Grating Excitation Monochromator

Entrance port B is fitted with a computer controlled shutter. Although typically configured for the Xe900 continuous xenon lamp, it may also be used for the µF920H microsecond flashlamp.

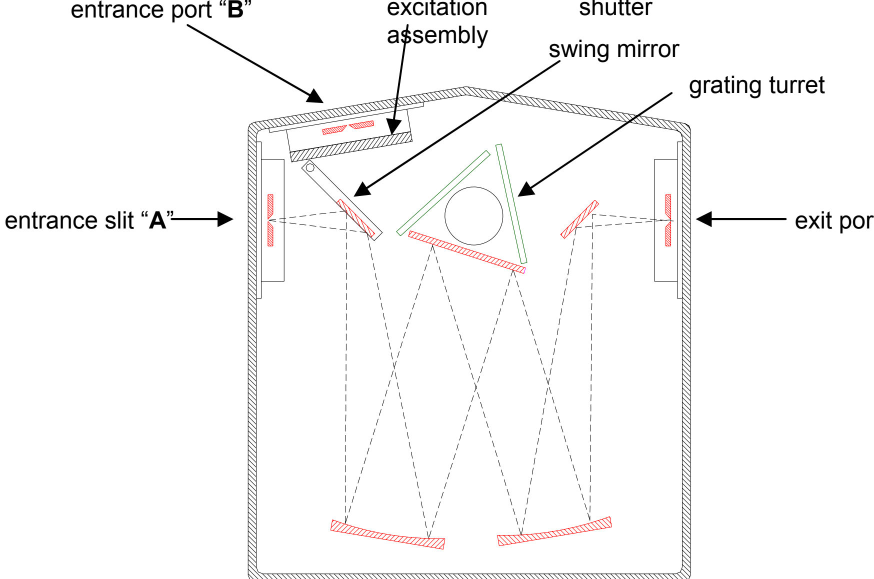


Figure 2-10: Schematic of Single-Grating Excitation Monochromator

Swing Mirror Assembly

If three lamps are required, a swing mirror assembly is attached to the monochromator as shown in Figure 2-11 below, allowing for a third entrance port.

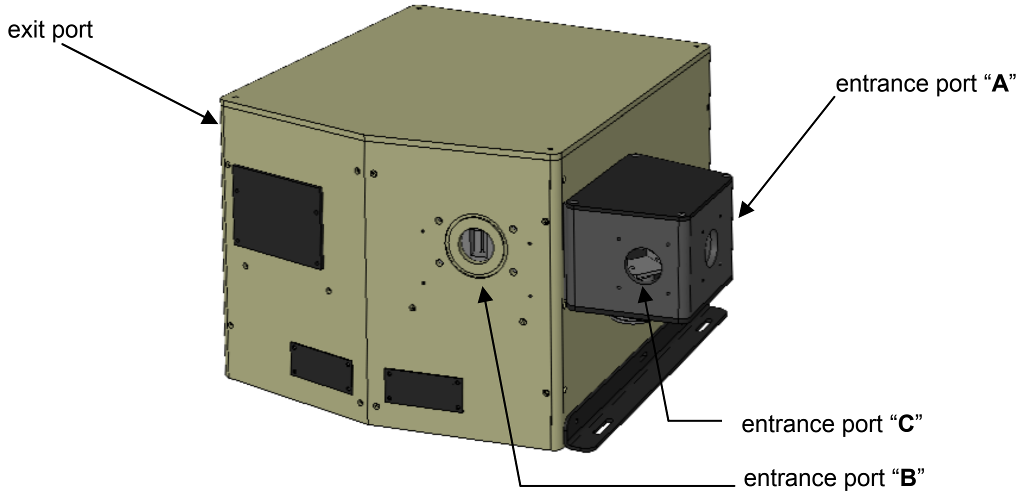


Figure 2-11: TMS300-X with Swing Mirror Assembly

Emission Monochromator

The single-grating emission monochromator has one input port connected to the sample chamber and two exit ports that can be connected to different detectors.

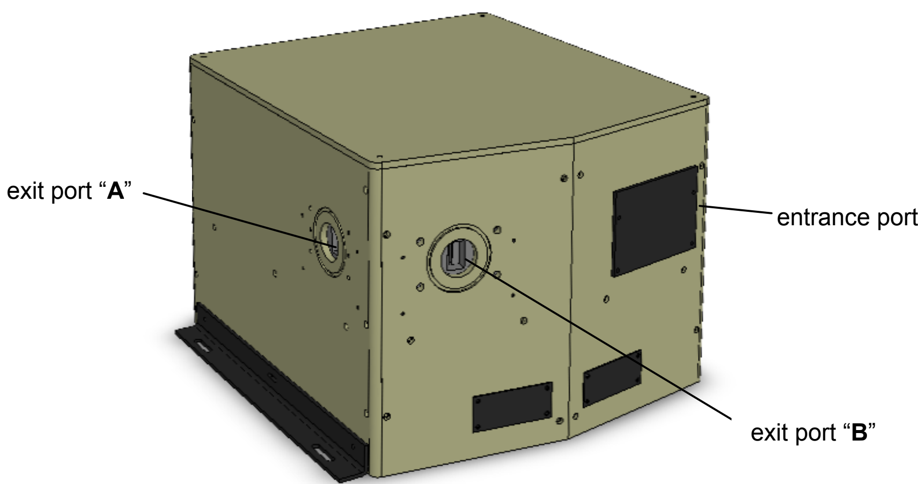


Figure 2-12: TMS300-M Single-Grating Emission Monochromator

Unlike excitation monochromators, the emission monochromator's shutter is not computer-controlled. Instead, it is directly interlocked to the sample chamber lid and front hatch. This ensures the detectors are protected against ambient light exposure.

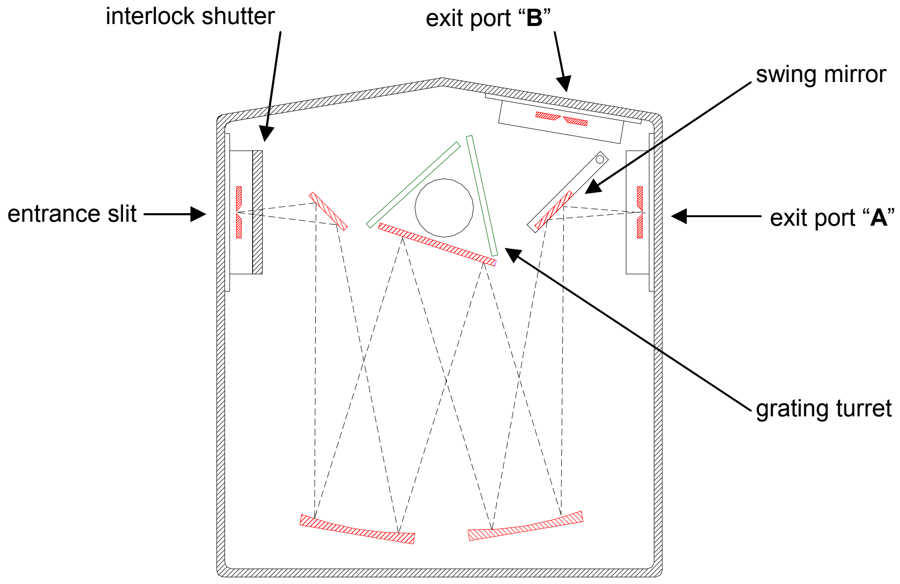


Figure 2-13: Schematic of Single-Grating Emission Monochromator

A computer-controlled swing mirror selects the detector in use. The standard emission monochromator can be fitted with two detectors.

A special single-grating emission monochromator is available for imaging applications. This monochromator has exit port B fitted with the standard slit assembly, whereas exit port A has no slit and is reserved for a CCD camera. The swing mirror assembly is in a different position so that the image for port A is outside the monochromator body, at the active area of the CCD camera.

2.2.2. Monochromator Gratings

The monochromator grating determines the spectral coverage and spectral resolution (grating groove density), the efficiency of the optical throughput (grating optimisation wavelength / blaze), stray light and polarisation effects (type of grating – holographic or ruled). The ratings for the standard gratings for excitation and emission monochromator, respectively, are shown in the table below:

Monochromator	Groove Density	Mechanical Wavelength Coverage	Optimisation Wavelength	Dispersion
single-grating excitation monochromator	1800 grooves/mm	200nm - 900nm	250nm (holographic)	1.8nm/mm
single-grating emission monochromator	1800 grooves/mm	200nm - 900nm	500nm (ruled)	1.8nm/mm
optional double-grating excitation monochromator	1200 grooves/mm	200nm - 900nm	300nm (ruled)	1.35nm/mm
optional double-grating emission monochromator	1200 grooves/mm	200nm - 900nm	500nm (ruled)	1.35nm/mm

Some applications may require additional or alternative gratings. In particular applications in the ultraviolet or in the near infrared spectral range require gratings of different specifications. See the ***FLSP920 Series Reference Guide*** for details on optional gratings.

2.3. Sample Chamber

The central component of the FLSP920 Series spectrometer is the sample chamber. This is a large hexagonal box sub-divided into the upper sample chamber, and a lower section containing control electronics.

The sample chamber has two access ports for routine access to the sample and mechanical/optical components inside the compartment: A circular lid that covers a 230mm diameter round access hole on the top of the chamber and a 260mm wide front hatch. Both access ports are interlocked, i.e. as soon as one of the two lids is lifted the shutter in the emission monochromator will close to protect the detector(s) against ambient light.

A third slot on top of the sample chamber accepts a filter holder for the excitation beam. This is not interlocked as the aperture is small and the ambient light entering the sample compartment is minimal.

To minimise stray light, the interior of the sample chamber is coated with a highly absorbing optical grade black paint. Take care not to damage the fragile paint or spill liquids or powders into the sample chamber as this will be difficult to clean off without adversely affecting the paint.

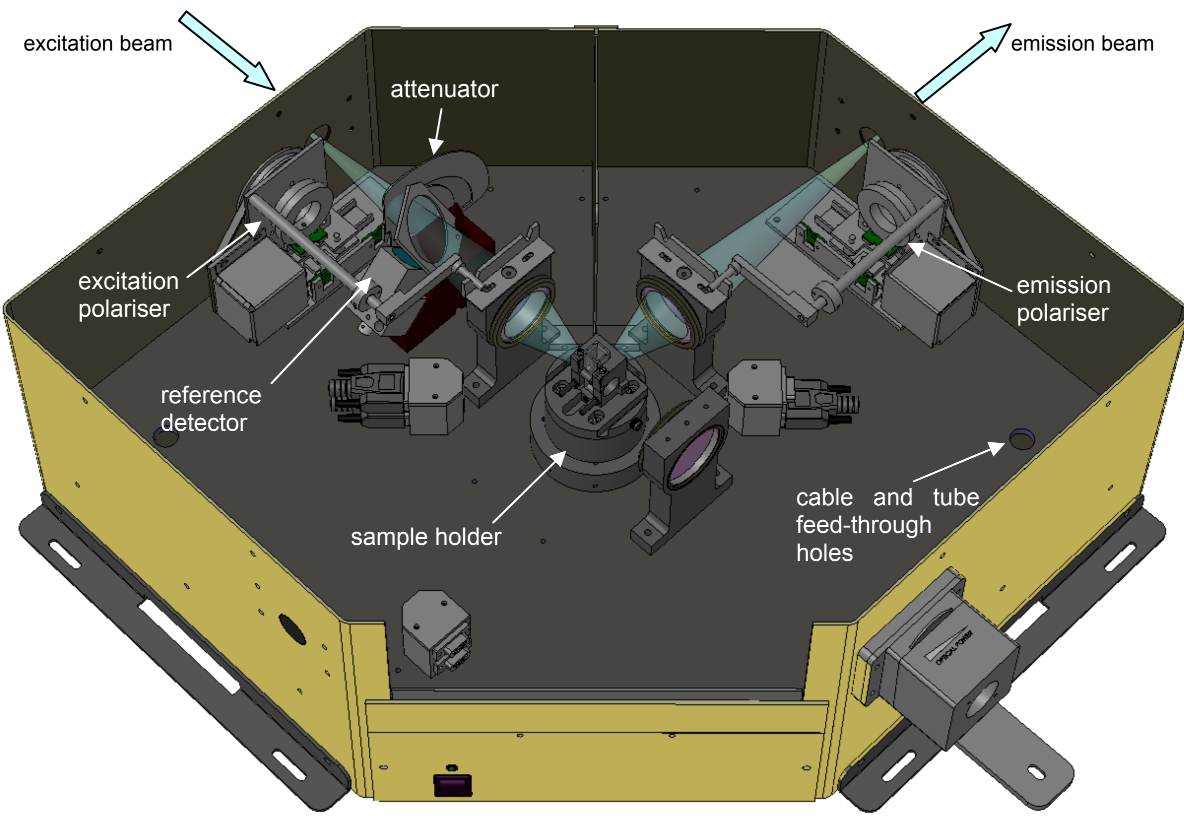


Figure 2-14: Sample Chamber, shown with optional polarisers and laser coupling flange

The sample chamber has four beam access ports, each 90° apart. This allows for a variety of configuration options such as “T”- geometry (with two emission arms pointing in opposite directions) or “X”-geometry with two excitation arms in addition to the two emission arms. The optical beam height within the sample compartment is 82mm.

Attenuator

The standard sample chamber contains an attenuator. This is a horizontal slit of variable width to attenuate the amount of light reaching the sample. The location of this arrangement is so that the effect on changing the spot size at the sample is minimal.

Reference Detector

The standard sample chamber contains a reference detector behind the attenuator. The reference detector monitors a fraction of the light incident at the sample position. The output of the reference detector is used to correct excitation spectra.

The beam splitter that diverts a fraction of the excitation beam to the reference detector is specially mounted in a way that the angles in respect to the vertical polarisation plane and to the horizontal beam polarisation plane are identical. This eliminates polarisation effects that would otherwise affect the correction.

Sample Chamber Options

The sample chamber in Figure 2-14 shows the standard sample chamber, fitted with standard components as well as two popular options: *polarisers* for the excitation and emission beam path and *an EPL laser coupling flange* (including a lens for laser beam focussing).

Two special versions of the sample chamber are available: One with a 120mm diameter hole in the base of the sample chamber. This allows the use of special cryostats. This hole can be plugged so that the sample chamber can be used as normal with the standard set of sample holders. For this sample chamber option a standalone spectrometer controller is provided.

A second special version of the sample chamber has an enlarged beam access port. This is needed to mount a vacuum UV refocusing flange for spectroscopy with VUV excitation.

See the *FLSP920 Series Reference Guide* for details on sample chamber options.

Accessing the Optical Compartments Inside the Sample Chamber

Usually there is no need to access the optical components inside the large hexagonal sample chamber. However you may sometimes want to rearrange components for special experiments. To do this:

Remove the round lid, front hatch and filter holder.

Unscrew the four screws on the top of the large sample chamber cover plate.

Remove the cable with the 9-way “D”-type connector in the sample chamber (on the left side behind the front sample chamber hatch).

You can now carefully lift up the large cover plate.

2.3.1. Standard Lens Optics

The standard sample chamber has lens optics for focussing the excitation light onto the sample and collection of the emission light (see Figure 2-14). The lens assembly has two plano-convex fused silica lenses. In normal operation the lens surface facing the sample is 70mm away from the centre of the sample position. The demagnification of the lens assemblies is 2.3. A typical slit opening of the excitation monochromator of 0.55mm (~1nm band pass for a standard grating) would produce a 0.24mm wide beam at the sample position. The typical height of the excitation spot is 3-4mm.

The lenses in the sample chamber are adjustable, although for standard applications re-adjustment should not be necessary.

Before the lens assembly can be moved towards or further away from the sample, loosen the grub screw on top of the lens holder. You can then screw the lens assembly into or out of the mount.

2.3.2. Mirror Optics and Other Beam Steering Options

Some applications require special beam focussing and beam steering options. These are available, refer to the ***FLSP920 Series Reference Guide*** for more details.

The table below gives a summary of those applications. Note that the listed applications also require other options and accessories (beyond the mirrors or prisms in the sample chamber), such as lamps, lasers, special detectors.

Application	Suitable Optics	where ?
VUV excitation (<200nm)	beam re-focussing vacuum flange	excitation
use of external lasers for sample excitation (not EPL and EPLED)	beam steering prism	excitation
NIR detection (>1700nm)	mirror optics or lens optics with special lens material	emission
NIR detection (>2500nm)	mirror optics	emission
opaque samples in front face geometry	mirror optics	excitation and emission

2.4. Sample Holders

There are various different types of sample holder. All sample holders are “plug compatible” and have a common socket interface. Their cylindrical bases fit into a slotted doughnut-shaped socket piece located in the centre of the sample chamber. A pointed tip knurled thumb screw on the base of each sample holder provides guidance for positioning and a way to fix the sample holder securely in place.

2.4.1. Standard Single Cuvette Holder

The standard sample holder is a single cuvette holder as shown below.

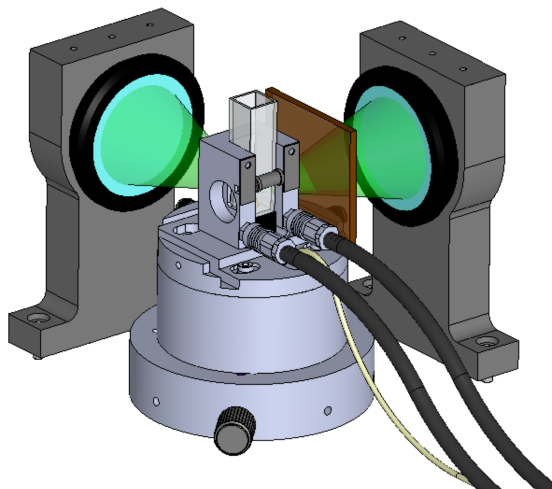


Figure 2-15: Single Cuvette Sample Holder with Lens Assembly, Light Cones and Filters

This holder is suitable for liquid samples measured in the spectrometer’s standard right angle geometry. The sample holder has a socket for quick exchange with other optional sample holders, a thermally isolated base, a sample holder body with feed-through pipes for liquid cooling and a temperature sensor. A spring loaded roller ensures exact positioning of the sample and avoids scratching of the cuvette. The socket of the sample holder is mounted to the base by four M3 screws recessed in slotted holes, providing some freedom for adjustment along the optical axis of excitation. The socket also has slots to hold filters.

The sample holder assembly is released by loosening the knurled knob on the bottom. The sample holder can be rotated and secured in multiples of 45 degrees.

This sample holder can be “hot-swapped” (in other words, it can be removed and replaced at any time without switching off the spectrometer). The cable for the temperature sensor (terminated with a D-type connector) can be removed and refitted to the upper of the two D-type sockets next to the emission channel lens assembly.

To completely remove the sample holder from the instrument, disconnect the coolant tubes (quick release fittings), the 9-way connector and – if present – the magnetic stirrer. For a temporary repositioning, simply move the sample holder to the right side inside the sample chamber.

2.4.2. Other Sample Holders and Sample Positioning Options

Other sample holders include 3-position sample turrets, front-facing sample holders for liquids, powders and films, holders with magnetic stirrers and a variety of sample cooling and heating options. Additionally, the sample chamber can accept an integrating sphere, adapters for multi-well plate readers, stopped flow assemblies titrators, computer-controlled sample positioners and fibre accessories. For details on these and other options, refer to the ***FLSP920 Series Reference Guide***.

2.5. Detectors

The FLSP920 Series usually includes a high-gain photomultiplier (PMT) detector, suitable for photon counting in both steady state and time-resolved applications.

Photomultipliers operating in photon-counting mode provide the highest sensitivity in the spectral range from 200nm to 1700nm; for measurements above around 1700nm analogue detectors are required.

A variety of photo-detectors can be fitted to the FLSP920 Series. When comparing detectors for sensitivity, two factors need to be considered:

- *Detector Responsivity*, usually published by the detector manufacturers as spectral responsivity of quantum yield curves
- *Detector Noise*, determined by the background signal (dark count rate or dark current)

For the sensitivity of the whole spectrometer (as opposed to that of the detector alone) it is important to consider other factors such as the efficiency of the monochromators (grating curves), efficiency of the optics, and power and brightness of the light sources in the spectral range of interest.

For details on detectors, alone and in combination with other spectrometer components, refer to the ***FLSP920 Series Reference Guide.***

2.5.1. Red Sensitive Photomultiplier in Cooled Housing

The standard photomultiplier is a side window photomultiplier with a spectral coverage from 200nm to ~870nm (Hamamatsu, R928P). This detector comes in a cooled housing that provides an operation temperature for the detector of -20°C to reduce the dark count rate to a minimum.

The cooled housing comprises the optics for re-focussing the radiation from the exit of the monochromator to the photoactive area of the detector, the dynode chain socket, thermo-electrical cooling elements and a fan for heat dissipation. A separate power supply, CO1, provides power and controlling circuits for the cooling elements of the detector head.

The detector bias voltage is supplied by the spectrometer controller. This is typically -1200V.

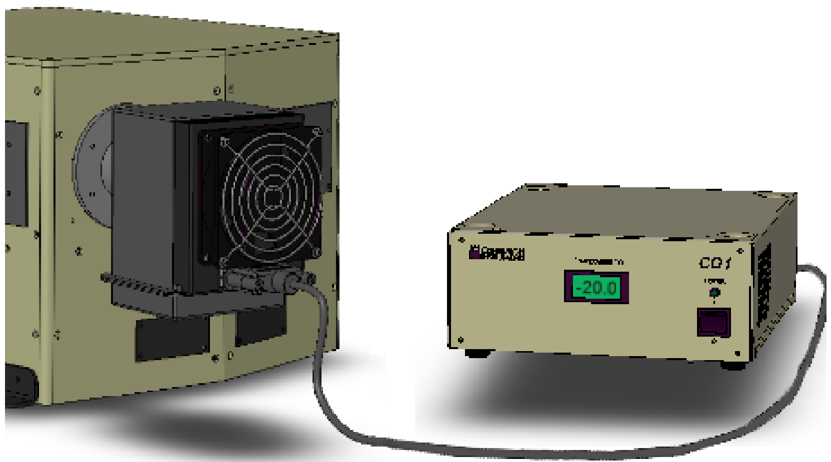


Figure 2-16: Detector assembly, comprising cooled head and power supply

CAUTION!

Do not disconnect the CO1 power supply from the detector when is use, as this can permanently damage the thermo-electrical circuit.

Detector Specification

The spectral response of this detector is specified to extend to 870nm. At that wavelength the quantum efficiency is reduced about 300 times compared to the maximum at 300nm.

The dark count rate at the operating temperature of -20 °C is typically 100cps.

The detector's minimum response width for time-resolved (TCSPC) measurements is 600ps. This is the characteristic width of the detector only. Additional contributions for broadening the temporal profile (such as the pulse width of the exciting light source) may also have an effect on the measured pulse width.

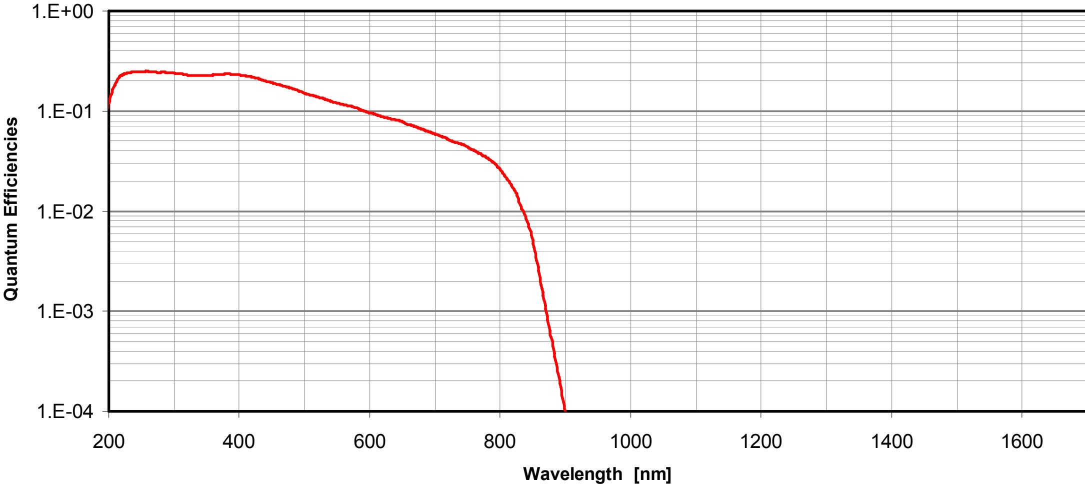


Figure 2-17: Quantum Efficiency of Red-Sensitive Photomultiplier

2.5.2. Other Detectors

Other detectors include extended-range red detectors, microchannel plate photomultipliers (MCP-PMTs), thermo-electrically cooled InGaAs detectors, and liquid nitrogen cooled infrared detectors. For details on these, refer to the ***FLSP920 Series Reference Guide***.

3. Introduction to F900 Software

The *F900* software application controls the FLSP920 Series of Fluorescence Spectrometers. Use it to set up measurement modes and parameters, configure spectrometer components, run scans and display and manipulate scan results. You can also import and export scan data to a variety of other standard applications.

The following sections give an introduction to F900. For more details, refer to the F900 Online Help (Help > Help Topics) and the ***FLSP920 Series Reference Guide***.

3.1. Starting F900

To start F900, double-click the F900 icon on your desktop or select Start > Programs > Edinburgh Instruments > F900 > F900.

The F900 splash screen is displayed while the software initialises spectrometer components. This auto-calibration takes a minute or so, depending on how many computer-controlled components are connected to the spectrometer.

Once initialisation is complete, the F900 main window is displayed with an open Signal Rate window (see Section 3.3 *Signal Rate* Window on page 29).

3.2. General Features of the F900 Main Window

The F900 Main Window has three areas: a large ***Display Area*** for measurement results, a ***Tool Bar*** on the top of the screen and a ***Status Bar*** on the bottom, see Figure 3-1 for example.

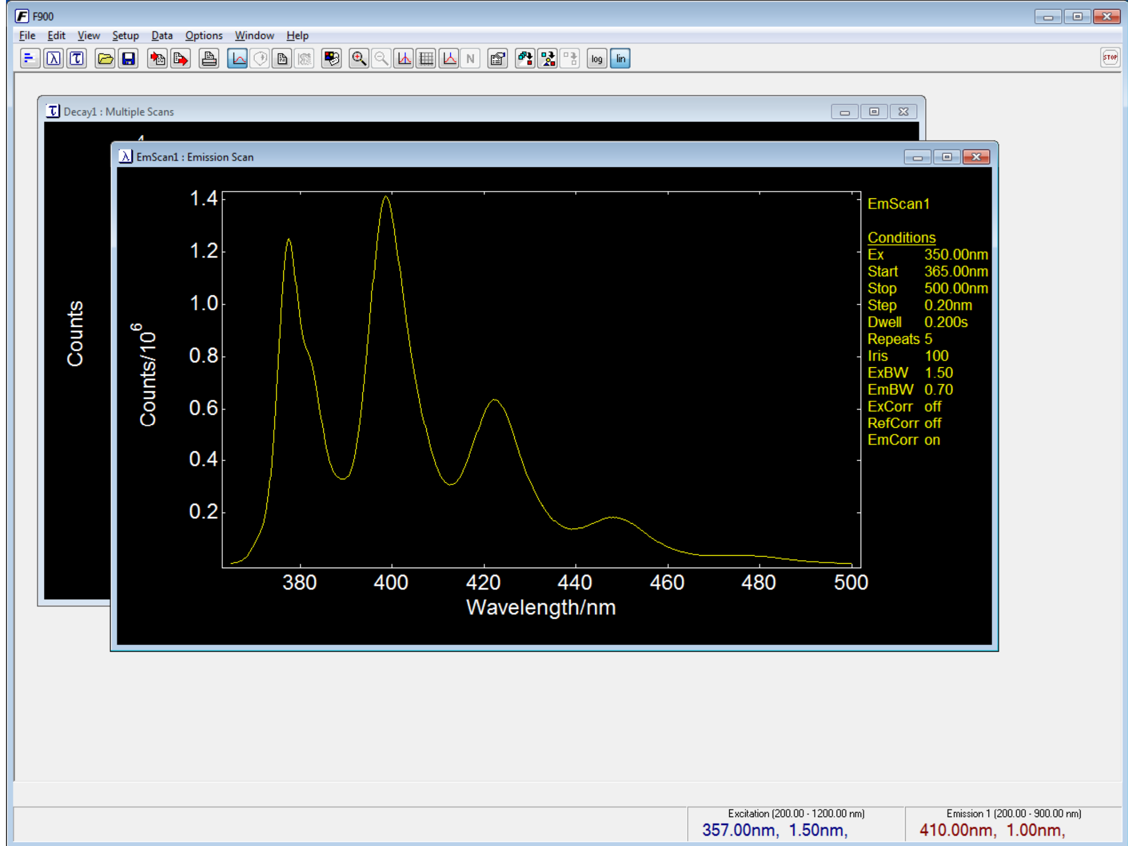
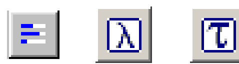


Figure 3-1: F900 main software window

Tool Bar

The tool bar has a row of icons for commonly-used commands. Some icons will be greyed-out, depending on the currently selected display area window.

- Scan Icons



Opens the Signal Rate, New Spectral Measurement, or New Lifetime Measurement windows. See the following sections for details.

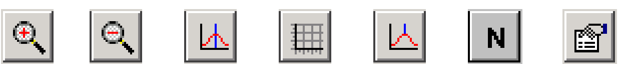
- File Icons

Open, save, import, export or print files.

- Scan View Icons

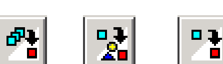
Change the view style for data windows. Depending on the type of a selected scan, switch the plot between 2D, 3D, text, or contour plot. You can also set plot options such as axes fonts and units, screen colours, and labels.

- Measurement Plot Icons



Zoom in to or out of a plot or show the cursor so that you can select and get data on a specific area. Other icons enable a plot grid, view plot peaks, normalise the graphs (in a contour plot) and view or edit plot properties.

- *Measurement Container Icons*



Join scans into a single measurement container (multiple scans), split multiple scans or extract individual scans from measurement containers.

- Scale Icons

View the Y-axis in logarithmic or linear format.

Scan Display Area

The Scan Display Area is where F900 scans and windows are displayed. You can have multiple scans open at the same time: use the Window menu to manage how they are displayed.

Right-click on a scan to display a pop-up menu. From here you can view the scan's properties, change the view and set multiple scan options.

Status Bar

The Status Bar at the bottom displays information about the current scan and spectrometer settings. What is displayed varies depending on whether a scan is in progress and on your particular component configuration.

Both Tool Bar and Status Bar can be disabled using the View menu options.

3.3. Signal Rate Window

The Signal Rate Window (Setup > Signal Rate) is where you setup the wavelength and other parameters before starting a scan. It is displayed automatically whenever you start F900.

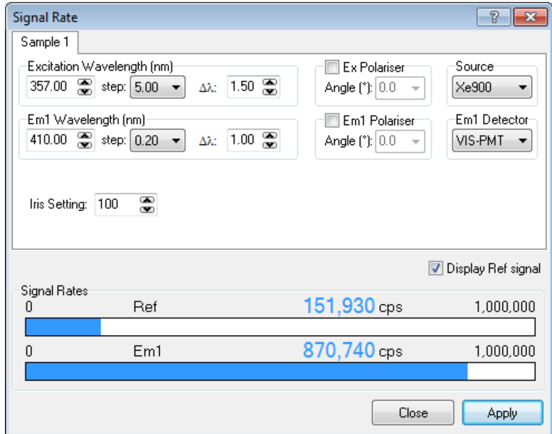


Figure 3-2: Signal Rate Window – principle layout

Property	Description
Excitation Wavelength	Set the excitation wavelength, step size* and spectral bandwidth.
Emission Wavelength	Set the emission wavelength, step size* and spectral bandwidth.
Iris Setting	Optically attenuate the light incident to the sample.
Ex Polariser	Enable the excitation polariser (if present) and set the polarisation angle.
Em Polariser	Enable the emission polariser (if present) and set the polarisation angle.
Source	Select the light source to use for the excitation.
Detector	Select the detector to use.
Display Ref Signal	Enable the display of the reference signal.
Signal Rates	Displays the current signal rates in counts-per-second (cps).

* The "Step Size" is not the step size that is used for spectral scanning. The step size that can be selected here only applies when the up and down arrow in the wavelength selection box are used.

The Signal Rate Window may have additional options depending on your spectrometer's geometry configuration, number of monochromators and type of sample holder.

3.4. Setup Menus

The Setup menu contains options to set up computer-controlled light sources (nF920, μF920H), computer controlled sample holders (sample positioners, plate reader attachments, titrators, etc) and sample cooling options (temperature controlled sample holders, cryostats). Computer controlled sample holders and cooling/heating devices are optional spectrometer accessories and are not covered by this user guide. Refer to the ***FLSP920 Series Reference Guide***.

This section only outlines the layout of the dialogue boxes for the nF920 nanosecond flashlamp and the μF920H microsecond flashlamp setup. Details on the Operation of these lamps are given in sections 5.3.1 and 5.2.1 on pages and 71 and 68, respectively.

3.4.1. nF Lamp Setup

Select ***Setup >> nF Lamp Setup*** to display the setup window for the nanosecond flashlamp. For details on the operation of the nF920 refer to section 5.3.1 on page 71.

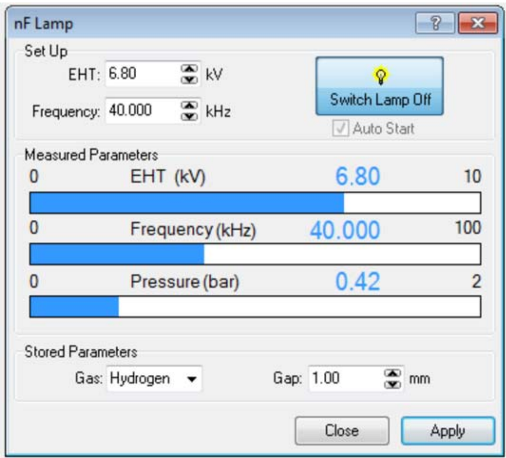


Figure 3-3: nF920 lamp setup dialogue box

Property	Description
Set Up	Specify the lamp's extra-high tension (EHT) and frequency, dependant on the filler gas and electrode gap size in use.
Switch Lamp On/Off	Click to switch the lamp on or off. By default, the lamp is started with Auto Start enabled. This ramps up the frequency during start-up.
Measured Parameters	Monitors the EHT, the lamp requency, and the gas pressure in the lamp.
Stored Parameters	Specify the lamp's coolant gas and electrode gap size. These parameters have no impact on the operation, they are here for records that will appear in the properties of a measurement

3.4.2. µF Lamp Setup

Select ***Setup >> µF Lamp Setup*** to display the setup window for the microsecond flashlamp. For details on the operation of the µF920H refer to section 5.2.1 on page 68.

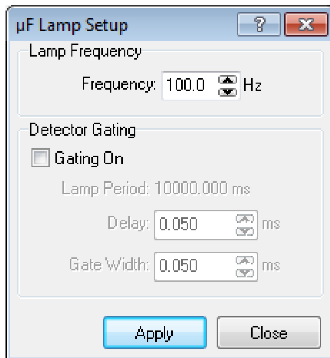


Figure 3-4: µF900H lamp setup dialogue box

Property	Description
Lamp Frequency	Set the lamp's frequency from 0.1 to 100 Hz.
Detector Gating	Enable gating (where applicable) to set the trigger delay and gate width.

3.5. Scan Types and Scan Properties

The F900 software produces two different file types, annotated with the file extensions .FS and .FL, respectively, depending on whether the x-axis is wavelength or time.

Each of these file types contains one or more measurement data or analysed data. These are called ***Scans***. A variety of different scan plots are available and can be grouped into either spectral scan plots or time resolved scan plots. A file type may only contain scans of the same group of scan types, i.e. spectral data or time resolved data.

This section of the manual briefly describes the scan plot types and outlines the scan properties that are attached to each scan. The scan properties are available via ***File >> Properties***, or by right mouse click on the active data window and selection of ***Properties***. If the active window contains more than one scan a property container is displayed first and a scan must be selected from the list of scans to view the scan properties.

3.5.1. Spectral Data

Excitation Scans

Spectral scans, measured with fixed emission wavelength and scanning excitation wavelength.

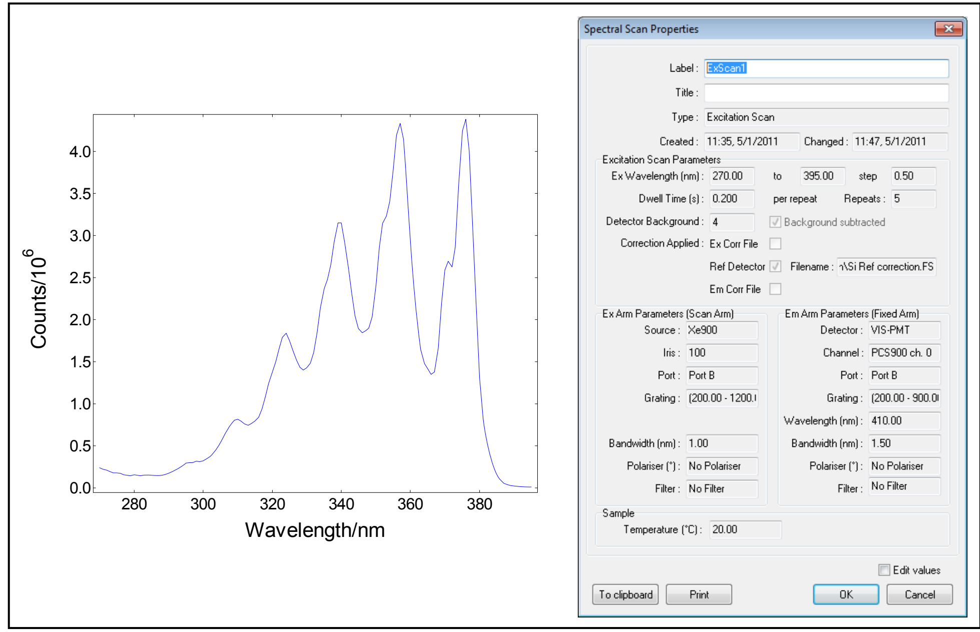


Figure 3-5: Excitation Scan, typical display and scan properties

Emission Scans

Spectral scans, measured with fixed excitation wavelength and scanning emission wavelength.

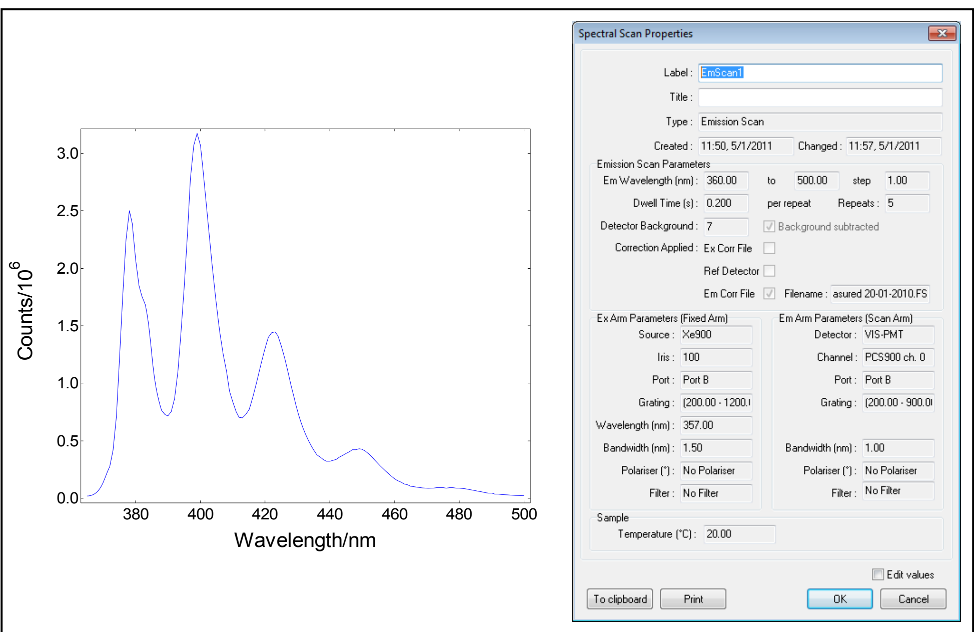


Figure 3-6: Emission Scan, typical display and scan properties

Synchronous Scans

Spectral scans, measured with excitation and emission monochromators scanning simultaneously, using a fixed offset between excitation and emission wavelengths.

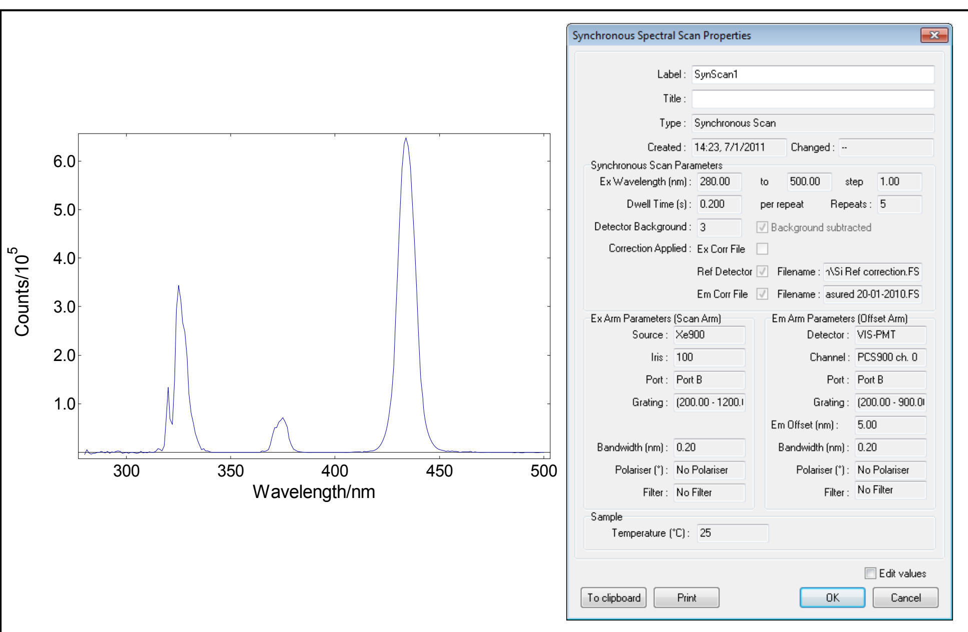


Figure 3-7: Synchronous scan, typical display and scan properties

Excitation Correction Scans

Spectral measurement of the reference detector (inside the sample chamber): This measurement records the spectral output of the excitation beam path and is therefore used to spectrally correct uncorrected excitation scans. The step size is typically 1nm and the scan is normalised to 1. Note that only Excitation Correction Scans can be used for spectral correction of Excitation Scans. Conversion of normal Excitation Scans into Excitation Correction Scans (using the file property dialogue box) is not possible.

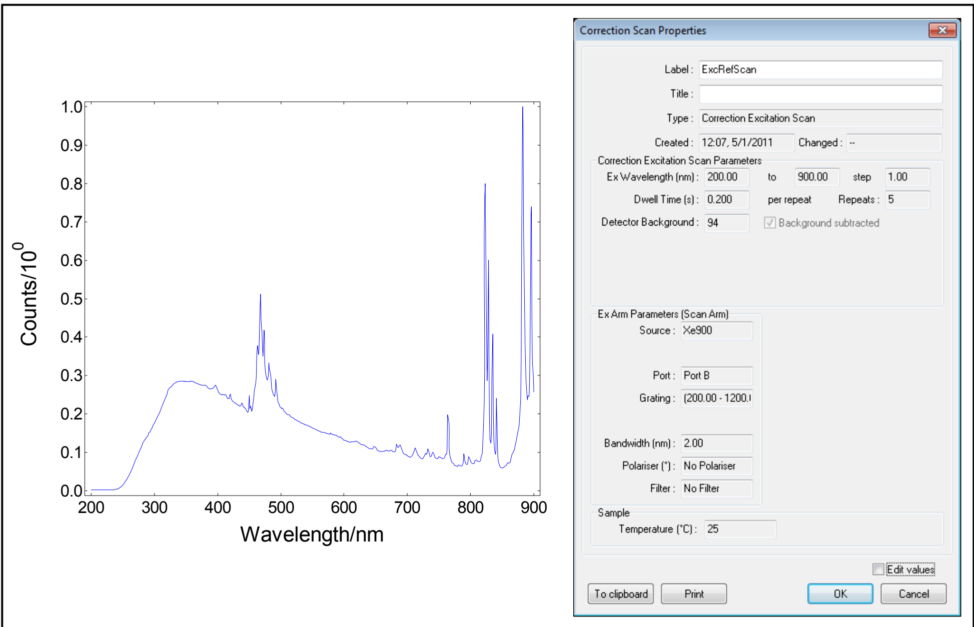


Figure 3-8: Excitation Correction Scan, typical display and scan properties

Emission Correction Scans

Spectral measurement of the emission beam path: This scan contains the spectral information of the emission monochromator and the detector using calibrated light source(s) and is therefore used to spectrally correct uncorrected emission scans. The step size is typically 1nm and the scan is normalised to 1. Note that only Emission Correction Scans can be used for spectral correction of emission scan data. Conversion of normal Emission Scans into Emission Correction Scans is not possible.

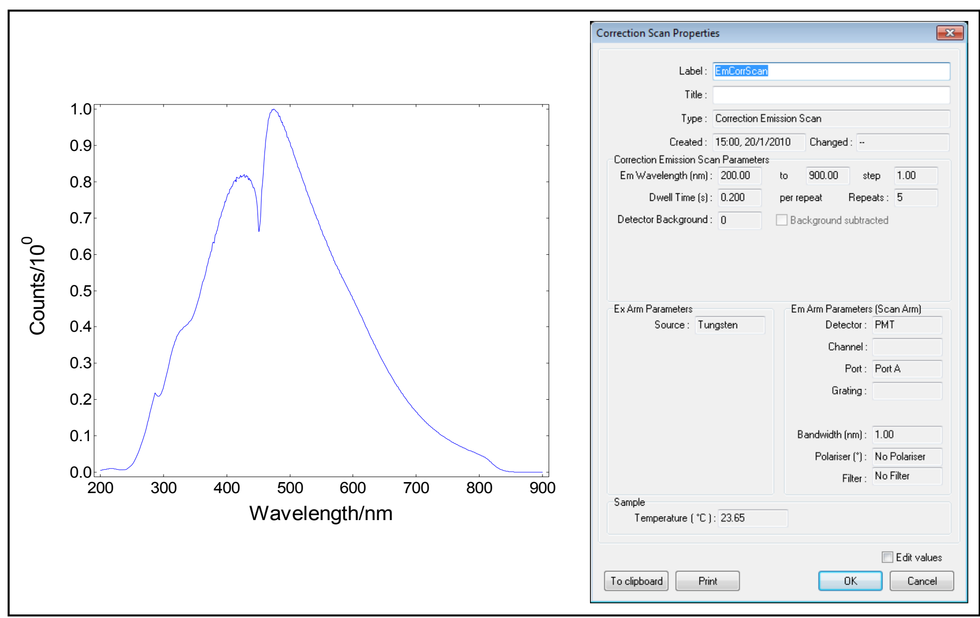


Figure 3-9: Example of an Emission Correction Scan, typical display and scan properties

Excitation Anisotropy Scans

Scans that result from automatically or manually acquired polarised excitation scans: The polarised excitation scans are used to calculate the Excitation Anisotropy function. An example is shown in Figure 3-10.

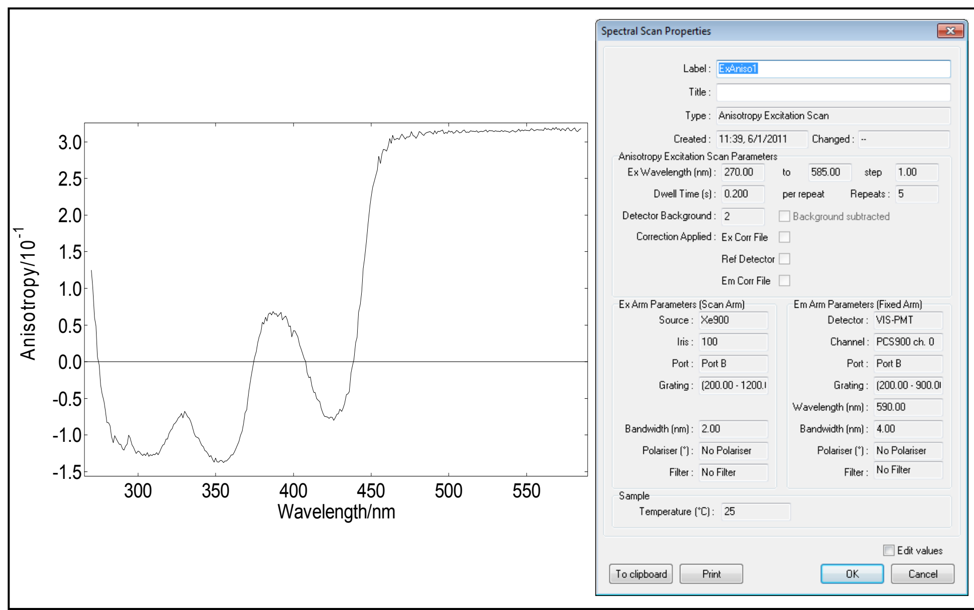


Figure 3-10: Excitation Anisotropy, typical display and scan properties

Emission Anisotropy Scans

Scans that result from automatically or manually acquired polarised emission scans. The polarised emission scans are used to calculate the Emission Anisotropy function. An example is shown in Figure 3-11. The plot in Figure 3-11 also contains the G-factor curve.

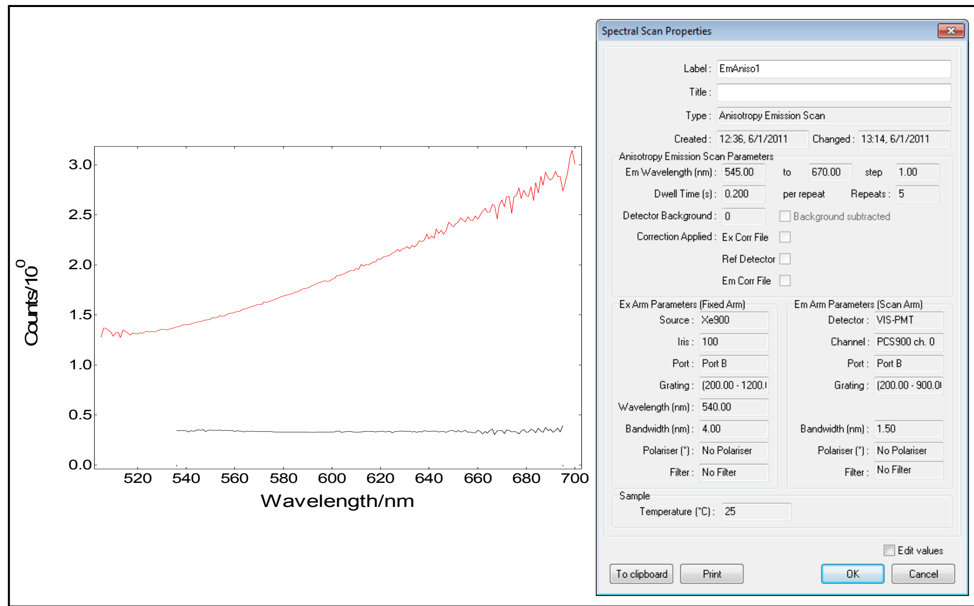


Figure 3-11: Emission Anisotropy, display of anisotropy function (black) and G-factor curve (red), scan properties

3.5.2. Time-Resolved Data

Time resolved measurements are data that were acquired by either TCSPC, MCS, Oscilloscope or standard single photon counting (kinetic scan). This scan type is sub-divided into decay measurements and measurements of the instrumental response function (IRF). If the measurement is an IRF then the box “Is Instrument Response” must be ticked (refer to the property box, bottom left: ***Is Instrument Response***). Windows containing a single decay measurement can be analysed using ***Exponential Tail Fit*** analysis; windows containing a single decay and a IRF can be analysed using ***Exponential Reconvolution Fit*** analysis.

Time Scan

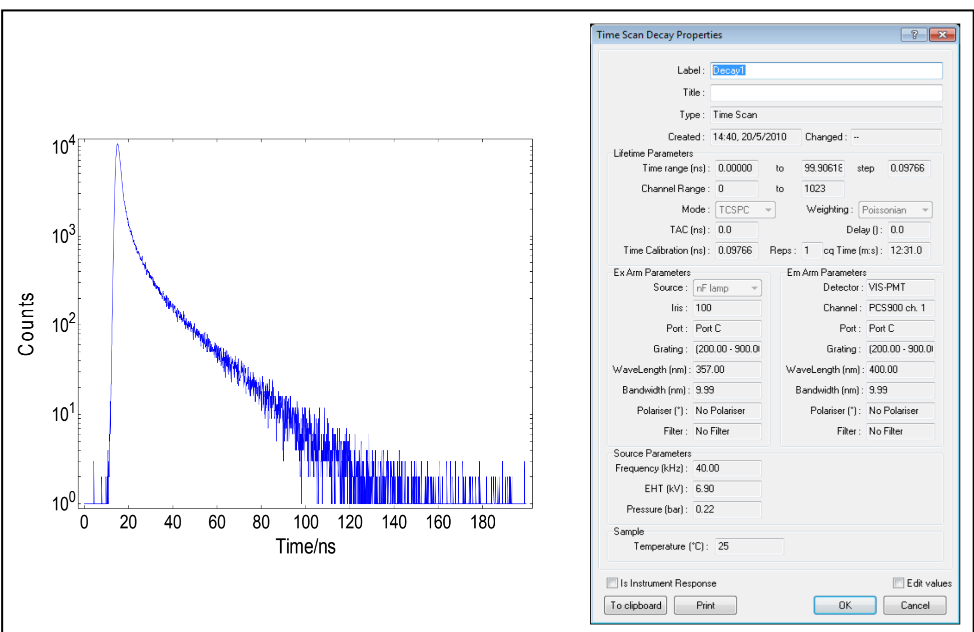


Figure 3-12: Time Scan, typical display and scan properties

Fitted Time Scans and Residual Time Scans

The result of a numerical fit is a set of lifetime parameters, as well as two different types of Time Scans: The fitted function and the residual function. These time scans are typically not shown separately (although they can be) but together with the (raw data) IRF and decay.

The properties of the ***Fitted Time Scan*** reveal the fit parameters. The properties of the ***Residual Time Scan*** present additional information, such as the Durbin Watson parameter.

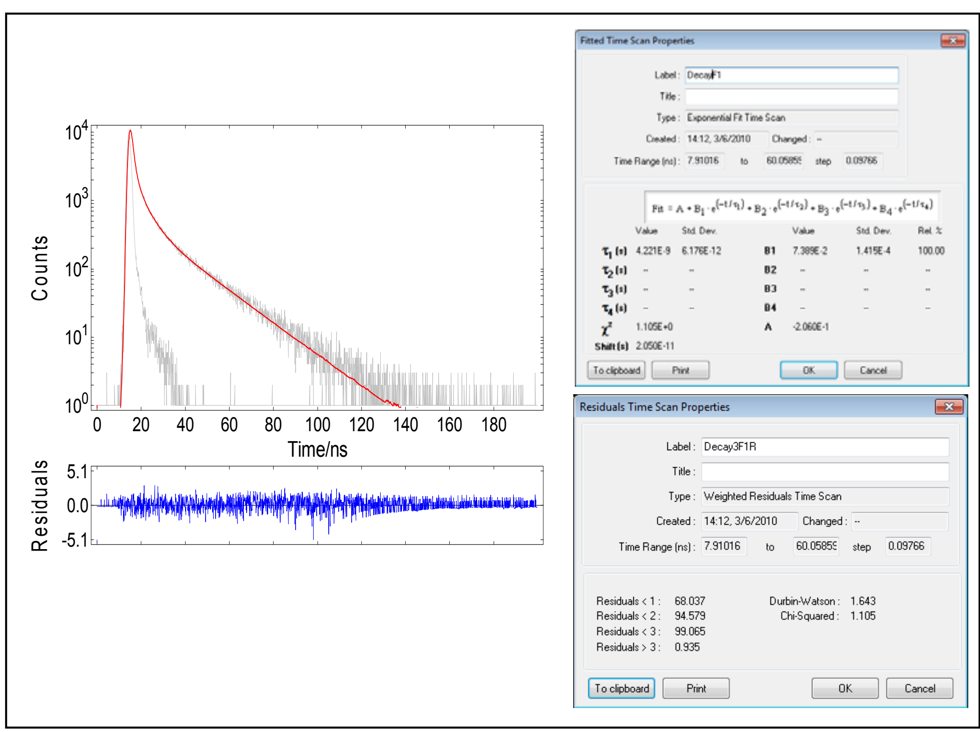


Figure 3-13: Fitted Time Scans and Residual Scans, typical display and scan properties

There are additional time scan types, e.g. ***Anisotropy Time Scans*** and ***Autocorrelation Time Scans.*** These types are similar to the Time Scan types outlined above and can be shown in the same graphical format with similar scan property boxes. ***Anisotropy Time Scans*** require the optional polarisers.

3.6. Graphical Presentations and Plot Options

F900 provides 2D graphics, 3D graphics, and contour plots for data visualisation, as well as a display of the numerical values for viewing and editing purposes.

2D, 3D, and contour graphics have their own colour, font style and line style sets. These can be set up via the ***Plot Options***. The three styles are used for the display of data within the F900 and when the graph is copied into the clipboard for further use in different computer applications (***Ctrl + C***). For 2D graphics, a separate set of colours and line styles may be set up for printing purposes (***File >> Print***).

3.6.1. 2D Data Presentation

Nine different 2D profiles can be independently set up in respect to their labels on X- and Y- axes, whether the Y-axis should be scaled from Zero or not and whether the Y-axis is displayed in linear or logarithmic scale as default. The use of different 2D profiles eases the handling of different data types. For example, 2D graphics for spectral scans and kinetic data may be set up with linear Y-axis as default, whereas time resolved data and plots of fitted decay curves are with a logarithmic Y-axis.

The nine different 2D plot defaults can be set up via ***Options >> Plot defaults >> 2D***. The plot of each individual 2D graph may also be changed ***right mouse click >> Plot options***.

Figure Figure 3-15 and Figure 3-16 below show typical 2D displays for illustration, a "Spectral" 2D plot and a "Fit Plot", respectively.

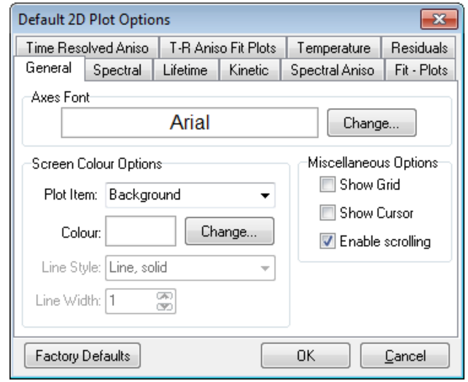


Figure 3-14: Setup of default 2D plot options

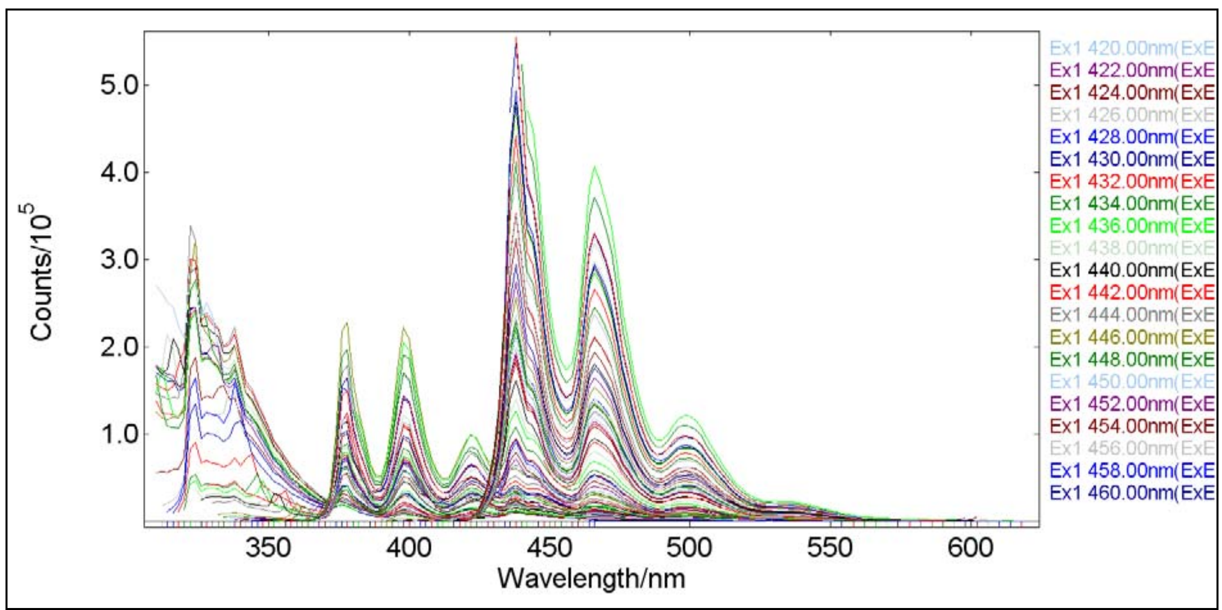


Figure 3-15: Example for 2D display of spectral data

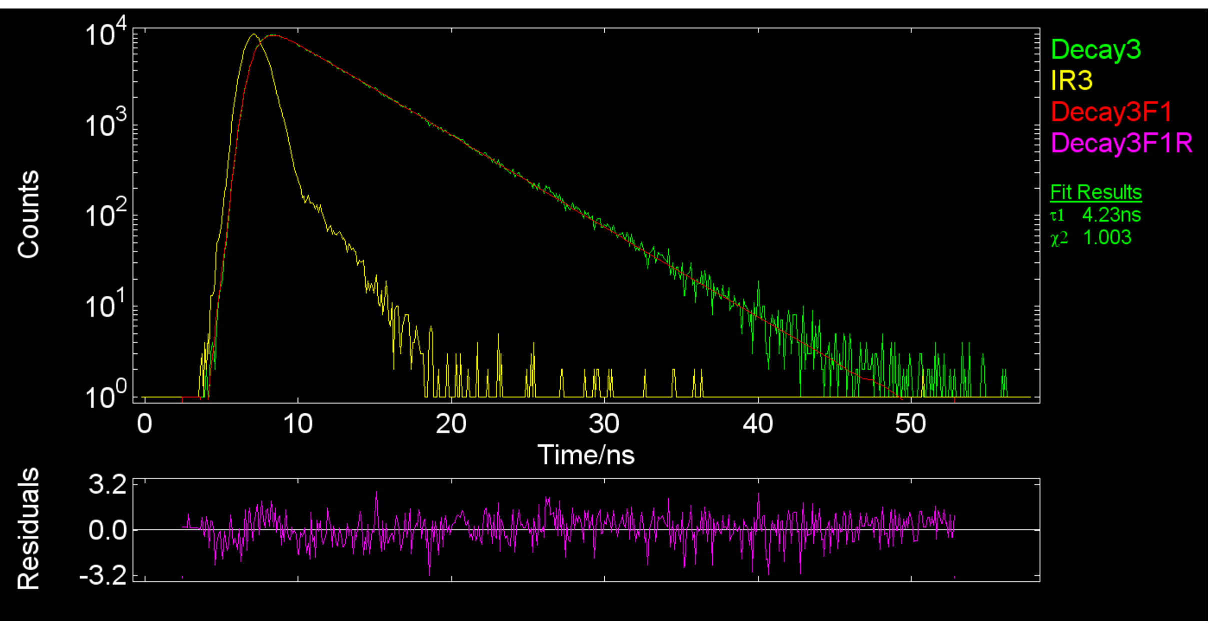


Figure 3-16: Example for 2D display of time resolved data

Task	Operation
Set up 2D defaults	***Options >> Plot defaults >> 2D...***
Modify colours and line styles	***Plot options >> General***,
Modify axes labels, scaling from Zero, swap between wavelength/time and channels	***Plot options >> graph type tab***
Switch between linear and logarithmic Y-scale	use lin and log from the tool bar
Switch cursor on or off	***View >> Show cursor***, or . By setting the curser the X- and Y- values will also be displayed.
Switch grid on or off	***View >> Show grid***, or
Show maxima, minima, or both	***View >> Show peaks***, or . Only available for spectral data.
Modify the sensitivity of the peak search	View >> Peak settings... Use this dialogue box also to specify, whether you want to display peaks, troughs, or both.
Zoom	use ***View >> Zoom In*** / ***Zoom Out*** or /

3.6.2. 3D Data Presentation

Data display in 3D is available if multiple scans or scan maps are contained in the same active window.

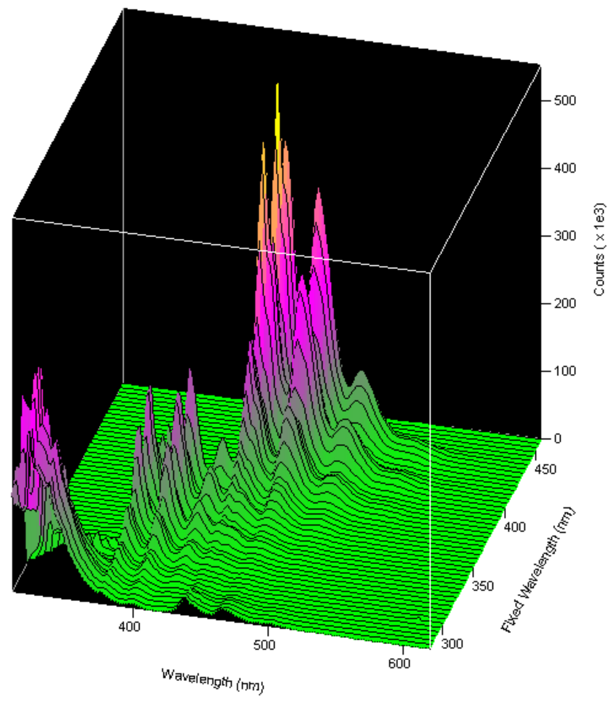


Figure 3-17: Example of a 3D display

Task	Operation
Set up 3D defaults	***Options >> Plot defaults >> 3D...***
Change colours	***Plot options >> Appearance***
Change the kind of mesh	***Plot options >> Mesh***
Zoom on X, Y, and Z axes	***Plot options >> Zoom***
Rotate 3D display	left mouse click and drag

3.6.3. Contour Plots

The contour plot option is available, if the active display contains more than one spectral scan or time resolved measurement.

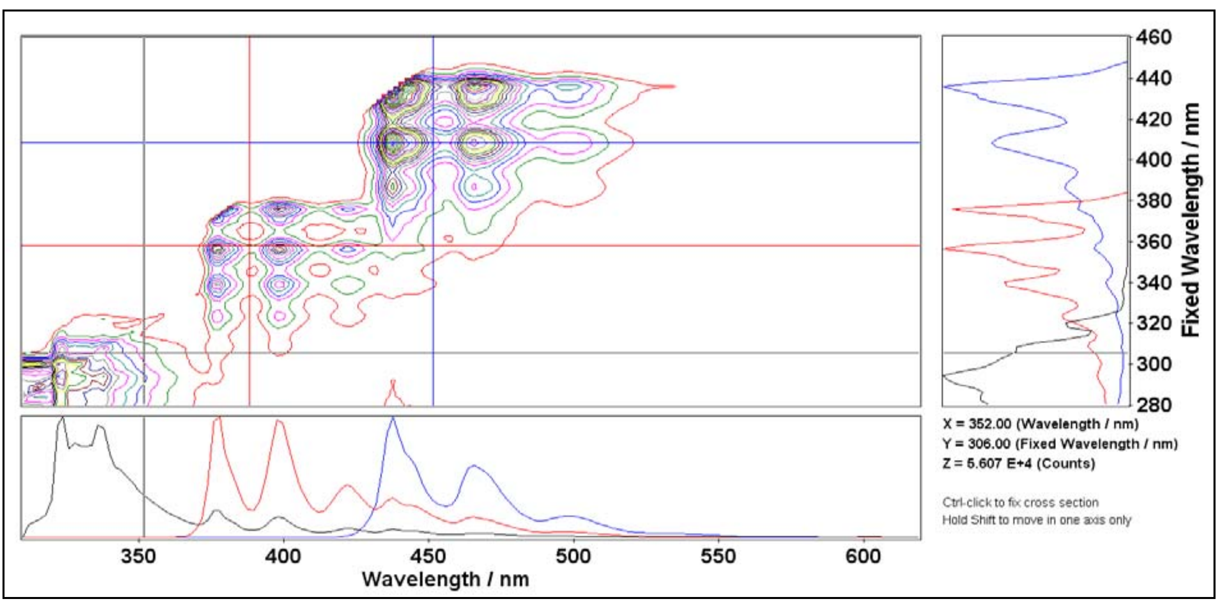


Figure 3-18: Example for Contour plot of spectral data

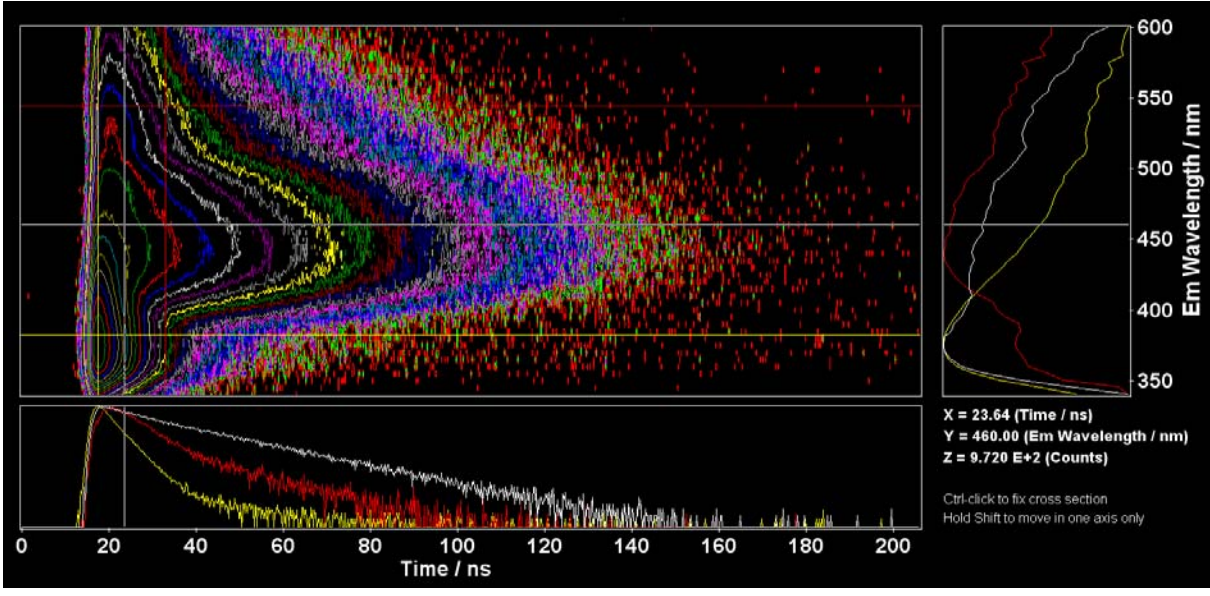


Figure 3-19: Example for Contour Plot of time resolved data

Task	Operation
Set up 3D defaults	***Options >> Plot defaults >> Contour...***
Change colours, add/remove contour lines, make contour lines invisible	***Plot options***
Change resolution on time axis	***Plot options***
Zoom	use ***View >> Zoom In*** / ***Zoom Out*** or [icon] / [icon]
Show live cross-hair and axes bars	***View >> Show cursor***, or [icon]. X,Y and Z values are shown when cross hair is live.
Freeze cross-hair and display in bars	hold Ctrl and click left mouse click
release fixed cross-hair	hold Ctrl, then click left mouse click on cross-hair
move cross-hair along one axis	hold Shift
Normalise data in display bars	click [N] in tool bar

3.6.4. View Numerical Data

This facility enables you to view the numerical values of your scan. You can also edit individual data points.

EmMap_mixture_2(Excitation Emission Map)

Experimental Conditions

Scan : Emission Scan : Ex1 290.00nm(ExEmMap)
Wavelength: 310.00 to 574.00 step 2.00 nm

Ex WaveLength: 290.00 nm
Em Slit Size: 2.00 mm — Ex Slit Size: 2.00 mm
Em Polariser: No polariser — Ex Polariser: No polariser
Dwell Time: 0.500 s — Temperature:

Data / 10e5 (Counts)

nm	0	2	4	6	8	10	12	14	16	18
310	1.778	1.694	1.746	2.096	1.968	1.682	2.840	2.903	2.235	2.238
330	2.171	2.120	1.926	1.929	2.023	1.733	1.484	1.399	1.276	1.157
350	1.006	0.867	0.780	0.681	0.583	0.512	0.451	0.393	0.346	0.290
370	0.245	0.217	0.212	0.223	0.204	0.164	0.142	0.114	0.092	0.075
390	0.067	0.063	0.072	0.087	0.098	0.090	0.072	0.056	0.045	0.037
410	0.029	0.025	0.023	0.025	0.031	0.036	0.038	0.039	0.038	0.044
430	0.054	0.071	0.122	0.197	0.227	0.193	0.159	0.150	0.139	0.108
450	0.090	0.076	0.068	0.065	0.066	0.073	0.101	0.121	0.132	0.128
470	0.120	0.112	0.098	0.081	0.069	0.062	0.050	0.041	0.038	0.034
490	0.032	0.033	0.038	0.040	0.040	0.039	0.039	0.035	0.032	0.030
510	0.024	0.024	0.020	0.017	0.016	0.014	0.013	0.011	0.011	0.010
530	0.010	0.011	0.009	0.009	0.010	0.008	0.009	0.006	0.007	0.006

Figure 3-20: Display of numerical values of a scan

3.7. Data Operation and Analysis with F900

F900 includes a suite of tools for operating and manipulating your data, accessed from the Data menu.

The Data menu is displayed when the scan display area has at least one scan window open. Options available depend on whether the currently selected scan window contains spectral or lifetime measurements.

Option	Spectral Data	Lifetime Data	Description
Combine	✓	✓	Combines data by adding, subtracting, multiplying, dividing, or (for spectral data) appending.
Scale	✓	✓	Multiplies measurements by a specified factor.
Normalise	✓	✓	Scales measurements to specified peak.
Subtract Baseline	✓	✓	Subtracts a stationary background.
Crop Range	✓	✓	Crop measurements down to a specified range.
Smooth	✓		Smoothes spectral data using a binomial technique.
Differentiate	✓		Differentiates spectral data using first, second, or higher order derivatives.
Integrate	✓		Integrates spectral data, from the shortest wavelength side to the longest.
Correction	✓		Apply manual spectral correction to existing scans.
Reverse	✓	✓	Reverses data in a scan along the X-axis.
Anisotropy Analysis		✓	Recalculate lifetime anisotropy based on a suitable measurement container and a specified G Factor.
Exp. Reconvolution Fit		✓	Reconvolutes a sample decay measurement based on an IRF, specified fitting range, and specified lifetime parameters.
Exp. Tail Fit		✓	Used to analyse scans containing a single decay measurement.
Autocorrelation		✓	Calculate an autocorrelation (residuals) curve.
TRES Data Slicing		✓	Slice TRES data based on a specified start time, stop time and number of slices.

For more details, see Section 6, Data Operation, on page 85 and the ***FLSP920 Series Reference Guide***.

3.8. Software and Hardware Options

The F900 Options menu lets you configure various aspects of the software and spectrometer components.

Option	Description
Correction Files	Set the spectral correction files to be used for correction of spectral scans. See Section.
Windows Options	Set various options for what to do when opening, appending, or closing scan files.
Measurement Options	Set up whether to allow temperature map creation, and the temperature tolerance value for automatic map creation
Analysis Options	Enable background subtraction (lifetime data) and whether to enable integrating sphere and absorption calculations for spectral data.
Plot Defaults	Set plot and plot axis options for 2D, 3D, and contour plots.
Sample Holder Options	Select the type of sample holder to use.
Select Plate Type	Select, modify, or create new plate configurations when using a Well-Plate Reader for samples.
Grating Change	Change the monochromator gratings to match the detectors spectral range, or for optimising the spectral coverage.
Oscilloscope Options	Select whether a connected oscilloscope is active or inactive.
PCS900 Configuration	Set the signal thresholds for each channel on the PCS900 card.

For more details se the ***FLSP920 Series Reference Guide***,

4. Making Spectral Measurements

FS920, FLS920, FSP920, and FLSP920 spectrometers can measure steady state photoluminescence spectra in the ultraviolet to near-infrared spectral range with single photon counting sensitivity. Other instruments of the FSLP920 series can measure spectra, too, but overall sensitivity/performance is somewhat reduced due to the lack of the appropriate excitation source.

Typical scans are excitation or emission scans where the wavelength is the scanning parameter. Other possible scans are scans for producing correction files, maps, and multiple scans. The table below summarises the scan types available for a standard spectrometer. The table also includes spectral anisotropy scans, as polariser upgrades are popular, the acquisition of those spectral anisotropy measurements is included in this paragraph.

Scan Type	Scan Parameter	Second (Map) Parameter
Emission Scan	emission wavelength	excitation wavelength (fixed)
Excitation Scan	excitation wavelength	emission wavelength (fixed)
Synchronous Scan	excitation and emission wavelengths, simultaneously	excitation-emission offset (fixed)
Excitation Correction Scans	excitation wavelength	n/a
Emission Correction Scans	emission wavelength	n/a
Excitation Anisotropy Scans	excitation wavelength, measurement of vertical polarisation plane (horizontal polarisation plane optional for G-factor)	emission wavelength (fixed), measurement of vertical and horizontal polarisation plane
Emission Anisotropy Scans	emission wavelength, measurement of vertical and horizontal polarisation plane	excitation wavelength (fixed), measurement of vertical polarisation plane (horizontal polarisation plane optional for G-factor)
Emission Map	emission wavelength	excitation wavelength, stepwise increased or decreased
Synchronous Map	excitation and emission wavelengths, simultaneously	excitation-emission offset, stepwise increased
Multiple Scans	repeated excitation, emission, or synchronous scans with optional waiting time between repeats	

The recognition of optional sample holders and accessories by the F900 software will automatically increase the type of scans available. For instance, temperature maps will be possible if cryostats or Peltier cooled sample holders are detected, spatial (sample) maps will be possible with sample X-Y stages or well plate reader attachment, maps of concentrations will be available with the optional titrator,

The operating instructions in this section assume that the instrument has been started, the spectrometer is in full operating conditions and samples have been prepared. If this is not the case, follow steps 1-5 below:

1. Switch on the PH1 power supply, the spectrometer controller, cooler power supply, and the Xe900 xenon lamp power supply.
2. Switch on the computer.
3. Double-click the F900 icon or select F900 from your Start menu to start the software.
4. Wait for the software to finish its initialisation process and component calibration. Once complete, the Signal Rate window is displayed:
5. Setup the sample for measurements. (This might include the use of special sample holders and mounts.)

The following sections will instruct how to perform the different types of spectral scans listed in the table above, using the F900 spectrometer operating software. The following rule applies to all spectral scans:

Use the ***Signal Rate*** dialogue box to prepare for a spectral scan, then use the scan type dialogue box (quick access via) to set up or modify scan parameters and to start the scan.

It is important to routinely use the ***Signal Rate*** dialogue box, as this contains some items that can only be setup there and not in any other spectral scan dialogue box. This applies to bandwidth settings, attenuator settings, polariser settings.

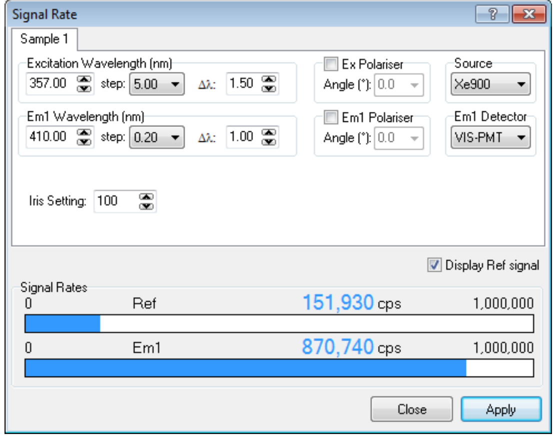


Figure 4-1: Signal Rate window for setting up spectral measurements

During spectral acquisitions the status bar on the button of the screen informs about the running activities, such as the number of the current scan, the sample temperature, the current monochromator settings, grating in use, spectral band width, angle of polarisation, etc.

Active spectral scans can be aborted by either pushing the ***Esc*** button or by clicking the tool button on the top right of the F900 screen. Note that the ***Esc*** button will only be active if the window containing the scan is in focus, not if a different data window has been highlighted.

Details of the parameter settings of a completed scan can be viewed in the ***Spectral Scan Properties***. The properties can be accessed via ***right mouse click >> Properties*** if the active window contains only one measurement or via ***right mouse click >> Properties >> Measurement List*** if more than one scan is present in the active data window.

Section 4.8 on page 60 provides some best-practice advice on making measurements.

4.1. Emission Scans

Scans with a fixed excitation wavelength and variable emission wavelength are called *Emission Scans*. The emission scan range is typically at a longer wavelength than the fixed excitation.

To set-up and perform an emission scan...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelength for which the signal is expected to be at a maximum. Ensure that the correct source (typically the steady state lamp Xe900) and the right detector have been selected. The photon count rate can be controlled by means of the attenuator (values between 1 and 100 are possible, note that the scale is not linear) and by means of the spectral band width in both the excitation and the emission beam path. In particular for solid or film samples it is important that the impact of the sample positioning and orientation on the signal rate is to be checked at this point.

2. Close the Signal Rate dialogue box and open the dialogue screen ***Emission Scan Setup…***. Upon opening this dialogue box all scan parameters of the last emission scan will be shown as default, apart from those that have been changed in the Signal Rate dialogue since the last scan. Typically this might be the excitation wavelength and spectral band pass (to be confirmed checking the Excitation tap)

and the spectral band pass in the emission (to be verified checking the Emission tap). The Emission Scan Parameters in the lower section of the dialogue box may be changed now.

Figure 4-2: Emission Scan Setup dialogue box

3. The tap General offers a variety of correction options for the spectral scan. The correction methods are described in detail in section 8 of this manual. For less experienced users it is recommended to start with the boxes ***Recording Ref. Data*** in the excitation and ***Em1 File '...'*** in the emission being ticked. However, it is also strongly recommended that the user measures his sample fully uncorrected, so that the impact of the correction on the data can be studied. The tick-box ***Display Ref. Data*** is only present if this option has been chosen in ***Options >> Misc. Options***. If this box is checked the data from the reference detector will be displayed in a separate window. This might be useful for subsequent correction. The function ***Subtract Background*** is switched on by default as soon as one of the automated correction methods has been selected. With the Background subtraction option being selected the background signal will be recorded prior to each scan and before each repeated scan.

4. All emission scan parameters can be saved by checking the Apply button. This will not start the scan, but it saves the parameter settings should the user decide to close and re-open this dialogue box. By checking the Start button the demonstrated scan parameters will be saved, the dialogue box will close and the spectral scan will begin.

The Emission Scan graph is displayed with points plotted in real-time. The auto-scaling Y-axis shows the number of photon counts for the total dwell time measured. Once the measurement is finished, you can right-click in the scan window to display a pop-up menu to change view settings and to view the scan properties.

Monitor the scanning process from the status bar. This shows the actual excitation and emission wavelengths, the current scan repeat number, the number of counts of subsequent scans and other information.

5. Select ***File >> Save*** to save the scan.

4.2. Excitation Scans

Scans where the emission wavelength is fixed and the excitation wavelength is scanned over a pre-defined range are known as *Excitation Scans*.

The scan range is typically at shorter wavelength than the fixed emission. Excitation scans reveal the absorption properties of a sample. They are useful in particular for the investigation of sample mixtures, as by means of the fixed emission wavelength species can be selectively measured.

To set-up and perform an emission scan...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelength for which the signal is expected to be at a maximum. Ensure that the correct source (typically the steady state lamp Xe900) and the correct detector have been selected. The photon count rate can be controlled by means of the attenuator (values between 1 and 100 are possible, note that the scale is not linear) and by means of the spectral band width in both the excitation and the emission

beam path. In particular for solid or film samples it is important that the impact of the sample positioning and orientation on the signal rate is to be checked at this point.

2. Close the Signal Rate dialogue box and open the dialogue screen Excitation Scan Setup…. Upon opening this dialogue box all scan parameters of the last excitation scan will be shown as default, apart from those that have been changed in the Signal Rate dialogue since the last excitation scan. Typically this might be the emission wavelength and spectral band pass (to be confirmed checking the Emission tap) and the spectral band pass in the excitation (to be verified by checking the Excitation tap). The Excitation Scan Parameters in the lower section of the dialogue box may be changed now.

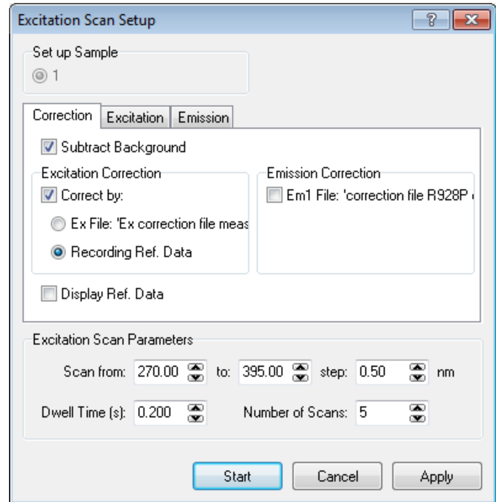


Figure 4-3: Excitation Scan Setup dialogue box

3. The tap General offers a variety of correction options for the spectral scan. The correction methods are described in detail in section 8 of this manual. For less experienced users it is recommended to start with the box ***Recording Ref. Data*** being ticked. However, it is also strongly recommended that the user measures his sample uncorrected, to study the impact of the correction on the data. The tick-box ***Display Ref. Data*** is only present if this option has been chosen in ***Options >> Misc. Options***. If this box is checked the data from the reference detector (corrected for the response of silicon) will be displayed in a separate window. This might be useful for subsequent correction. The function ***Subtract Background*** is switched on by default as soon as one of the automated correction methods has been selected. With the Background subtraction option being selected the background signal will be recorded prior to each scan and before each repeated scan and will then be subtracted from the raw scan data.

4. All excitation scan parameters can be saved by checking the Apply button. This will not start the scan, but it saves the parameter settings should the user decide to close and re-open this dialogue box. By checking the Start button the demonstrated scan parameters will be saved, the dialogue box will close and the spectral scan will begin.

4.3. Synchronous Scans

With synchronous scans both the excitation and the emission wavelengths are scanned simultaneously, with the offset between excitation and emission being either Zero or with the emission wavelength at a fixed offset above the excitation wavelength.

Synchronous scans are useful for the characterisation of complex fluorophore mixtures. For Zero-Offset synchronous scans, those species that have a non-zero overlap between the excitation and the emission spectrum will produce characteristic spectral bands. The width of the bands will depend on the width of the spectral overlap, while the amplitude of the bands is proportional to the absorbance and the fluorescence quantum yield of the species contained in the sample. For Non-Zero-Offset synchronous scans, species with shift between absorption and emission that is equal to the offset will produce the biggest signal.

To set-up and perform a synchronous scan...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelength for which the signal is expected to be at a maximum. Ensure that the correct source (typically the steady state lamp Xe900) and the correct detector have been selected. The photon count rate can be controlled by means of the attenuator (values between 1 and 100 are possible, note that the

scale is not linear) and by means of the spectral band width in both the excitation and the emission beam path. In particular for solid or film samples it is important that the impact of the sample positioning and orientation on the signal rate is to be checked at this point.

2. It is important to note that synchronous scans with an offset of Zero are only meaningful for samples that have negligible scattering properties. If the sample scatters significantly an offset different from Zero should be chosen with the spectral band width of both excitation and emission smaller than the offset.

3. Close the Signal Rate dialogue box and open the dialogue screen ***Synchronous Scan Setup***.. Upon opening this dialogue box all scan parameters of the last synchronous scan will be shown as default. The Excitation Scan Parameters and the lower section and the Ex to Em offset in the Emission tap may be changed now.

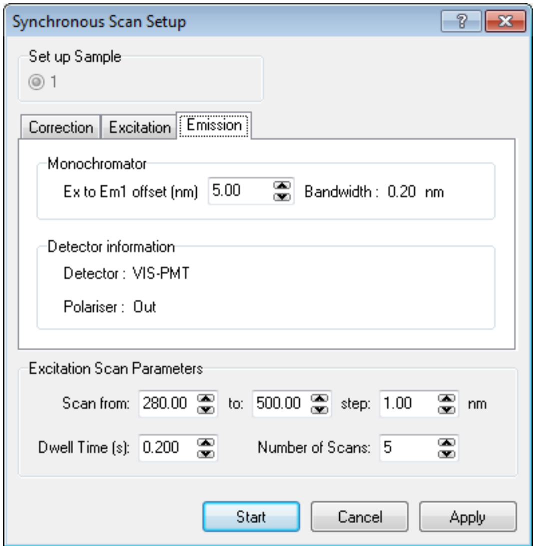


Figure 4-4: Synchronous Scan setup dialogue box

4. As for excitation and emission scans, the tap ***General*** offers a variety of correction options for this spectral scan. The correction methods are described in detail in section 8 of this manual. For less experienced users it is recommended to start with the boxes Recording Ref. Data in the excitation and EmCorr Scan in the emission being ticked. However, it is also strongly recommended that the user measures his sample fully uncorrected, so that the impact of the correction on the data can be studied. The tick-box Display Ref. Data is only present if this option has been chosen in Options > Misc. Options. If this box is checked the data from the reference detector will be displayed in a separate window. This might be useful for subsequent correction.

5. All synchronous scan parameters can be saved by checking the Apply button. This will not start the scan, but it saves the parameter settings should the user decide to close and re-open this dialogue box. By checking the Start button the demonstrated scan parameters will be saved, the dialogue box will close and the spectral scan will begin.

Note that for synchronous scans the parameter on the X-axis is the excitation wavelength. The offset of the emission is registered in the scan properties.

4.4. Correction Scans

Correction scans are spectral scans that are used to generate files suitable for spectral correction. The spectrometer comes supplied with a set of correction files, the number of these files is dependent on the number of gratings, light sources and detectors. For a standard spectrometer with one excitation source (Xe900), one detector (single photon counting PMT) and one grating (or grating set) for both excitation and emission monochromator, two correction files are required: an ***Excitation Correction File*** and an ***Emission Correction File***. The ***Correction Scan*** option allows to measure spectra that can be converted into correction files.

Correction scans are of a different file type than excitation, emission, or synchronous scans. Only correction scans can be converted into correction files that are then used for spectral correction. Correction files can be assigned for correction in ***Options >> Correction Files***.

4.4.1. Excitation Correction Scans

Excitation correction scans are measured using the calibrated reference detector. Excitation correction files are generated for each individual FLSP920 series spectrometer at the Edinburgh Instruments factory. The file is made with a fixed bandwidth (generally 1nm), therefore, strictly speaking the spectral correction provided is only valid when the same bandwidth is used to make the measurement. However the user is able to generate new correction files for the different bandwidths in use. A new correction file should also be generated if the Xe900 bulb has be re-aligned, e.g. after lamp replacement.

To set-up and perform an excitation correction scan...

1. Use the Signal Rate dialogue box to observe the signal of the reference detector. Set up parameters of the excitation arm, e.g. excitation bandwidth. Ensure that the correct source (typically the steady state lamp Xe900) has been selected. Select a wavelength where the excitation is at a maximum (this is grating and bandpass dependant but is normally 468nm). The signal rate on the reference detector can be controlled by means of the attenuator (values between 1 and 100 are possible, note that the scale is not linear), the signal level should not exceed 4,000,000.

2. Close the Signal Rate dialogue box and open the dialogue screen ***Correction Scan >> Excitation***…. Upon opening this dialogue box all scan parameters of the last excitation scan will be shown as default, apart from those that have been changed in the Signal Rate dialogue since the last correction scan. The Correction Scan Parameters in the lower section of the dialogue box may be changed now. Typical scan parameters for a standard visible grating and Xe900 are shown in Figure 4-5.

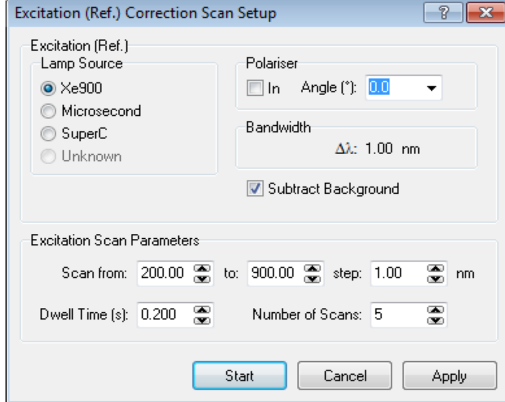


Figure 4-5: Excitation Correction setup dialogue box

3. Start the measurement (***START***). A typical scan result is shown in Figure 3-8.

Generating the excitation correction file from the excitation correction scan is straight forward: As the reference detector is a corrected detector (the correction file for this detector is called ***Si Ref Corr.FS***), no further file manipulation is required. For convenience the measurement may be normalised to Unity.

The reference detector is corrected in units of photon flux (or spectral radiance in photon units). Therefore any corrected excitation scan will have the same units.

To generate the Excitation Correction File from the Excitation Correction Scan ...

4. Normalise the measurement for convenience.

5. Save the file in an appropriate location, such as ***C:\DocumentsandSettings\AllUsers\ApplicationData\EdinburghInstruments\F900*** (WindowsXP) or ***C:\ProgramData\Edinburgh Instruments\F900*** (Windows 7)

6. In order for the F900 software to use this file as an excitation correction file, the file needs to be assigned as such. To do this go to ***Options > Correction files***, select the excitation tab and select 'modify' to allocate the newly saved file.

4.4.2. Emission Correction Scans

A calibrated light source of known spectral output is needed to generate emission correction files. Emission correction files are made for each individual FLSP920 series spectrometer at the Edinburgh Instruments factory using calibrated deuterium and tungsten lamps. For detectors with sensitivity beyond 2.5µm a calibrated Nernst source is used.

The calibrated light sources that are used to produce the Emission Correction File are calibrated in units of photon flux (or spectral radiance in photon units). Therefore any corrected emission scan will have the same units.

The emission correction scans are made with a fixed bandwidth (generally 1nm). The bandwidth dependence is not nearly as critical as for excitation correction scans, as the detector responsivity has no narrow spectral features, compared to those of the xenon lamp. Therefore the same emission correction file can be safely used for all spectral band widths. Re-calibration of the emission channel is only required when detectors or gratings are changed or replaced. An emission correction file produced once will last for years. Re-calibration is only be needed for the purpose of verification of the long term stability of the emission channel.

You can re-calibrate the emission channel using your own calibrated lamps, you can purchase a calibration set from Edinburgh Instruments, or contact the company for a loan of the calibration set.

A complete correction file for the spectral range between 200nm and 900nm is generated from four independent emission correction scans, as shown in the table below. This is necessary as the resulting correction file is supposed to be free of higher order artefacts caused by grating in the emission monochromator. Higher orders are blocked by the given short wavelength cut-off filter.

Scan Number	Calibrated Light Source	Filter	Scan Range
1	Tungsten	–	300nm – 420nm
2	Tungsten	350nm	400nm – 620nm
3	Tungsten	550nm	600nm – 900nm
4	Deuterium	–	200nm – 400nm

To set-up and perform an emission correction scan...

1. Install the tungsten calibration lamp and the scattering prism as described (refer to ***FLSP920 Series Reference Guide***).
2. Use the Signal Rate dialogue box to observe the detector signal. Set up emission parameters. Ensure that the correct detector has been selected. For more complex systems also check that the desired grating has been selected. Choose a wavelength where the emission is at a maximum (for a system with standard detector this is around 550nm). The signal rate can only be controlled by means of the iris that is part of the calibration lamp. The signal level should not exceed 2,000,000cps.
3. Close the Signal Rate dialogue box and open the dialogue screen ***Correction Scan >> Emission****…*. The Correction Scan Parameters in the lower section of the dialogue box may be changed now. The scan parameters for the first scan are shown in Figure 4-6.
4. Start the fist of the four correction scans (***START***).
5. Make the remaining two correction scans. Note that these are measured with wavelength filters in place (filter holder in emission channel near scattering prism) and with different scan ranges.
6. Now install the deuterium lamp, use the Signal Rate screen to optimise the signal for 400nm, then make than last spectral scan.

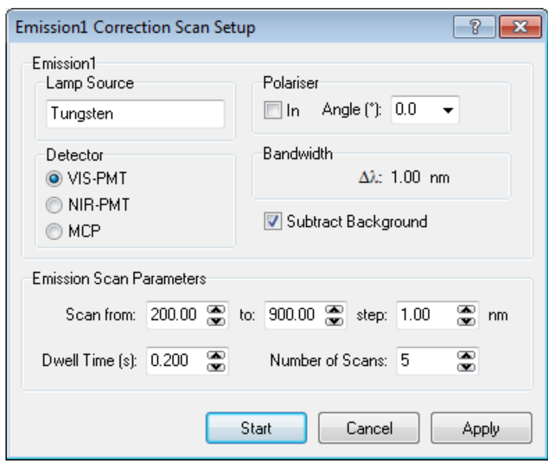


Figure 4-6: Emission Correction setup dialogue box

To generate the Emission Correction File ...

7. Subtract the background level from each of the four scans. This procedure is not necessary if the detector has a negligible background or if the background subtraction had been done automatically during the scans.

8. Overlay the tungsten measurements (scans 1-3 – ***Join Visible*** tool button) and verify a good match in the cross-over sections. The filters listed in the table above have a flat transmission in the given scan range, errors due to variation in the transmission in the stated scan range are negligible, although a scaling may be required.

9. Join the three emission scans (***Data >> Combine >> Append***), using the short wavelength scan first. Ensure the Scaling option isa applied. The result of this procedure is one emission correction scan.

10. Divide the remaining two correction scans (generated by deuterium and tungsten lamp) by the corresponding lamp file, i.e. the scans that was measured using the deuterium lamp by the deuterium file, the (joined) scan that was measured with the tungsten lamp by the tungsten file.

11. Overlay the corrected deuterium measurement with the corrected tungsten measurement (***Join Visible*** tool button) and verify a good match in the cross-over section. The files may have to be scaled for better visualisation. It is important to have a good match of the two files, typically in the range between 350nm and 380nm.

12. Join the two emission correction files in the centre of the matching region, using the append function (***Data >> Combine >> Append).*** The file may now be

13. Normalise the measurement for convenience. It might also be appropriate to use the smooth function, provided spectral features will not be distorted. A typical emission correction file is shown in Figure 3-9.

14. Save the file in an appropriate location, such as ***C:\DocumentsandSettings\AllUsers\ApplicationData\EdinburghInstruments\F900*** (WindowsXP) or ***C:\ProgramData\Edinburgh Instruments\F900*** (Windows 7)

15. In order for the F900 software to use this file as an emission correction file, the file needs to be assigned as such. To do this go to ***Options > Correction files***, select the emission tab and select ‘modify’ to allocate the newly saved file.

4.5. Spectral Anisotropy Scans

The measurement of spectral fluorescence anisotropy requires computer-controlled polarisers in both the excitation and the emission channels (optional accessory for all FLSP920 spectrometers). Linearly polarised light of vertical (V = 0^0) and horizontal (H = 90^0) orientation are used to excite the sample and detect the sample emission. See the ***FLSP920 Series Reference Guide*** for more details on the FLSP920 polariser accessory.

Anisotropy measurements reveal information about the ability of the average assembly of molecules to rotate during the short period of being in an excited state. For most fluorophores, anisotropy effects are only observed when the solvent has a high viscosity (e.g. glycerol and mineral oil) or when the fluorophores are attached to slowly rotating macromolecules (e.g. bound to proteins or embedded into membranes). In low viscosity solvents (such as water and ethanol) spectral anisotropy is generally not seen as the rotation effects are too fast.

Before performing anisotropy measurements, measure the sample's excitation and emission fluorescence spectra without polarisers. This way you can familiarise yourself with the sample's range of maximum absorption and emission.

Before you can start making the polarised measurements you must move the polarisers into the beam path. For this open the sample chamber lid and locate the handles for the polarisers (Figure 4-7). Then open the Signal Rate dialogue box and tick the box ***Ex Polariser***. A message will come up that asks you to move the excitation polariser into the beam (Figure 4-8). Do so by moving the leaver into the "IN" position (Figure 4-7), then quit the message with ***Retry***. Repeat the same procedure with the emission polariser.

The spectrometer is now ready for the anisotropy scans.

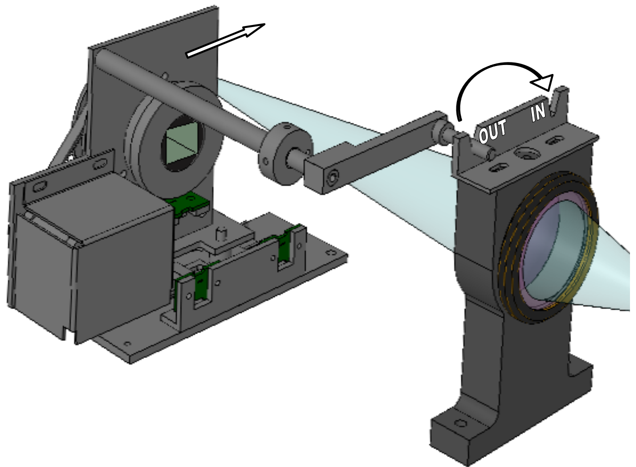


Figure 4-7: Polariser assembly

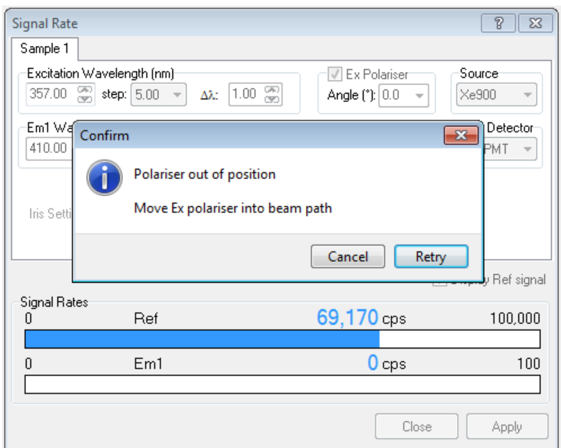


Figure 4-8: Signal Rate box with active polarisers

4.5.1. Excitation Anisotropy Scans

Excitation anisotropy functions are calculated from either four linear polarised excitation scan measurements (I_{VV}, I_{VH}, I_{HV}, I_{HH}) or from two measurements (I_{VV} and I_{VH}), depending on whether G-factor correction is applied or not. The equations for the calculation of the G-factor, the anisotropy free excitation scan, and the anisotropy function are given in the formula section on page 103, equations (1) – (3).

To setup and perform and Excitation Anisotropy Scans...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelengths for which the signal is expected to be at a maximum. Ensure that the correct source (steady state lamp Xe900) and the right detector have been selected.

2. With all other settings remaining unchanged, check the signal levels for all four combinations of polariser orientations: Excitation polariser = 0° and 90°, Emission polariser = 0° and 90°. Ensure that none of the signals exceeds 2 million cps. Adjust the monochromator slits and the attenuator accordingly.

3. Close the Signal Rate dialogue box and open ***Anisotropy Scan >> Excitation***. Enter appropriate scan parameters in the lower section of the dialogue box, then select the Anisotropy tab (Figure 4-9) to define the anisotropy specific settings.

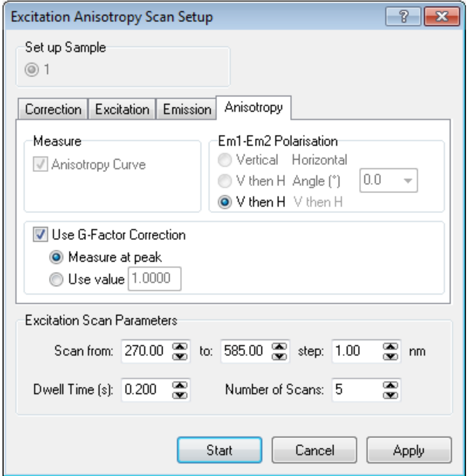


Figure 4-9: Excitation Anisotropy Setup Dialogue Box

4. The Anisotropy tap contains options for the G-factor corrections. Typically the G-factor correction is checked and ***Measure at peak*** is selected. With this option the two scans I_{VV} and I_{VH} will be measured. At the end of the two scans the software will move the excitation monochromator to the peak of the I_{VV} scan and will then set the excitation polariser to the horizontal position to measure I_{HH} and I_{HV} at this peak wavelength. Alternatively, if the G-factor is known the ***Use value*** can be selected and the known G-factor can be manually entered. A typical example for the set of polarised fluorescence excitation scans is given in Figure 4-10. Note that these curves are spectrally uncorrected. For the calculation of the fluorescence anisotropy functions correction of the individual polarised curves is not required. However, it is important to note that a possible detector background should be automatically subtracted, as a significant background would impact on the resulting anisotropy function.

5. At the end of the scans the G-factor will automatically be measured (if the option was selected - see above) and finally the excitation anisotropy scan will be generated in a separate data window. The G-factor can be viewed in file properties of this measurement result (***right mouse click >> Properties***). Typical raw polarised measurements are shown in Figure 4-10 overleaf. A typical anisotropy scan and the corresponding scan properties are shown in Figure 3-10 on page 35.

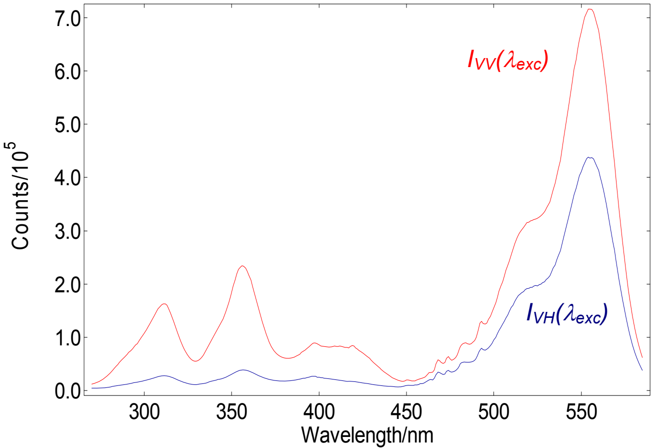


Figure 4-10: Raw polarised fluorescence excitation scans of rhodamin B in glycerol:

4.5.2. Emission Anisotropy Scans

Emission anisotropy scans are similar to excitation anisotropy scans in the way the polarised measurements $I_{VV}(\lambda)$ and $I_{VH}(\lambda)$ are made. However, they are different in respect to the G-factor correction. While for the excitation anisotropy only one G-value is required (as the emission wavelength is fixed), a whole curve of G-values is required for the correction of the emission anisotropy, i.e. each emission wavelength setting has a G-factor. The equations for the calculation of the G-factor curve, the anisotropy free emission scan, and the emission anisotropy function are given in the formula section on page 103, equations (4) – (6).

For single species, emission anisotropies are typically independent of the wavelength (as there is only one emission dipole involved) and provide therefore – compared to excitation anisotropy scans – only limited information.

To setup and perform and Emission Anisotropy Scans...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelengths for which the signal is expected to be at a maximum. Ensure that the correct source (steady state lamp Xe900) and the right detector have been selected.

2. With all other settings remaining unchanged, check the signal levels for all four combinations of polariser orientations: Excitation polariser = 0° and 90°, Emission polariser = 0° and 90°. Ensure that none of the signals exceeds 2 million cps. Adjust the monochromator slits and the attenuator accordingly.

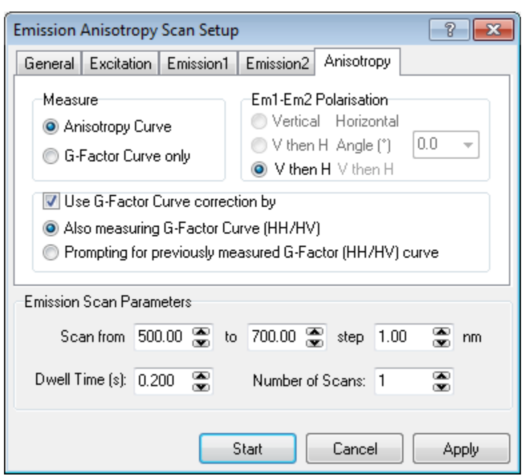


Figure 4-11: Emission Anisotropy setup dialogue box

3. Close the Signal Rate dialogue box and open ***Anisotropy Scan >> Emission***. Enter appropriate scan parameters in the lower section of the dialogue box, then select the Anisotropy tab to define the anisotropy specific settings.

4. The Anisotropy tab contains options for the G-factor corrections. On the top left of this dialogue tap you decide whether to measure a complete emission anisotropy scan (i.e. an anisotropy scan with G-factor correction) or to measure the G-factor curve only. Typically the (complete) ***Anisotropy Curve*** option will be selected and with this two options for the G-factor are given: If the first of the two G-factor options was selected (***Also measuring G-factor Curve (HH/HV)***) the full measurement will finally contain all four polarised curves, i.e. $I_{VV}(\lambda)$, $I_{VH}(\lambda)$, $I_{HV}(\lambda)$ and $I_{HH}(\lambda)$. If the second of the two G-factor options was selected only $I_{VV}(\lambda)$ and $I_{VH}(\lambda)$ will be measured and a previously measured G-factor curve can be loaded before the anisotropy calculation is performed. A typical example for the set of polarised fluorescence emission scans is given in Figure 4-12.

5. After scanning both the G-factor curve and the emission anisotropy curve will be displayed in different windows. Figure 3-11 shows an example of both curves.

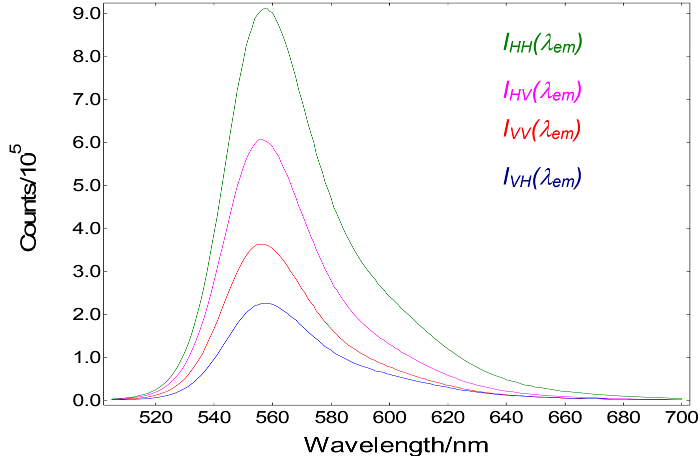


Figure 4-12: Raw polarised fluorescence emission scans of rhodamin 6G in glycerol

4.6. Emission Maps

Emission Maps are a series of emission scans (see Section 4.1 on page 46) with excitation wavelengths being systematically changed. Emission maps are often displayed by means of a contour plot graphic; this view is often referred to as EEM (Excitation-Emission-Map)

To set-up and perform an Emission Map measurement ...

Have sufficient background knowledge about the sample's emission properties. It is useful to perform one or more emission scans (refer to section 4.1 on page 46) with the aim to find the set of excitation and emission wavelengths at which the signal is at the maximum.

1. Open the ***Signal Rate*** screen, enter the set of excitation and emission wavelength for the expected maximum signal. Then select the spectral band widths and the attenuator setting so that the detector signal is appropriate, i.e. < 2 million cps). It might be appropriate to choose identical band widths for both excitation and emission.

2. Close the signal rate screen and open the ***Emission Map Setup*** dialogue box.

3. Use the lower section of the dialogue box to enter the appropriate excitation range and emission range, select the step sizes, the dwell time and the number of scans. Remember that the map is generated by performing emission scans with sequential increase (or decrease) of the excitation wavelength. For equal resolution on the X- and the Y axes of a future contour plot it would be appropriate to select identical wavelength step sizes for both excitation and emission. The overall measurement might take a long time, so keep ***Dwell Time*** and ***Number of Scans*** to a minimum, the step sizes to an affordable maximum.

4. Select the ***General*** tab and choose the method of spectral correction.

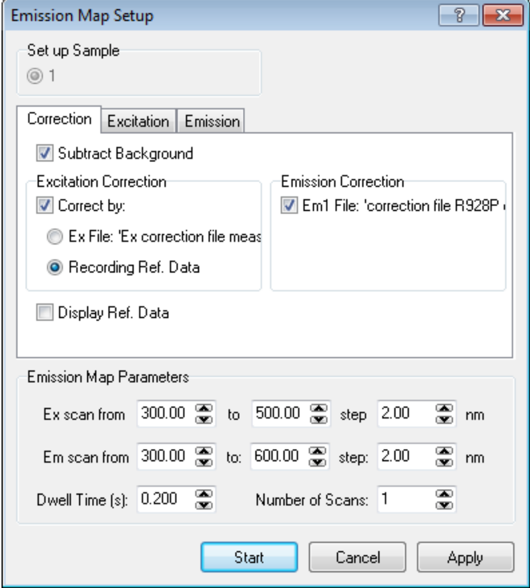


Figure 4-13: Emission Map Setup – General

5. Select the ***Excitation*** tab and decide whether the excitation should be stepwise increased – no tick, or stepwise decreased – tick the box ***Reverse Excitation Order*** (refer to Figure 4-14). Use the latter option when there is a risk for noticeable photo-degradation of the sample during the long measurement time. Starting at longer wavelengths (lower excitation energies) reduces the impact of photo-degradation on the measurement.

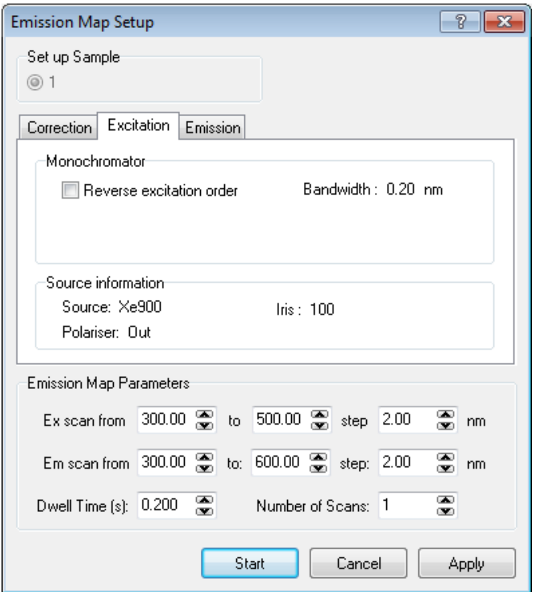


Figure 4-14: Emission Map Setup – Excitation

6. Select the ***Emission1*** tab. If none of the boxes in this tab are ticked, the scanning will cover the full range as specified in the lower section of the dialogue box. However, this potentially means that the emission will scan across the excitation. (In the given example of Figure 4-15 this applies to the cross-over section between 300nm and 500nm.) For scattering samples the cross-scanning is not desirable, in this case tick the upper of the two boxes. If you also want to avoid to pick up the second order scatter, tick the lower box, too. Once a box is ticked you have the option to decide, how many nanometers away from the actual excitation wavelength the emission scan should start.

7. Start the scan: ***Start***.

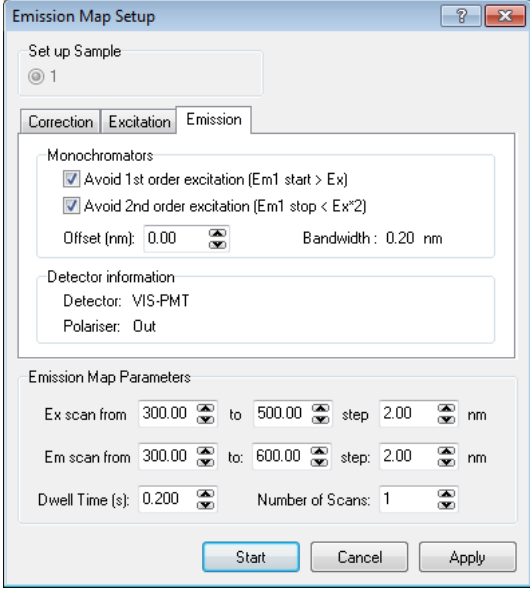


Figure 4-15: Emission Map Setup – Emission

4.7. Synchronous Maps

Synchronous Maps are a series of synchronous scans (see Section 4.3 on page 48) with the offset between excitation and emission being systematically increased.

To set-up and perform a Synchronous Map measurement ...

Gain sufficient background knowledge of the sample's emission properties. It is useful to perform one or more synchronous scans (refer to section 4.3 on page 48) with the aim to find the set of excitation wavelength and emission offset for which the signal is at the maximum.

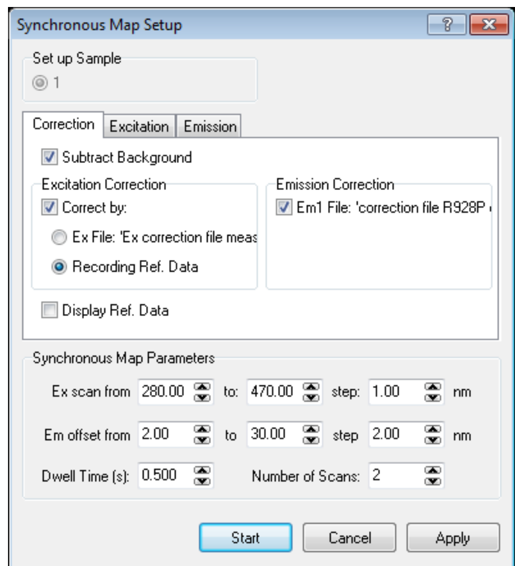


Figure 4-16: Synchronous Map Setup - General

1. Open the ***Signal Rate*** screen, enter the set of excitation and emission wavelength for the expected maximum signal. Then select the spectral band widths and the attenuator setting so that the detector signal is appropriate, i.e. < 2 million cps). It might be appropriate to choose identical band widths for both excitation and emission.

2. Close the signal rate screen and open the ***Synchronous Map Setup*** dialogue box.

3. Use the lower section of the dialogue box to enter the appropriate excitation range and the range for the emission offset, select the step sizes, the dwell time and the number of scans. Remember that the map is generated by performing synchronous scans with sequential increase of the emission offset. The overall measurement might take a long time, so keep ***Dwell Time*** and ***Number of Scans*** to a minimum, the step sizes to an affordable maximum.

4. Select the ***General*** tab and choose the method of spectral correction.

5. Start the map measurement: ***Start***.

4.8. Tips for Making Good Spectral Measurements

It is always useful to know your sample's absorption properties before attempting spectral emission measurements. If proper spectroscopic data are not available, even just looking at the sample can provide valuable information. For example, samples that are transparent will most likely absorb in the UV, and are likely to emit (if indeed there is any emission) in the blue spectral range. Samples that have yellow colour will absorb in the 400nm region and will probably emit green or orange, samples that are blue will absorb at around 600-700nm and will have dark red or even infrared emission.

You should also gain experience with sample concentration and alignment issues. Liquid samples are easier to measure than solid or film samples, as typically less scattering is involved and alignment effects are negligible. However, even with liquids concentration effects can seriously affect the quality of spectral scans. For samples with absorption and emission in the visible spectral range it is useful to physically observe the sample when it is excited:

1. Open the sample chamber lid. This will close the detector shutter (for protection) and will allow you to observe the sample.
2. Select the ***Signal Rate*** box. This will open the excitation shutter so that excitation light is permitted to the sample.
3. Set the excitation wavelength to a value that is consistent with the sample's main absorption.
4. Look at the sample! Observe the excitation, distinguish between excitation and emission (a filter in front of the eye might help), gain an insight into potential problems with sample alignment and concentration.

The following is a list of potential effects, problems and sources of errors, together with some tips on how to overcome or minimize them.

Tips for all types of spectral scans:

1.	Inner Filter Effect

When working with liquid samples, sample concentrations should be chosen that are small enough to avoid the inner filter effect. If the concentration is high, and a wavelength of excitation is selected that is in a range where the sample has high absorbance, only the cuvette surface facing the excitation beam is strongly emitting and no excitation light might reach the cuvette centre. However, the cuvette centre is imaged into the emission monochromator. Therefore not much light might reach the detector.

It is instructive to study this effect by observing the situation using your eyes, for example using a highly concentrated sample of fluorescein with excitation at about 490nm. The emission can be observed with the sample chamber lid open and the signal rate dialogue box open (so that the excitation shutter is open).

Tips:

- Reduce the concentration of the fluorophore(s).
- Chose an excitation wavelength that is in a range where the sample absorbance is reduced. (This can cause other problems, such as excitation of unwanted sample species or sample contaminations.)
- Use micro-cuvettes or triangular cuvettes.
- Use a front face sample holder, this allows to detect the emission at the same surface that also faces the excitation beam.

2. Higher Order Effect

A large spike may appear at a wavelength that has the doubled value as that of the excitation wavelength.

This is the second order of the excitation; the problem is a typical phenomenon of a grating monochromator. In this case the problem is generated in the emission monochromator which, when set to a specific wavelength, will not only transmit this selected wavelength, but also a wavelength with half (and third, and fourth…) of the numerical value. The problem also appears in the excitation monochromator, but there it is less frequently seen as the excitation wavelength is often shorter than the emission wavelength and often the second order is highly or even completely suppressed.

Tips:

- Use a wavelength cut-off filter (blocking at shorter, transmitting at longer wavelength) in the emission beam path (bind the sample). The filter's cut-off wavelength should be a little shorter than the starting wavelength of the scan.
- If a scan is to be performed over a wide range (say 300-800nm) one might even see the second order of the emission (in this case the emission from 300-400nm re-appearing between 600-800nm). In this situation there is no other way than measuring the emission spectrum in two steps, with two different cut-off filters, and subsequent joining (***Data > Combine .> Append***).

3. Weak Sample Emission

The sample shows very weak emission. What options are there to increase the signal?

Tips:

- The utmost important task is to check the positioning of the sample in the sample compartment. A visible inspection of the sample excitation is recommended (look at the sample when the ***Signal Rate*** screen is open). If the excitation is outside the visible spectrum it might be changed temporarily to 500nm for an assessment of the situation.
- The sample volume of a liquid sample might be too low. Increase the volume, use a micro-cuvette, or raise the cuvette.
- Bulk or film samples might have to be re-positioned.
- Increase the spectral band pass. If this is only done in the excitation it is still possible to obtain good spectral resolution for the emission scan. In the worst case both spectral band passes in excitation and emission have to be increased.

Please note: With very wide spectral band widths there is an increased risk to measure stray light that passes through the monochromators. There is also an increased risk to accidently over-expose the detector when scanning over the excitation or over the second order of the excitation.

4. Detector Saturation

If the detector is exposed to too much signal (either sample emission, or scattered excitation light), the spectrometer will be unable to "count all photon" that impact on the detector. Although this saturation effect is usually caused by more than one problem, the common term is "detector saturation".

If detector saturation occurs it will not necessarily harm the detector, but surely the data are distorted. It therefore is good practice to stay out of the range of detector saturation.

[The biggest cause for "detector saturation" is the randomness of the arrival times of photons at the detector and the subsequent randomness of the electrical pulses produced by the detector. A 100MHz counter would be able to count 100 million photon pulses that are all equi-spaced in time, but with random arrival times the narrow spaced pulses will be missed and therefore less than 100 million will be recorded.]

Detector saturation is easily overlooked when measuring with short dwell times. For example, a spectral scan that was acquired with a dwell time of 0.01s will be affected by saturation although the biggest signal is only 20,000 counts. Remember the typical saturation threshold is at 2 million counts per second!

Tips:

- Use the Signal Rate screen prior to measurements to check for the maximum possible signal
- Start with narrow spectral band widths before attempting bigger band widths.
- Use the iris attenuator, if appropriate
- Gain experience upper signal limits on scans with different dwell times.

5. Repeat Scanning

The dwell time, multiplied by the number of scans, results in the overall integration time per step (when the scan has finished). Is it better to use longer dwell times or a higher number of scans to improve the signal-to-noise ratio?

Tip:

- Use a shorter dwell time, (e.g. 0.1s or 0.2s) and instead a higher number of scans. This has the advantage that the overall spectrum is revealed at a fraction of the overall measurement time. An unwanted spectrum can therefore be aborted much earlier. Also, slow lamp fluctuations or potential detector background drift will be less significant in the measurement result as a result of multi-scan averaging. It is obvious that setting the dwell time to very short values has it's limitations. This applies in particular to analogue detectors with build-in Lock-in and the measurement of samples with very narrow spectral features. In those cases the dwell time should not be shorter than 0.3s.

6. Spectral Correction

Which type of spectral correction is best? Automated or manual? Which type of automated?

There is no general answer to those questions, it often depends how demanding the specific task is. However, in the majority of applications the full automated correction as described in the sections above is appropriate. Nevertheless, it is unavoidable to study the correction and check out the various options.

Tip:

- Do fully uncorrected measurements and use the manual correction (***Data >> Correction***) to correct your data.
- Study the impact of the background (high background, small signal levels).
- Data that are measured in spectral regions of reduced detector sensitivity are significantly modified (in a positive and negative sense) by the correction. Spectral correction in this spectral region is particularly demanding.

7. Samples with strong Absorption-Emission overlap

With many samples the emission spectrum overlaps the excitation spectrum to some degree.

Tip:

- In order to measure the full emission spectrum it might be appropriate to excite the sample not at the peak of the absorbance, but some 10nm to 50nm before the peak. This increases the range over which the emission can be scanned without distortions by scattered light.

Tips specific for spectral anisotropy scans:

8. Precision of G-factor

A correct G-factor is essential for a good anisotropy measurement.

Tips:
- Generally, a precision of the G-factor to 2 decimal place is required.
- For excitation anisotropies (where only one G-value is needed) the G-factor can be viewed and edited by selecting the window containing the original polarised scans and then selecting ***Data > Anisotropy (Ex)***.
- Modifying the G-factor manually allows to assess the impact of the G-factor on the final anisotropy curve. A G-factor that is too small will shift the anisotropy towards negative values, a G-factor that is too big will result in an anisotropy that is too positive.
- An unambiguous test for the correctness of the G-factor is to use a sample where the fluorophores are in a low viscous environment (e.g. water, ethanol). For those samples the anisotropy is expected to be Zero (the data are randomly distributed around Zero). If the G-factor is wrong the anisotropy curve will be offset from Zero.

9. Impact of background on anisotropy function

A high background in the scans of the raw polarised data will impair the anisotropy result, in particular in those regions where the sample signal is small.

Tips:
- Use the automated background subtraction.
- If possible, increase the sample signal (still avoiding saturation for each of the polarised scans).

10. Noise of anisotropy curves

Anisotropy curves will always look more noisy than the original polarised scans. This is in particular true for those regions where the emission or excitation scans are of low amplitude.

Tips:
- Measure spectral fluorescence anisotropy curves only in those regions where the signal is significant.
- The signal-to-noise ratio of anisotropy scans can be improved by increasing the signal and by increasing the number of scan repetitions and longer dwell times. When the signal is increased pay attention to avoid saturation of each of the polarised scans.

11. Manual measurements of spectral anisotropy

Anisotropy measurements can also performed manually, either by setting the polarisers to 0^0 and 90^0 in the signal rate screen and then making “normal” excitation or emission scans, or by even using non-standard polarisers such as dichroic sheet polarisers and then performing “normal” scans with the polarisers in the correct orientation.

Tips:
- If manual anisotropy measurements are made using the standard computer controlled polarisers, the polarisation angle is registered by the software and as long as at least the I_{VV} and the I_{VH} scans are contained in the same data window the anisotropy can be calculated using ***Data > Anisotropy***.
- If polarisers are used that are not recognised by the software, the polariser orientation can be edited manually in the properties of the measurement. Access the file properties (right mouse click on the graph) and select ***Properties***. On the bottom right side of the scan properties tick the button ***Edit Values***, then change the polarisation angle from none to either **0** or **90**. Once the polarisation angle is edited and provided the active window contains at least I_{VV} and the I_{VH}, the anisotropy can be calculated using ***Data > Anisotropy***.

5. Making Time Resolved Measurements

All spectrometers of the FLSP920 series can acquire time resolved data, with different time resolution. The time resolution depends on whether the spectrometer can perform standard Single Photon Counting (SPC), Multi-Channel Scaling (MCS) or Time Correlated Single Photon Counting TSCPC). All three techniques are single photon counting techniques. This guarantees highest detector sensitivity for all time ranges relevant for photoluminescence phenomena. Combined spectrometers (i.e. FLS920, FLP920, FSP920, and FLSP920) use more than one of the above techniques and therefore have and increased range of time resolution. You can find out which of the techniques is applicable to your spectrometer by studying section 1.1 FLSP920 Series Models on page 5.

F900 automatically selects one of three techniques to acquire time resolved measurements. Which of the techniques is in use depends on the type of light source that has been selected.

Technique	Time Range						
	pico	nano	micro	mili	sec	min	hour
Standard Single Photon Counting (SPC)				✓	✓	✓	✓
Multi-Channel Scaling (MCS)			✓	✓	✓		
Time-Correlated Single Photon Counting (TCSPC)	✓	✓	✓				

[FLSP920 spectrometers can also be upgraded to lifetime data acquisition by means of an oscilloscope (for performing time resolved measurements with analogue detectors in the near infrared spectral region). This fourth data acquisition method is optional, refer to the ***FLSP920 Series Reference Guide*** for details.]

Standard Photon Counting for Kinetic Scans (Milliseconds – Hours)

Kinetic Scans refer to measurements with a fixed excitation and a fixed emission wavelength, while the signal, in photon counts per unit time, is recorded over a time period of typically seconds to hours. Data acquisition is made using the PCS900 card in the computer.

Throughout the measurement the sample excitation may be continuously on, for instance in the study of photo-degradation, or the sample excitation may be on for a selected period of time, for instance in the study of after-glow processes or for the study of both rise and decay kinetics.

The “Kinetic Scan” option is available for all type of instruments of the FLSP920 series. The typical excitation source is the Xe900.

MCS (Microseconds – Seconds)

Multi-Channel Scaling (MCS) is a single photon counting technique that is used for recording luminescence decays in the time range between microseconds and seconds. A pulsed light source with low repetition rate is used to excite the sample and the photons emitted by the sample are recorded using the PCS900 card in the computer.

Standard FLSP920, FP920, FLP920 and FSP920 spectrometers use the microsecond xenon flashlamp (µF900H) as light source for multi-channel scaling experiments. This lamp has a pulse width of <2µs and a pulse repetition rate between 0.1Hz and 100Hz.

For a more detailed description of the MCS technique and alternative excitations sources refer to the ***FLSP920 Series Reference Guide***.

TCSPC (Picoseconds – Microseconds)

Time-Correlated Single Photon Counting (TCSPC) is a single photon counting technique that is used for recording fluorescence data in the time range from picoseconds to microseconds. A high repletion pulsed light source is used to excite the sample and the photons emitted by the sample are processed using the TCC900 card in the computer.

Standard FLSP920, FL920, FLP920 and FLS920 spectrometers use the nanosecond flashlamp (nF920) as the default light source for TCSPC acquisitions. This lamp has a pulse width of <2ns and a pulse repetition rate of typically 40 kHz.

Picosecond pulsed diode lasers and picosecond pulsed light emitting diodes (EPLs and EPLEDs), mode locked lasers and supercontiuum lasers are also increasingly used as light sources for TCSPC measurements with the range of FLSP920 spectrometers.

For a more detailed description of the TCSPC technique and alternative excitation sources refer to the ***FLSP920 Series Reference Guide***.

The following measurement types are available:

Measurement Type	Technique	Parameter	Second (Map) Parameter
Kinetic Scan	SPC	time (millisec. – hours)	
Time Resolved Measurement	MCS	time (microsec. – seconds)	
Time Resolved Measurement	TCSPC	time (picosec. – microsec.)	
Multiple TR Measurements	MCS	time (microsec. – seconds)	
	TCSPC	time (picosec. – microsec.)	
Excitation TRES	MCS	time (microsec. – seconds)	excitation wavelength
	TCSPC	time (picosec. – microsec.)	excitation wavelength
Emission TRES	MCS	time (microsec. – seconds)	emission wavelength
	TCSPC	time (picosec. – microsec.)	emission wavelength
Time Resolved Anisotropy	TCSPC	time (picosec. – microsec.)	measurement of vertical and horizontal polarisation plane

The recognition of optional sample holders and accessories by the F900 software will automatically increase the measurement types available. For instance, temperature maps will be possible if cryostats or Peltier cooled sample holders are detected, spatial (sample) maps will be possible with sample X-Y stages or well plate reader attachment.

The operating instructions in this section assume that the instrument has been started, the spectrometer is in full operating conditions and samples have been prepared. If this is not the case, follow steps 1-5 below:

1. Switch on the PH1 power supply, the spectrometer controller, cooler power supply, and the Xe900 xenon lamp power supply.
2. Switch on the computer.
3. Double-click the F900 icon or select F900 from your Start menu to start the software.
4. Wait for the software to finish its initialisation process and component calibration. Once complete, the Signal Rate window is displayed:
5. Setup the sample for measurements. (This might include the use of special sample holders and mounts.)

The following sections will instruct how to perform the different types of time resolved measurements listed in the table above, using the F900 spectrometer operating software. The following rule applies to all spectral scans:

Use the ***Signal Rate*** dialogue box to prepare for a lifetime measurement, then use the dialogue box for time resolved measurements (quick access via) to set up or modify scan parameters and to start the scan.

It is important to routinely use the ***Signal Rate*** dialogue box, as this contains some items that can only be setup there and not in any of the dialogue boxes for time resolved measurements. This applies to bandwidth settings, attenuator settings, polariser settings.

Even more than for spectral measurements it is important to pay attention to the signal rate levels, as for time resolved measurements different (lower!) saturation limits apply. These saturation limits depend on the rate of the exciting pulsed excitation source, For TCSPC the signal rate screen therefore contains the information about the repetition rate of the source.

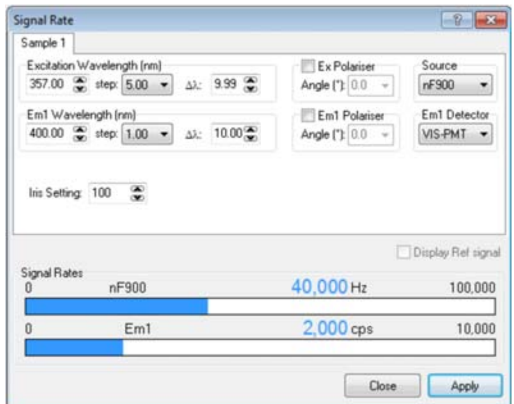


Figure 5-1: Signal Rate window for setting up time resolved measurements

During the time resolved measurements the status bar on the button of the screen informs about the running activities, such as wavelengths and bandwidths settings, the sample temperature, angle of polarisation, etc.

Active lifetime measurements can be aborted by using the "***Stop***" button of the active acquisition dialogue box.

Details of the parameter settings of a completed measurement can be viewed in the ***Spectral Scan Properties***. The properties can be accessed via ***right mouse click >> Properties*** if the active window contains only one measurement or via ***right mouse click >> Properties >> Measurement List*** if more than one scan is present in the active data window.

5.1. Kinetic Measurements

With ***Kinetic Measurements*** the time course of the signal is followed over a period of time, typically minutes, with a time resolution of a fraction of a seconds to seconds. Excitation and emission wavelengths remain fixed for the time of the measurement.

The light from the excitation source (which is controlled by the excitation shutter) may be permanently on, or off, or may be switched on and off during the kinetic scan.

To set-up and perform an emission scan...

1. Use the Signal Rate dialogue box to set up measurement parameters, i.e. excitation and emission wavelength and band width. Ensure that the correct source (typically the steady state lamp Xe900) and the right detector have been selected. The photon count rate can be controlled by means of the attenuator (values between 1 and 100 are possible, note that the scale is not linear) and by means of the spectral band width in both the excitation and the emission beam path. For solid or film samples it is important that the impact of the sample positioning and orientation on the signal rate is to be checked at this point.

2. Close the ***Signal Rate*** dialogue box and open the dialogue screen ***Kinetic Scan Setup….*** Set up the (overall) ***Scan Time*** and the ***Time Resolution***. The combination of both will dictate the total number of data points, which is displayed for reference. This number can not exceed 10,000.

3. Set up the options for the excitation shutter. If you select the option ***Timed***, you can also decide when the shutter will open, and for how long it will remain open.

4. Kinetic measurements may be corrected for fluctuations of the excitation light. Select the option ***Correct by Ref. Detector*** if you require this correction. The reference signal may also be recorded and displayed by ticking the box Display Ref. Data. The Display Ref. Data option is only available, if activated in ***Options >> Misc. Options***.

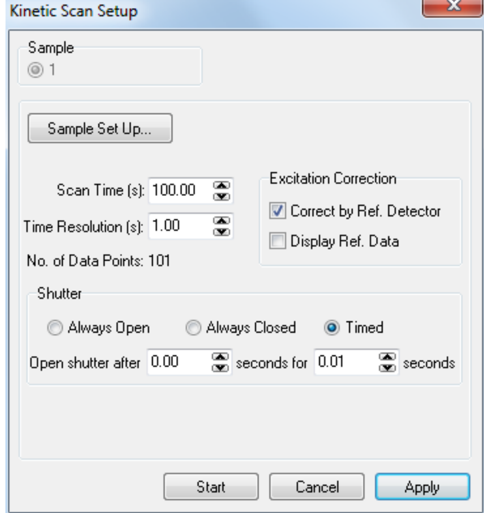


Figure 5-2: Kinetic Scan Setup

5. Start the map measurement: ***Start***.

A active kinetic measurement can be stopped by either pushing the ***Esc*** button or by clicking the STOP tool button on the top right of the F900 screen. Note that the ***Esc*** button will only be active if the window containing the scan is in focus.

As wilh all other time resolved measurements, kinetic scans can be analysed by exponential fitting. This is available via ***Data >> Exp. Tail Fit***. However, kinetic processes in the time regime of seconds or minutes often follow have more complex models than standard exponentials, the tail fit option might only provide a “crude” time constant for the overall decay (or ride) process.

5.2. Manual Time Resolved Measurements – MCS

This section describes how to make a time resolved measurement in multi-channel scaling mode. This mode requires an excitation source with low pulse repetition. The µF900H is the standard light source for this. Alternative light sources are available, refer to the ***FLSP920 Series Reference Guide*** for details.

5.2.1. Operation of the µF900H

The µF900H microsecond flashlamp is computer controlled in the way that it is triggered by the spectrometer controller. The unit has an integrated mains power supply that needs to be switched on separately (switch on the rear panel of the µF900H).

The lamp will only provide optical output (flashes with the repetition rate that has been selected in the µF Setup window) when measurements are in progress, i.e. when the Signal Rate dialogue box is open or while performing a lifetime measurement (or a spectral scan).

The lamp repetition rate can be set up via ***Setup >> µF Lamp Setup*** (refer to chapter 3.4.2 on page 31). This pulse repetition rate needs to be matched to the samples decay time. For samples with decay times in the millisecond range a repetition rate less than 100Hz may have to be selected to avoid background build-up. For

samples with decay times in the upper millisecond range, or even with seconds, selection of a very low repetition rate (<1Hz) is required.

Detector gating is an optional accessory. Refer to the ***FLSP920 Series Reference Guide*** for further details.

5.2.2. Measurement of a Phosphorescence Decay

Photoluminescence decays in the time range of microseconds to seconds are called here (for convenience) phosphorescence decays although this might not always be scientifically correct. One of the biggest application of phosphorescence decays of this time range is the time resolved emission of lanthanides, and this is actually a special type of fluorescence.

To set-up and perform a time resolved phosphorescence measurement ...

1. Check the signal using the ***Signal Rate*** dialogue box. Ensure that the correct source has been selected (μF900H or alternative low repetition sources). The operation of a low repetition rate light source can usually be heart. Ensure excitation wavelength and emission wavelength have been appropriately selected.

2. Having an understanding of the expected lifetime (or estimating the expected lifetime), review the pulse repetition rate of the light source. The pulse repetition period of the light source should not be shorter that 10 times the longest lifetime to be measured. If the pulse repetition rate of the light source needs to be changed, go to ***Setup >> μF Lamp*** to do this.

3. Continue to use the ***Signal Rate*** dialogue box. Check the detector signal (in cps). Ensure that the signal is not saturated. This is not easy and requires experience, as the saturation limit is not only linked to the lamp repetition rate, but also to the phosphorescence decay time, which you are about to measure. For a completely unknown sample use no more than 2000-3000cps as a signal to start with. Change the amount of signal by adjusting the Iris Setting and/or the band width in excitation and emission.

4. Close the signal rate screen and open the dialogue box for manual lifetime measurements (τ >> ***Manual***). The dialogue box will appear on the right side of the F900 main panel and is divided into two dialogue fields. The upper filed will be required to start and stop measurements and provides an extract of Signal Rate options. The lower field is specific for the lifetime settings in multi-channel scaling mode (refer to Figure 5-3).

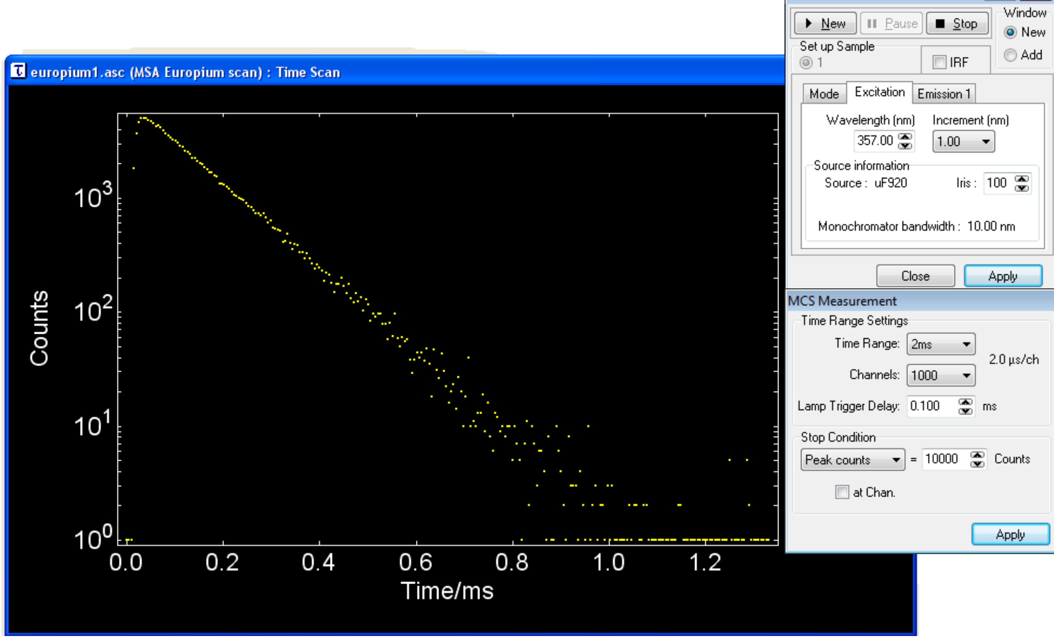


Figure 5-3: Dialogue box for a manual time resolved measurement by MCS

5. In the upper field you can start (New), Stop, and Pause measurements. You also decide whether the new lifetime measurement is to be shown in a previous data window (***Add*** – on the very right side), or in a new data window (***NEW*** – very top right). For measurements of the phosphorescence decay the box IRF must be left blank. The lower part in the upper dialogue field is tabbed and some parameters inherited from the signal rate screen can be changed, if needed. However, monitoring of the detector signal as a result of those changes is not possible.

6. The lower dialogue field is designed for set up the time-related measurement parameters. ***Time Range*** and ***Channels*** effect the full time window and the number of time bins within the full time range, respectively. Changing one of these two parameters will change the time calibration (in time/channel) that is displayed adjacent to these editable boxes.

7. The ***Lamp Trigger Delay*** parameter will shift the position of the rising edge of the measurement along the time axis. An increase of the trigger delay value will cause a shift to the right.

8. The ***Stop Conditions*** can be chosen between stop after a predefined number of ***Sweeps*** (light source flashes), stop after a predefined measurement ***Time*** (in seconds) or stop after a predefined number of ***Counts***. If the latter stop condition has been selected, the option will be given to select a certain channel for which this stop condition will be valid. If this option is not chosen (box not ticked), the measurement will be stopped when the peak of the overall measurement has reached the stop condition. Note that with this stop condition option the measurement may take a little longer as the computer will become busy to search through each data sweep to find the maximum peak count, select one of the other stop conditions to avoid this potential delay.

9. Start the measurement by clicking the ***NEW*** button. The measurement will stop when either the stop condition is reached, or when the ***STOP*** button is ticked.

10. Start the measurement by clicking the ***NEW*** button. The measurement will stop when either the stop condition is reached, or when the ***STOP*** button is ticked.

A time resolved phosphorescence decay measurement is typically analysed by fitting to a model function of one or more exponential parameters. This is available via ***Data >> Exp. Tail Fit***.

Time resolved phosphorescence decay measurements may also be analysed using numerical reconvolution (***Data >> Exp. Reconvolution Fit***). However, this is only possible, if the active window contains not only the phosphorescence decay, but also the corresponding instrumental response function (IRF).

5.2.3. Measurement of the Instrument Response Function

In a large number of cases, a measurement of the instrumental response function (IRF) will not be required for the analysis of phosphorescence decay measurements. The width of the IRF will be negligible small, compared to the intrinsic lifetime(s) of the decay, and analysis by the Tail fit routine will be sufficient.

However, for the correct determination of short (<50µs) lifetimes, reconvolution will be necessary, and thus the IRF needs to be measured.

To set-up and perform a measurement of the IRF ...

1. Open up the ***Signal Rate*** screen, reduce the attenuator (***Iris***) to the minimum (***1***), then change the emission wavelength to a value identical to that of the excitation. This way you will be detecting the (Rayleigh) scattered light from the sample. It is important to note that attenuator is set to a minimum, and only if no light is detected then it should be stepwise increased while observing the detector signal rate. Reducing the attenuator alone might not even be sufficient to attenuate the signal: For a correct measurement of the IRF the signal should be no more than 1000cps (for a lamp operating at 100Hz). A scatterer (such as Ludox) may be used instead of the sample. This way the risk is eliminated that a fraction of photoluminescent light “contaminate” the IRF measurement.

2. Close the signal rate screen and open the ***Manual*** lifetime measurement screen. After step 1. above it is not recommended now to change any of the parameters. Changing sample parameters (such as excitation or emission wavelength) would result in a change of the signal rate that had been critically set up, changing time-related parameters must not be done, as the IRF must be measured under identical time-related conditions as the phosphorescence decay.

3. Before starting the IRF measurement pay attention that the box ***IRF*** has been ticked. This way the software will recognise the measurement as an IRF measurement (and not a sample decay). This identification is important for the subsequent data analysis. It is also recommended to add the IRF measurement to the data window that was used for the sample decay measurement, as for the analysis the window must contain the decay and the IRF.

If you forget to tick the box ***IRF*** before you start to acquire data, then you can still identify the IRF after the measurement has been completed. Access the measurement properties (***right mouse click >> Properties >> (Properties)***) and tick the box ***Is Instrument Response*** on the bottom left of the property display.

4. The IRF is typically measured to the same peak height as that of the decay measurement. But this is not absolutely necessary for the subsequent data analysis. Start the measurement (***New***).

5. On completion of the IRF measurement this can be used in the analysis of the phosphorescence decay. Refer to chapter 7.6 on page 98.

5.3. Manual Time Resolved Measurements – TCSPC

This section describes how to make a time resolved measurement in Time Correlated Single Photon Counting mode. This mode requires an excitation source with high pulse repetition rate. The nF920 is the standard light source for this. Alternative light sources are available, refer to the ***FLSP920 Series Reference Guide*** for details.

5.3.1. Operation of the nF920

Principle features of the nF920 nanosecond flashlamp are described in section 2.1.3 of this manual, starting at page 15. The lamp operation is computer controlled, this is made via the setup dialogue box ***nF lamp setup*** that is described in section 3.4.1 on page 30.

This section assumes that the nF920 is in full working conditions. This means:

The lamp has been recently cleaned and all instructions have been followed to put the lamp back in operating conditions. If this is not the case the lamp will most likely not run properly. Refer to the ***FLSP920 Series Reference Guide*** for instructions on the cleaning, re-alignment and refilling procedure.

Start-up of the Nanosecond Flashlamp

1. If it has not been done yet, switch on the nF920 power supply and wait at least 5min, allowing the thyratron trigger within the lamp head to heat up. This is a good time to re-check the gas pressure, cables, gas tubes and optical fibre. The gas pressure is viewed in the nF920 lamp setup menu, this is accessed via ***Setup >> nF Lamp Setup***.

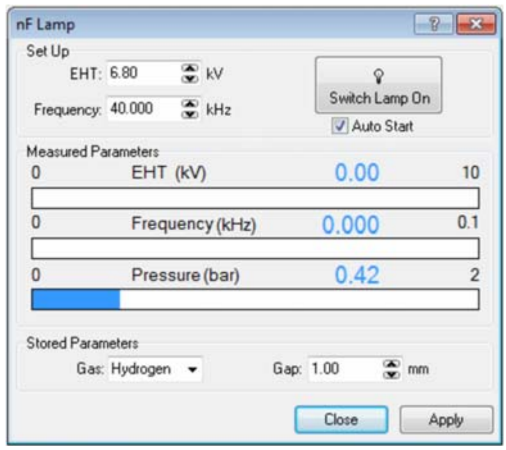


Figure 5-4: nF Lamp setup screen before start-up

2. The operational parameters displayed will not have changed from the last operating session. Double check that these are the parameters you want to use this time again. For standard operating conditions this is EHT=6.8kV and Frequency=40kHz, refer to the FLSP920 Series Reference Guide. For normal start-up, the Auto Start option located beneath the ON/OFF button is selected.

3. A critical parameter at this point is the gas pressure. Ensure the gas pressure is as required (0.45bar ±0.05bar for standard operating conditions, refer to the ***FLSP920 Series Reference Guide***). If the gas pressure is incorrect the lamp will not operate, a gas refill or even a complete maintenance cleaning might be required.

4. When the warm-up period of approximately 5 min (from the time the nF920 power supply was switched on) has expired, switch the lamp on by clicking on the ***Switch Lamp ON*** button. This will switch the EHT on and will stepwise ramp up the frequency (Auto-Start). After 2-3 seconds the auto-start-up has been completed and measured lamp parameters should be displayed accordingly, refer to Figure 3-3 on page 30.

The lamp is in proper operating conditions when the measured frequency is stable within the range of ±50Hz. Initially this tolerance interval may be exceeded, but after 2-3 min of operation the lamp should be stable. If it is not, re-gas filling, or even re-servicing the lamp will be required.

5.3.2. Measurement of a Fluorescence Decay

Photoluminescence decays in the time range of picoseconds and nanoseconds are called here (for convenience) fluorescence decays although this might not always be scientifically correct. As heavily quenched phosphorescence decay processes might also fall into this time range.

To set-up and perform a time resolved fluorescence measurement ...

1. Check the signal using the ***Signal Rate*** dialogue box. Ensure that the correct source has been selected (nF920 or alternative high repetition rate sources). Correct operation of the high repetition rate light source can be verified by the frequency that is displayed on the upper of the two signal rate bars, refer to Figure 5-1 on page 67 Ensure excitation wavelength and emission wavelength have been appropriately selected.

2. Having an understanding of the expected lifetime (or estimating the expected lifetime), review the pulse repetition rate of the light source. The pulse repetition period of the light source should not be shorter that 10 times the longest lifetime to be measured. For sample excitation with the standard nF920 flash lamp (operating at 40kHz) the repetition rate is – for the vast majority of lifetime measurements – low enough and there will be no need to further reduce the repetition rate. However, for alternative TCSPC light sources with much higher pulse repetition rates the issue of selecting an appropriate pulse rate is of significant relevance.

3. Continue to use the ***Signal Rate*** dialogue box. Check the detector signal (in cps). Ensure that the signal is below saturation and pulse pile-up level. With TCSPC the signal count rate from the detector should not exceed more than 5% the rate of the exciting light source. For operation with the standard nanosecond flashlamp (40kHz) this means the detector signal must not exceed 2000 counts per second. Change the amount of signal by adjusting the ***Iris*** setting and/or the band width in excitation and emission.

4. Close the signal rate screen and open the dialogue box for manual lifetime measurements (**>> *Manual***). The dialogue box will appear on the right side of the F900 main panel and is divided into two dialogue fields. The upper filed will be required to start and stop measurements and provides an extract of ***Signal Rate*** options. The lower field is specific for the lifetime settings in multi-channel scaling mode (refer to Figure 5-5).

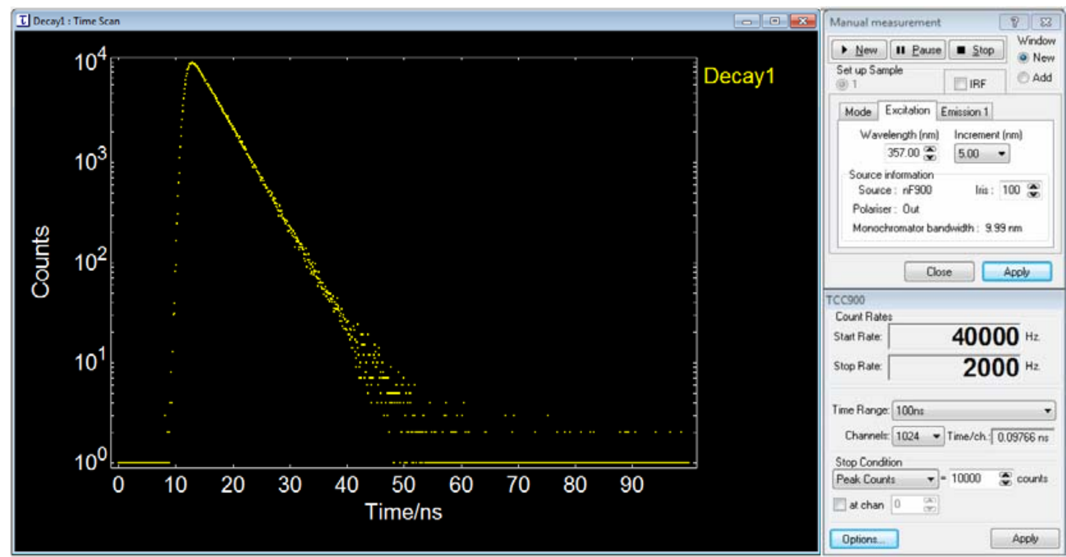


Figure 5-5: Dialogue box for a manual time resolved measurement by TCSPC

5. In the upper field you can start (***New***), ***Stop***, and ***Pause*** measurements. You also decide whether the new lifetime measurement is to be shown in a previous data window (***Add*** – on the very right side), or in a new data window (***NEW*** – very top right). For measurements of the fluorescence decay the box IRF must be left blank. The lower part in the upper dialogue field is tabbed and some parameters inherited from the signal rate screen can be changed, if needed. Changes will be made after the ***Apply*** bottom has been ticked.

6. The lower dialogue field has a display of the two rates: ***Start Rate*** = pulse repetition rate from the exciting source; ***Stop Rate*** = signal count rate from the detector. Use this dialogue field also to set up the time-related measurement parameters. ***Time Range*** and ***Channels*** effect the full time window and the number of time bins within the full time range, respectively. Changing one of these two parameters will change the time calibration (in time/channel) that is displayed adjacent to these editable boxes. The Options box is for an advanced setup of detector thresholds, delays, etc. For standard spectrometers there should be no need to change any of those settings.

7. The ***Stop Conditions*** can be chosen between stop after a predefined measurement ***Time*** (in seconds) or stop after a predefined number of ***Counts***. If the latter stop condition has been selected, the option will be given to select a certain channel for which this stop condition will be valid. If this option is not chosen (box not ticked), the measurement will be stopped when the peak of the overall measurement has reached the stop condition. This is perhaps the most commonly used stop condition in TCSPC measurements, most measurements will be stopped with the value set to 10000 counts.

8. Start the measurement by clicking the ***NEW*** button. The measurement will stop when either the stop condition is reached, or when the ***STOP*** button is ticked.

A time resolved fluorescence decay measurement may be analysed by fitting to a model function of one or more exponential parameters. This is available via ***Data >> Exp. Tail Fit***.

Note that the tail fitting procedure is only applicable for fluorescence decays that are not affected by the finite width of the instrumental response function. With a width of the IRF of 1-2ns (for operation with the standard nF920 lamp), tail fitting will only provide satisfactory results for lifetimes greater than 10ns.

Lifetimes shorter than this must be analysed using numerical reconvolution (***Data >> Exp. Reconvolution Fit***). However, this is only possible, if the active window contains not only the fluorescence decay, but also the corresponding IRF.

5.3.3. Measurement of the Instrument Response Function

For the analysis of a fluorescence decay by numerical reconvolution an instrument response function needs to be obtained. The IRF contains the information about the time response of the overall optical and electronic system. Only a correct measurement of this information, and the use of this information in the process of analysis, will result in accurate results and lifetimes that can be trusted.

To set-up and perform a measurement of the IRF ...

1. Open up the ***Signal Rate*** screen and change the emission wavelength to a value identical to that of the excitation. This way you will be detecting the (Rayleigh) scattered light from the sample. For a measurement of the IRF the rules of saturation apply, too. This means the scattered light signal should not exceed 4000cps, if the standard light source nF920 is being used. The level of signal can be controlled by adjusting the signal attenuator (***Iris***) Sometimes a change in the bandwith setting is required to further adjust the scattered light level. For an accurate measurement of the IRF a scatterer (such as Ludox) may be used instead of the sample. This way the risk is eliminated that a fraction of fluorescent light "contaminate" the IRF measurement.

2. Close the signal rate screen and open the ***Manual*** lifetime measurement screen. After step 1. above it is not recommended now to change any of the parameters. The IRF must be measured under identical conditions compared to the previous measurement of the fluorescence decay.

3. Before starting the IRF measurement pay attention that the box ***IRF*** has been ticked. This way the software will recognise the measurement as an IRF measurement (and not a sample decay). This identification is important for the subsequent data analysis. It is also recommended to add the IRF measurement to the data window that was used for the sample decay measurement, as for the analysis the window must contain the decay and the IRF.

If you forget to tick the box ***IRF*** before you start to acquire data, then you can still identify the IRF after the measurement has been completed. Access the measurement properties (***right mouse click >> Properties >> (Properties)***) and tick the box ***Is Instrument Response*** on the bottom left of the property display.

4. The IRF is typically measured to the same peak height as that of the decay measurement. But this is not absolutely necessary for the subsequent data analysis. Start the measurement (***New***).

5. On completion of the IRF measurement this can be used in the analysis of the fluorescence decay. Refer to chapter 7.6 on page 98.

5.4. Multiple Time Resolved Measurements

This measurement option provides a tool to automatically acquire a series of time resolved measurements (TCSPCS or MCS) by repeating the measurements with or without a preset delay (waiting time). All data is stored together in a single container file.

Typically a Multiple Time Resolved Measurement is made to establish a possible change in the decay process over a longer period of time. It is recommended that single measurements on this or a similar sample have been made before (refer to section 5.2 on page 68 for MCS measurements and section 5.3 on page 70 for TCSPC measurements). This way you have a background knowledge on the sample specific settings (such as wavelength and bandwidth) and on time range specific settings (such as the required time range and suitable stop conditions).

To set-up and perform a Multiple Time Resolved Measurement ...

1. Open up the dialogue box for multiple time resolved measurements (>> Multiple). For repeated measurements of an IRF tick the box ***Instrument Response***. For a repeated measurement of a sample decay you leave this box un-checked. Define the number of measurements you want to do (***Measure this sample ...***). The box ***Save data each time*** will most likely remains un-ticked. This way each of the measurements will start from Zero. (With the box checked a new measurement cycle would add the new data to the previous result, effectively continuing the old measurement.)

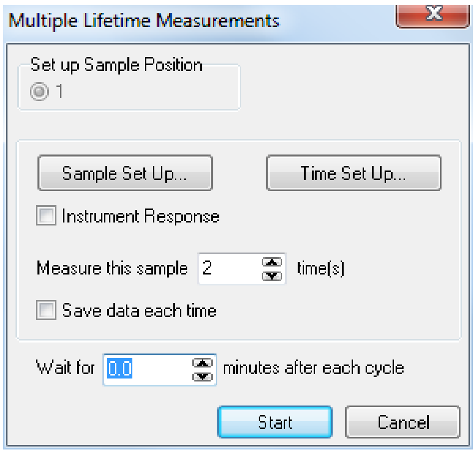


Figure 5-6: Setup of multiple time Resolved measurements

2. Do not start the multiple measurement yet! It is important to review sample and time settings before you start. For Multiple Time Resolved Measurements the sample specific settings (such as excitation and emission wavelengths and band width, iris settings) are inherited from the Signal Rate window. These settings can be reviewed and changed in the Sample Set Up box. In contrast, time specific settings (such as time range, number of channels, stop conditions) are inherited from the Manual measurement dialogue box. These settings can be reviewed and changed in the Time Set Up box. A typical oversight is the setup of appropriate stop conditions that applies for the individual measurements.

3. Now start the multiple measurement: ***Start***.

A ongoing multiple measurement can be stopped by either pushing the ***Esc*** button or by clicking the STOP tool button on the top right of the F900 screen. Note that the ***Esc*** button will only be active if the window containing the scan is in focus.

5.5. TRES-Map Measurements

Time Resolved Emission (or Excitation) Map Measurements is a powerful tool in time resolved fluorescence spectroscopy. TRES-Map measurements consist of a series of time resolved measurements (TCSPC or MCS) with the emission (or excitation) wavelength systematically stepwise changed. This Map measurement can be converted to time resolved emission (or excitation) spectra (TRES). These spectra can provide information that goes far beyond that from (conventional) spectra by steady state fluorescence spectroscopy.

Time resolved emission measurements are also used for more detailed analysis by numerical curve fitting. For example, systematically changes of the lifetime when changing the wavelength may be revealed. Alternatively, the set of lifetimes may remain constant when scanning over the wavelength, but the amplitude of the lifetime components may systematically change instead. Refer to that advanced data analysis FAST provided by Edinburgh Instruments.

5.5.1. Excitation TRES-Map Measurements

TRES-Map measurements with the excitation wavelength systematically changing are only available when a TCSPC source is used that has a broad spectrum and the wavelength for excitation is controlled by the F900 software. The standard nF920 is such a source.

To set-up and perform an Excitation TRES Measurement ...

1. Prior to setting up a TRES-Map measurement use the facility for Manual time resolved fluorescence measurements to perform test measurements (refer to section 5.2 on page 68 for MCS measurements and section 5.3 on page 70 for TCSPC measurements). This way you gain knowledge on the sample specific settings (such as wavelength and bandwidth) and on time range specific settings (such as the required time range and suitable stop conditions) and the time it takes for a single curve to be measured.

2. Open the dialogue box for setting up Excitation TRES-Map measurements (>> ***TRES*** >> ***Excitation***). Select the range of excitation you want to cover and choose the step size. Select a step size that is consistent with the spectral band width in the excitation channel. Also, be aware of the number of individual lifetime measurements that is to be performed within the selected spectral range. The emission wavelength is inherited from the Signal Rate box, if required, this might be changed here. Leave the tick-box ***Instrument Response*** un-checked.

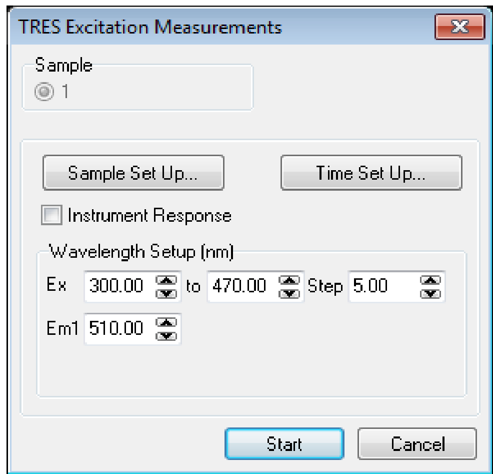


Figure 5-7: Excitation TRES-Map setup dialogue box

3. Do not start the TRES-Map measurement before you have reviewed the sample and time settings. For the TRES-Map measurement the sample specific settings (such as excitation and emission band width and iris) are inherited from the ***Signal Rate*** window. These settings can be reviewed and changed in the ***Sample Set Up*** box. In contrast, time specific settings (such as time range, number of channels, stop conditions) are inherited from the ***Manual*** measurement dialogue box. These settings can be reviewed and changed in the Time Set Up box.

4. Now start the TRES measurement: ***Start***.

A ongoing TRES-Map measurement can be stopped by either pushing the ***Esc*** button or by clicking the tool button on the top right of the F900 screen. Note that the ***Esc*** button will only be active if the window containing the scan is in focus.

5.5.2. Emission TRES-Map Measurements

Emission TRES-Map measurements are probably more common than Excitation TRES-Map measurements, as they can be converted into time resolved emission spectra. In difference to Excitation TRES-Maps they also do not require a broad band excitation source.

The setup of Emission TRES-Map measurements is similar to those of Excitation TRES. Refer to Figure 5-8 and the instructions given in the previous section. Note that stop conditions for the individual lifetime measurements (via ***Time Set Up***) must be set to ***Time***, if you want to generate time resolved emission spectra from your set of TRES data.

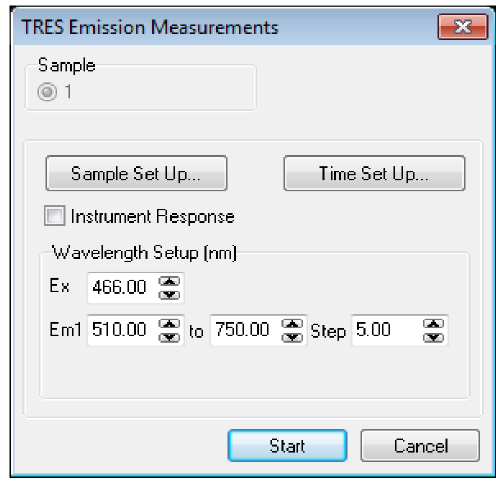


Figure 5-8: Emission TRES-Map setup dialogue box

5.6. Time Resolved Anisotropy Measurements

The measurement of time resolved fluorescence anisotropy requires computer-controlled polarisers in both the excitation and the emission channels (optional accessory for all FLSP920 spectrometers). Linearly polarised light of vertical (V = 0^0) and horizontal (H = 90^0) orientation are used to excite the sample and detect the sample emission. See the ***FLSP920 Series Reference Guide*** for more details on the FLSP920 polariser accessory.

Either four ($I_{VV}(t), I_{VH}(t), I_{HV}(t), I_{HH}(t)$) or two ($I_{VV}(t)$ *and* $I_{VH}(t)$) time resolved measurements are required for the calculation of the anisotropy function, depending whether G-factor correction is applied or not. The equations and definitions used in the calculations are given in the formula section on page 103, equations (7) – (9). See also the ***FLSP920 Series Reference Guide*** for further details on the time resolved fluorescence anisotropy.

Before performing time resolved anisotropy measurements, it is required that you have a background knowledge of the sample's excitation and emission properties. It is also important that you know the spectral excitation anisotropy, as a bad choice of the excitation wavelength can result in a Zero anisotropy, even if the sample has strong absorption and rotational correlation times in the nanosecond time range. It is also recommended that you know the time scale of the fluorescence decay (not affected by anisotropy). This is ideally done by time resolved fluorescence measurements, as described in section 5.3, on page 71, with the polarisers in magic angle configuration. But even "normal" fluorescence lifetime measurements will provide good background information.

Time resolved fluorescence anisotropy measurements reveal information about the ability of the average assembly of molecules to rotate during the short period of being in the excited state. For most fluorophores anisotropy effects are only observed when the solvent is of high viscosity (glycerol, mineral oil), or if the fluorophores are attached to slowly rotating macromolecules (bound to proteins or embedded into membranes). For these systems the rotational diffusion time and the fluorescence lifetime are similar – a condition for anisotropy effects to be observed. In low viscosity solvents (water, ethanol, etc) or in frozen samples time resolved fluorescence anisotropy is generally not seen as the rotation effects are either too fast or to slow, respectively.

Due to these time range limitations, time resolved fluorescence anisotropy measurements are practically exclusively made in TCSPC mode with high repetition light sources for sample excitation.

Before you can start making the polarised measurements you must move the polarisers into the beam path. This is described in section 4.5 on page 53.

Performing Time Resolved Fluorescence Anisotropy Measurements

1. Prior to performing time resolved fluorescence anisotropy measurements, use the Signal Rate dialogue box to set up measurement parameters such as excitation and emission wavelength, spectral band width of excitation and emission. Ensure that the correct source (typically the nF920 nanosecond flashlamp or an EPL) and the right detector have been selected.

2. Still using the Signal Rate dialogue box, check the signal levels for all four combinations of polariser orientations: Excitation polariser = 0°, 90°, Emission polariser = 0°, 90°. Ensure that non of the signal count rates exceeds the pile-up limit of about 5% of the source' start rate; adjust the attenuator and/or monochromator slits accordingly.

3. Open the dialogue box for time resolved anisotropy measurements (***>> Anisotropy*** - Figure 5-9) From within this dialogue box you can set up and start single and multiple time resolved anisotropy measurements. For a single anisotropy measurement, set the ***Measure this Sample*** value to 1. For multiple measurements set this value to the total number of anisotropy measurements you intend to make. If you do multiple scans, you can define a waiting time between the individual measurements.

4. The value ***Polariser Repetition*** applies to both single and multiple anisotropy measurements. A value of 5 (as shown in Figure 5-9) means the set of polarised time resolved measurements will be repeated 5 times, with each repeat the acquisition will continue to add the counts to the previous data.

5. The lower section of the dialogue box is for setting up the type of G-factor correction. With the option ***Fixed value of*** chosen, no additional measurements will be made for obtaining G-factor correction. This option is best (as it is time saving) if you know the G-factor from previous measurements – you simply enter the known value. You can also select the option to measure the G-value, either by doing the two additional lifetime measurements $I_{HH}(t)$ and $I_{HV}(t)$ and subsequent integration and ratioing (***Measure now by integrating TCSPC***), or by acquiring the signal amplitude for the I_{HH} and I_{HV} settings for a pre-defined duration of time. The latter of the two options is typically faster, but might not be as accurate.

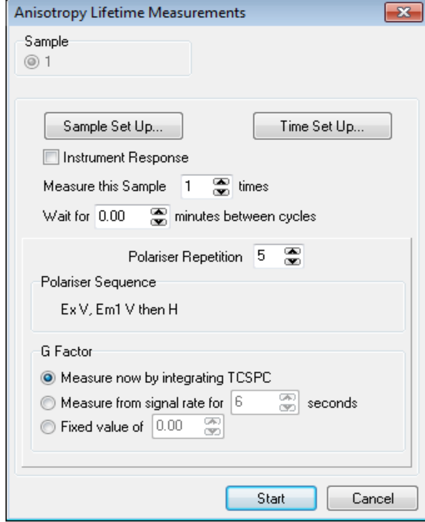


Figure 5-9: Setup for automated time resolved fluorescence anisotropy measurements

6. Do not yet start the measurement! Review first the sample specific settings (***Sample Set Up***) and the time specific settings (***Time Set Up***). The sample specific settings (such as excitation and emission wavelength and band width, iris setting) are inherited from the Signal Rate window and should therefore be all right, provided you have followed step 1. above. The time specific settings are inherited from your last ***Manual*** time resolved measurement. A typical oversight is the setup of the appropriate stop conditions: For time resolved anisotropy measurements these must be set to ***Time*** to get meaningful results.

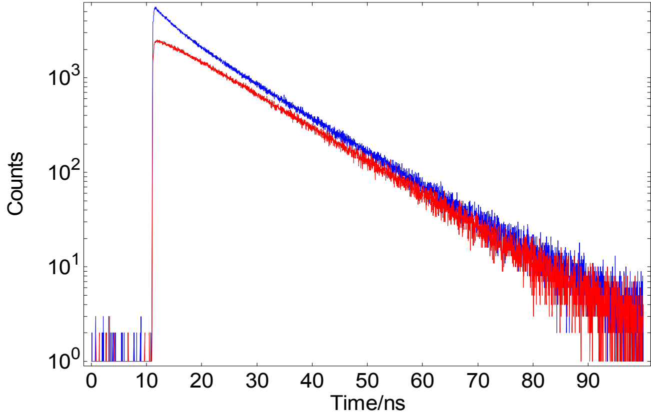


Figure 5-10: Raw polarised time resolved measurements

7. During the acquisition process of the raw polarised time resolved data the software will automatically acquire two or four curves, depending on the mode of G-factor correction. The status of the measurement will be shown in the status bar the bottom of the screen. At the end of the measurement the anisotropy function will be calculated and displayed in a separate window. The G-factor can be viewed in file properties (right mouse click . Properties).

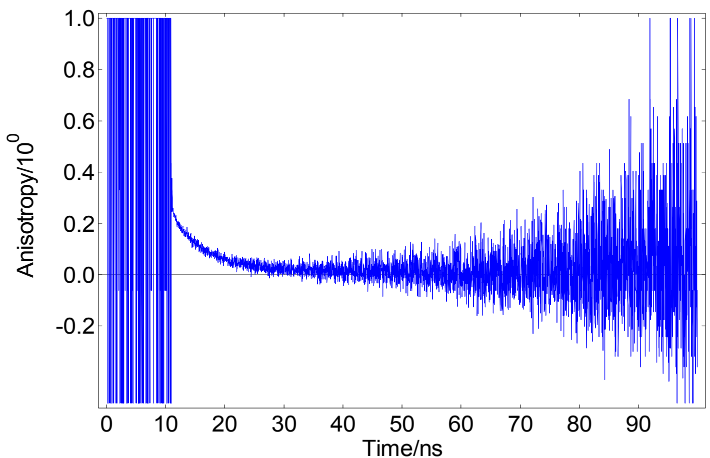


Figure 5-11: Example of the final time resolved anisotropy curve

The anisotropy curve can now be numerically analysed to obtain the characteristic rotational diffusion time(s). For the analysis please bear in mind that only exponential tail fit is applicable (select **Data > Exp. TailFit…**). Anisotropy analysis models that also allow numerical reconvolution are available in the advanced data analysis software FAST.

For proceeding with tail fit analysis it is important to select the correct fitting range. In particular the start channel should not be allowed to be in the noise before the decay, this inevitably will result in unsuccessful fits. It further should be noted, that, together with the calculation of the anisotropy curve the variances for each data channel were calculated and they are (invisibly) attached to the anisotropy data. The correct variances will be used in the analysis to produce the correct weighting factors for the data.

5.7. Tips for Making Good Lifetime Measurements

Good time resolved measurements require background knowledge of the sample to be measured, i.e. knowledge about the absorption properties and emission properties. It therefore is desirable that you measure excitation and emission scans first, before attempting lifetime measurements. You are also referred to section 4.8 for tips on making good spectral measurements, starting on page 60 of this user guide.

Time resolved measurements, however, have specific issues, and they are outlined below:

Tips for time resolved measurements:

1.	Inner Filter Effect

As for spectral measurements, the inner filter effect should be avoided. This effect will reduce the signal that is available for the lifetime measurement.

If inner filter effect is present, and the sample has a strong overlap between absorption and emission, re-absorption may occur. This can artificially increase the value of the measured lifetime (in the nanosecond time scale).

The same tips apply as outlined in section 4.8 for the inner filter effect.

2.	Scatter from the Source

Scattered light from the source might passes the emission monochromator in first or second order, and this interferes with the time resolved measurement.

This type of interference is recognised by a narrow initial spike “on top” of the decay. With measurements in the microsecond and millisecond regime this narrow initial scatter spike might be just discarded in the data analysis. With decay measurements in the nanosecond time scale and (unwanted) IRF superimposes the fluorescence decay. The region of the IRF might either be discarded in the data analysis, or the additional IRF is numerically treated in the reconvolution process (by adding an additional component with a fixed very short lifetime).

Tip:

- Use a wavelength cut-off filter (blocking at shorter, transmitting at longer wavelength) in the emission beam path (behind the sample) to block the scattered spike from the source.
- Discard the “contaminated” region during the data analysis process.

3.	Weak Sample Emission

The sample shows very weak emission. What options are there to increase the signal?

Tips:

- The utmost important task is to check the positioning of the sample in the sample compartment. A visible inspection of the sample excitation is recommended (look at the sample when the ***Signal Rate*** screen is open). If the excitation is outside the visible spectrum, or the light is too weak to be seen by the naked eye, you might change the excitation temporarily to 500nm for an assessment of the situation. You might even change to another light source (such as the Xe900) for the assessment of the alignment situation.
- The sample volume of a liquid sample might be too low. Increase the volume, use a micro-cuvette, or raise the cuvette.
- Bulk or film samples might have to be re-positioned.
- Increase the spectral band pass.

Please note: With very wide spectral band widths there is an increased risk to measure stray light that passes through the monochromators. There is also an increased risk to accidently over-expose the detector.

It is worth noting that TCSPC measurements (although they might take very long) can still yield good results even if the signal is as low as the background (a few counts per second). The reason for this is that background photons will be distributed over the full second, whereas the TCSPC data will only be acquired when the time window has started by trigger pulse from the source.

4. Pulse Pile-Up on Signal (TCSPC)

Pulse Pile-Up is only a problem in TCSPC measurements, where only one detector count can be accepted for a flash cycle of the excitation source. If more than one detector count arrive at the electronics in a measurement cycle, only the first one is “seen” and the others are ignored. This will skew the decay measurement towards shorter lifetimes. To avoid this effect the stop rate should never be higher than 5% of the start rate. [For very accurate determinations of lifetimes one should not go beyond 2%, for “quick and rough” acquisitions 10% might be acceptable.]

Tips:

- Attenuate the signal to a level that avoids pulse pile-up (below 5% of the start rate).
- Use a start repetition rate that is sufficiently high

5. Pulse Pile-Up on Background (TCSPC)

Pulse Pile-Up may also occur on background, if the background count rate is very high, for example with modern NIR-PMTs. The consequence of this type of pulse pile-up is a sloping background level in the resulting TCSPC measurement.

Tips:

- Try to use the detector in a mode that gives the lowest detector background count rate.
- Use a start repetition rate that is sufficiently high.

6. Signal Saturation (MCS)

Multi-channel scaling measurements, particularly those short decays and scatter in short time ranges, can be severely affected saturation. The reason for this is that the number of photon pulses that can “fit into each time bin” is limited. For very narrow time bins this number can be as low as 5 per lamp flash, and with the lamp flashing at 100Hz, the limit for a single bin would be 500 per second.

Tip:

- Measure an unknown sample with no more than 2000-3000 cps. The signal may then be increased if the sample decays in milliseconds. The signal might even have to be decreased if the sample decay time is only a few microseconds.

Tips specific for time resolved anisotropy measurements:

7. G-Factor

A correct G-factor is essential for a good anisotropy measurement.

Tips:

- A precision of the G-factor to 2 decimal place is required.
- The G-factor can be viewed and edited by selecting the window containing the original polarised lifetime measurements and then selecting ***Data > Anisotropy Analysis***.
- Modifying the G-factor manually allows to assess the impact of the G-factor on the final anisotropy curve. A G-factor that is too small will shift the anisotropy towards negative values, a G-factor that is too big will result in an anisotropy that is too positive.
- An unambiguous test for the correctness of the G-factor is to use a sample where the fluorophores are in a low viscous environment (e.g. water, ethanol). For those samples the anisotropy is expected to be Zero (the data are randomly distributed around Zero). The G-factor is correct when the time resolved data are distributed around Zero.
- In some cases even the positioning of the sample or the replacement of filters can have an effect on the G-factor. Careful handling and/or alternative methods for the G-factor determination are required.

8. Weak Signal

As time resolved anisotropy measurements consist of a series of time resolved measurements, with several cycles and sometimes reduced signal levels due to the additional optical elements polarisers, acquisition times can be significantly longer than normal fluorescence lifetime measurements.

Tip:

- Use a higher number of cycles (polariser repetitions) instead of long acquisition times for the individual lifetime acquisitions. This averages potential sample or light source fluctuations, but also allows an early assessment of the polarised measurements.

9. Alternative Ways for G-Factor Measurements

If time resolved fluorescence anisotropy measurements are made by using EPLs or titanium sapphire lasers as excitation sources, the polarisation plane of excitation can not be computerised rotated for G-factor acquisitions.

Tips:

- EPLs can be manually rotated. Once the emission is in the horizontal plane a full anisotropy measurement with G-factor correction can be performed. The resulting anisotropy will be meaningless and has to be rejected, but the G-factor that can be accessed via the properties can then be used for further anisotropy measurements (when the EPL is operating in the vertical plane again.
- The polarisation plane of a Titanium Sapphire laser can be rotated by means of a Soleil Babinet compensator or by a lambda-half plate. Similar to the procedure given for EPLs above the G-factor can be obtained.
- An alternative way to obtain the G-factor is to use a sample where the fluorophores are in a low viscous environment (e.g. water, ethanol). For those samples it is expected that the time resolved anisotropy is randomly distributed around Zero for all data channels. Using the ***Data > Anisotropy Analysis*** dialogue an estimate for the G-factor can be iteratively obtained.

10. Manual Measurement of Time Resolved Fluorescence Anisotropy

Time resolved anisotropy measurements can also performed manually, either by setting the polarisers to 0^0 and 90^0 in the signal rate screen, or by even using non-standard polarisers such as dichroic sheet polarisers.

Tips:

- If manual anisotropy measurements are made using the standard computer controlled polarisers the polarisation angle is registered by the software and as long as at least the I_{VV} and the I_{VH} measurement are contained in the same window the anisotropy can be calculated using ***Data > Anisotropy Analysis***.
- If polarisers are used that are not recognised by the software the polariser orientation can be edited manually in the properties of the measurement. Access the file properties (right mouse click on the graph) and select ***Properties***. On the bottom right side of the scan properties tick the button ***Edit Values***, then change the polarisation angle from none to either **0** or **90**. Once the polarisation angle is edited and provided the active window contains at least I_{VV} and the I_{VH} the anisotropy can be calculated using ***Data > Anisotropy Analysis***.

6. Data Operations on Spectral Data

A variety of the data operation tools and data analysis options is available to spectral data. Some data manipulation options are common to both spectral and lifetime data, others are specific. Therefore, the list of options that is available by clicking the pull down menu ***Data*** will vary depending on the data file type.

A summary of the data operation tools and data analysis options for spectral and time resolved data is given in section 3.7. “Data Operation and Analysis with F900” on page 42 of this User Guide.

This section describes in detail how to use the standard data operation tools for spectral data. A range of non-standard data operation tools is available. They may be activated via ***Options >> Analysis Options >> Spectral Data***. These advanced options require data that can only be measured with special hardware (integrating sphere, absorption accessory). For a description on these tools refer to the ***FSLP920 Series Reference and Maintenance Guide***.

6.1. Add, Subtract, Multiply, Divide

With these data operation options a single spectral scan (of a set of spectral scans) can be added to (subtracted from, multiplied or divided) to another spectral scan.

For example, to add two different curves together, open the dialogue box ***Data > Combine > Add…*** (refer to Figure 6-1. The list that is provided within this dialogue box contains all data windows that are open or minimised within the F900 program. The sequence is in order of time accessed. In the example of Figure 6-1 the spectral scan ***Example Data 2*** will be added to the scan ***Example Data 1***. Instead of ***Example Data 2*** another measurement may be picked that may also be in the data container ***Example Data Set***. Double click on the container to open it. To return to the original list of files and containers click on the icon that will have opened up on the top right of the dialogue box.

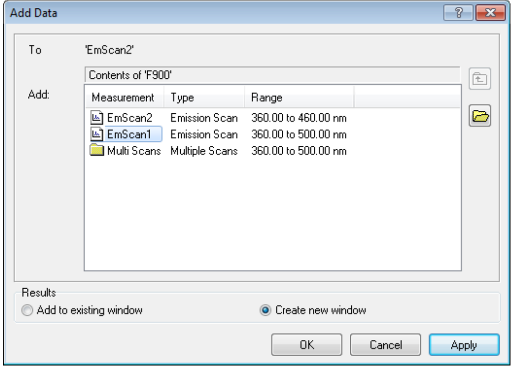


Figure 6-1: Add spectral scans

The spectral scans to be added must have the same step size. It is not necessary that they have the same spectral coverage. If the spectral ranges do not fully overlap the resulting file will extend from the minimum of the two files to be added to the maximum of those files. The data that are not available are automatically treated as Zero.

The resulting curve(s) can be added to the original window, or they can be shown in a new data window. This is selected by the corresponding radio buttons on the lower part of the dialogue box.

By clicking the **Apply** button the numerical operation will be processed and the new data will be demonstrated. The dialogue box will remain open for further data adding. Selecting **OK**, in contrast, will perform as with Apply, but will automatically close the dialogue box.

6.2. Append

The ***Append*** operation is only available to spectral data and correction scans. It has been designed for the purpose of joining spectral scans of different spectral ranges into one. The ***Append*** function will only work if the two different spectral scans to be joined have at least one overlapping wavelength point. The spectral scan that is to be appended on must have a spectral coverage that is at shorter wavelengths than the one that will be appended with.

In contrast to the other ***Combine*** operations described in sections 6.1 to 6.2, only a single spectral scan can be appended on. If the active data window contains more than one scan the ***Append*** option will not be available.

Layout and functionality of the ***Append*** dialogue box are similar to the those described in sections 6.1. The difference is additional information and select options that are given beneath the list of data.

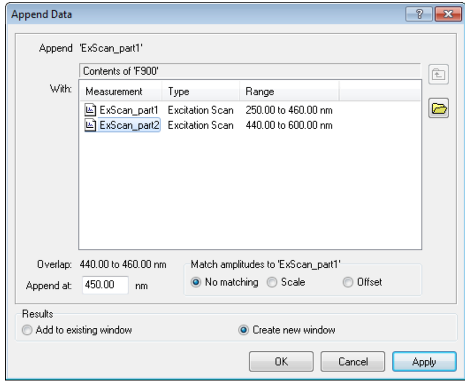


Figure 6-2: Append dialogue box

If the selected file fulfils the conditions that are required to append it to the original file the overlap range will be displayed and the option will be given to select a wavelength within the overlapping range (centre of the overlap as default). The selected wavelength in the ***Append at*** box has no function if the ***No matching*** option has been selected. However, if either ***Scale*** or ***Offset*** have been selected, the data that will be appended to the original curve will be scaled or offset so that they match the original curve at that selected wavelength. The resulting spectrum will have the data of the original curve up to the wavelength selected in ***Append at*** and will have the scaled of offset data of the second curve from there onwards. An averaging in the overlapping range is not provided.

6.3. Scale, Normalise, Subtract Baseline, Crop Range

Scale

The ***Scale*** operation multiplies a single measurement or a set of measurements contained in a single data window by a factor.

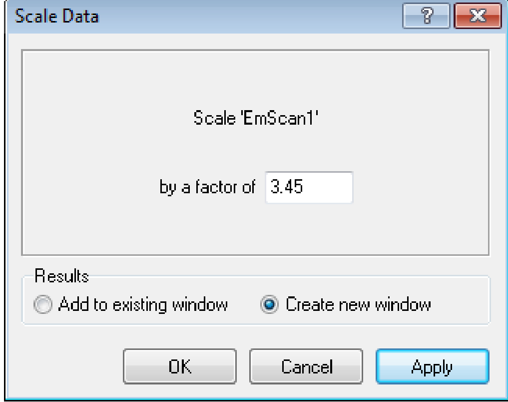


Figure 6-3: Dialogue box for scaling data

By selecting ***Scale*** from the ***Data*** menu the dialogue box as shown in will open up. The scaling factor may be edited and the option for the display of the resulting curve may be selected.

Note that a negative scaling factor is possible, this can be used to invert the data. Note that a scaling factor of Zero is not possible.

This scaling will apply to all curves that are contained in the data window. ***Apply*** will process and display the data, but will keep the dialogue box open. ***OK*** will process the data and will automatically shut down the dialogue box.

Normalise

The ***Normalise*** operation scales a single measurement or a set of measurements contained in a single data window by a factor, so that the resulting curve(s) have a peak value that is given in the "***to***" edit box, see Figure 6-4. For a set of scans, the peak of the individual scans will not necessarily be a the same wavelength.

The "**to**"-value can be manually edited. Alternatively a curve is selected in the list of files below. As soon as a single measurement is highlighted, the maximum of this measurement will be found and will be displayed in the "**to**" box. If a measurement container is highlighted the maximum of all curves in that container will be shown.

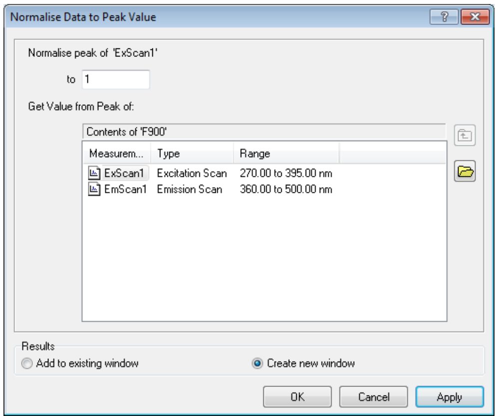


Figure 6-4: Normalise dialogue box

Normalisation to negative numbers will invert the data and all data will have a common minimum value. Normalisation to Zero will set all curves to Zero.

Note that when original data are displayed in the logarithmic scale, the resulting data may not be suitable for demonstration in this way, e.g. when curves are normalised to ≤ 1. The user may have to change the graph to linear scale. In some cases even that may not be sufficient to view the data and the ***Scale from Baseline*** box might have to be un-checked (***right click on the graph / Plot Options / Spectral / …***)

Subtract Baseline

This option is for subtraction a stationary background, form a single spectral scan, or from a set of spectral scans.

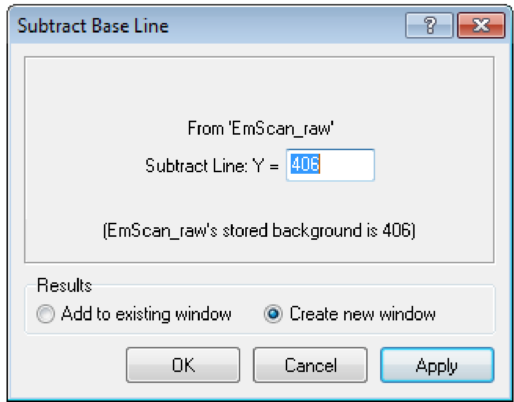


Figure 6-5: Subtract baseline

The number that is displayed in the editable filed will be subtracted from the data of the active data window. Upon opening the dialogue box the editable filed is normally blank. An exception is a raw single scan that was measured without automatic background subtraction. As the background had been recorded, it is now displayed as a default value for manual background subtraction.

The function may be use to artificially add a background to the data. In this case the Baseline value would be a negative number.

Crop Range

Spectral scans may be cropped, i.e. the spectral range may be cut back. This function is useful for removing unwanted data from the left or the right side of the graph, in particular if the **Append** function (see section 6.2) is to be used afterwards.

To crop the data range of a single curve or a number of curves in the active data window, open the dialogue box and edit the default values. The default values are the far left and the far right extremes of the current data range.

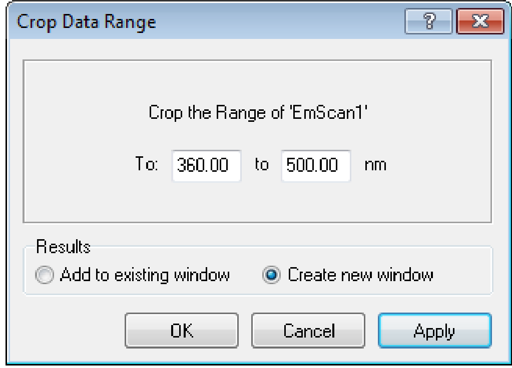


Figure 6-6: Crop Range dialogue box

6.4. Smooth

Spectral data can be smoothed. The binomial smoothing technique is applied, with the option to include between 1 and 4 neighbouring data points on either side of each point to be smoothed (≡ ***Number of Points*** between 3 and 9, respectively).

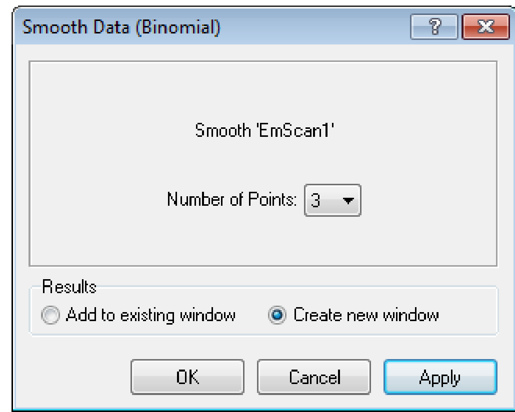


Figure 6-7: Smooth dialogue box

Note, excessive smoothing may alter real spectral features. Smoothing is best performed on oversampled data, i.e. curves with high number of data points for the spectral features involved.

6.5. Differentiate and Integrate

Differentiate

Use this data operation to differentiate the spectral scans. Derivatives of the first order, the second order, even up to the 4th order can be performed. For the reduction of noise, smoothing can be applied prior to differentiation.

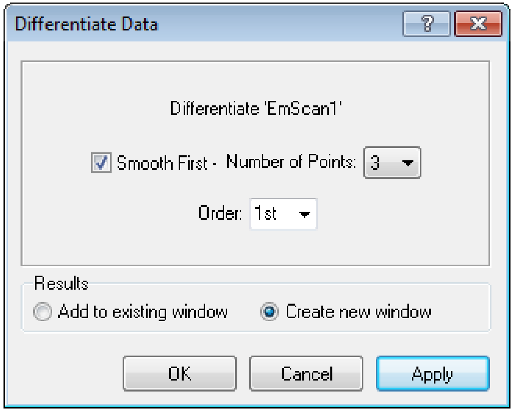


Figure 6-8: Differentiate dialogue box

Integrate

The **Integrate** operation is only available for spectral data. The integration of the whole spectral scan will be performed, starting at the short wavelength side and ending at the data point of the longest wavelength. Thus, the longest wavelength data point of the resulting integrated curve represents the integral number of the whole original curve.

If an integral number is required of a range that starts at longer wavelengths than the first data point of the original scan, then you must either crop the original scan on the short wavelength side before using the ***Integrate*** operation, or you subtracts the Y-value taken at the long wavelength side of the integrated curve minus the Y-value taken at the short wavelength side.

Note that the ***Integrate*** and ***Differentiate*** functions are not fully reversible. Subsequent application of integration and differentiation to a spectral scan will return the original data apart from a scaling factor and a possible Y-axis offset.

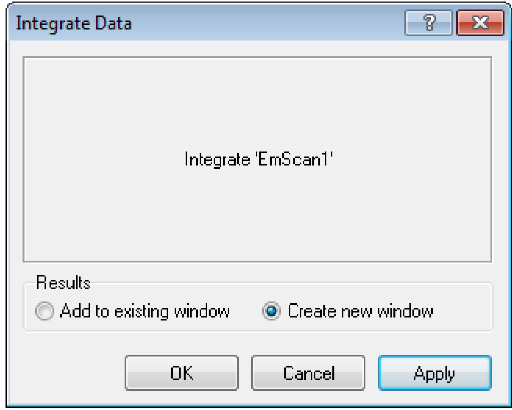
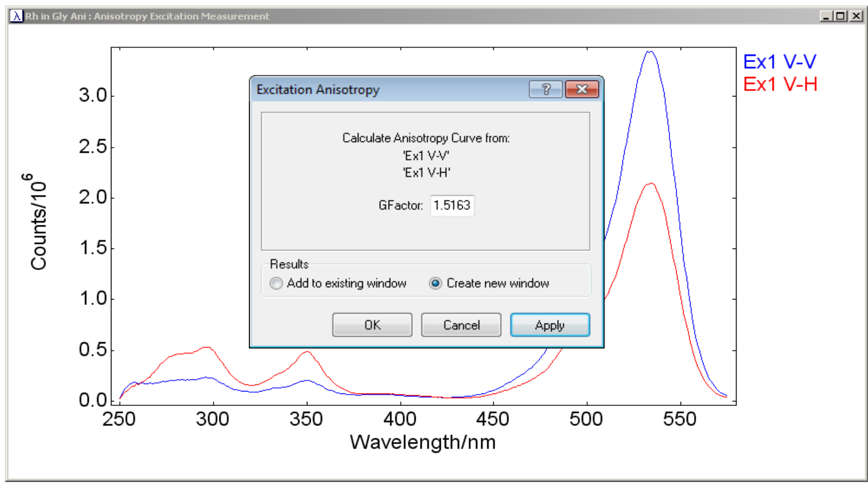


Figure 6-9: Integrate dialogue box

6.6. Anisotropy

This data operation enables you to re-calculate spectral fluorescence anisotropy functions, both for polarised excitation and emission scans.

Note that for calculating spectral anisotropy functions a measurement container is required that contains the original data measured with the automated anisotropy acquisition mode. If the original measurement container has been broken, e.g. by splitting and re-joining the data, anisotropy re-calculation will not be possible.



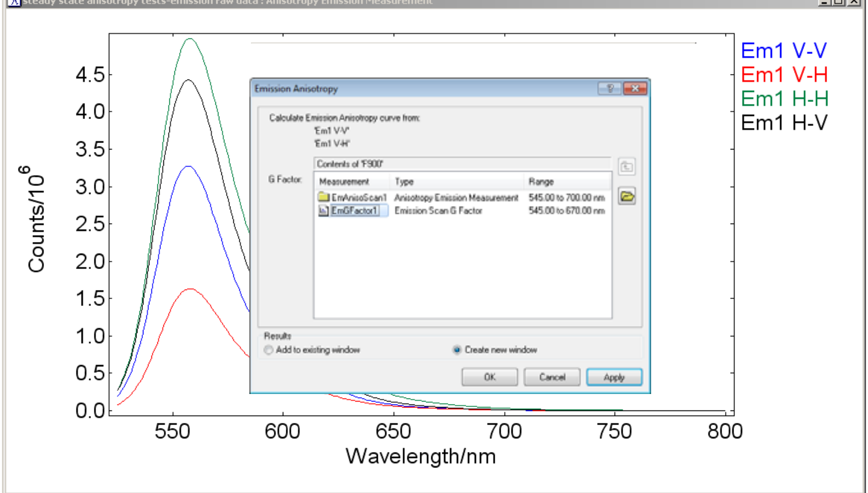


Figure 6-10: Dialogue boxes for manual excitation and emission anisotropy calculations

6.7. Spectral Correction

This dialogue enables you to perform spectral correction post acquisition, using stored correction files of your spectrometer. The correction files that are being used have been produced at the Edinburgh Instruments factory, or you have re-produced them yourself, refer to the instructions given in section 4.4 on page 49.

Correction files are only available for correction, if they have been correctly assigned for. This assignment is made in ***Options >> Correction Files...*** . Maintaining the correct correction file assignment is critical for producing true spectra.

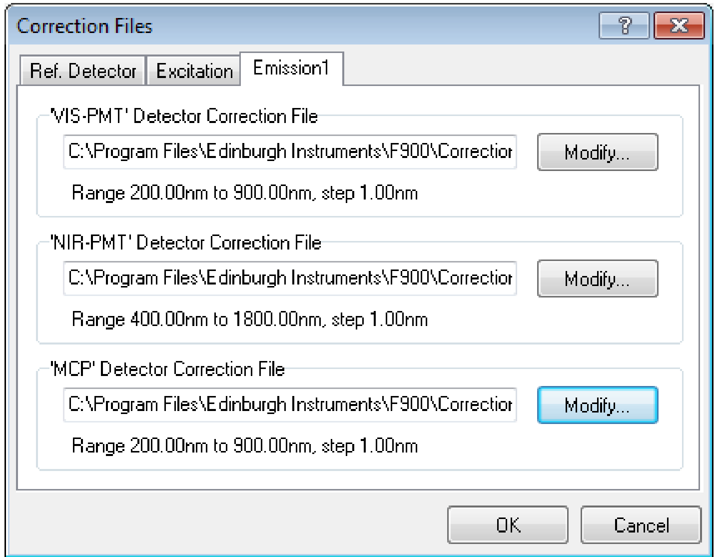


Figure 6-11: Assignment of correction files

When the active window contains one or more spectral scans, the option for manual post acquisition correction will be available (***Data >> Correction...***). The dialogue box shown in Figure 6-12 will open up. The box will show a list of the scans of the active data window on the left.

One of the scans of this list can be highlighted for correction. When highlighted the software will recognise whether the scan is an excitation scan, an emission scan, or a synchronous scan and the option for correction either excitation, or emission, or both will be provided. Note that for more complex spectrometers more than one emission correction files may be present. Remember to select the appropriate file before applying correction.

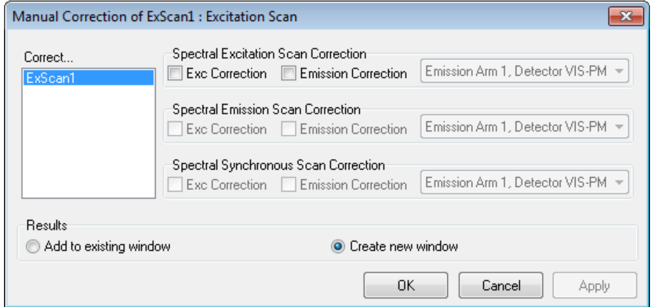


Figure 6-12: Dialogue box for manual correction

Although correction files are typically measured with 1nm step size, the scans to be corrected can have any step size. An algorithm is provided to compensate for the mismatch in step sizes.

7. Data Operations and Analysis on Time Resolved Data

A variety of the data operations and analysis tools is available to time resolved data. Some data tools are common to both spectral and lifetime data, others are specific. Therefore, the list of options that is available by clicking the pull down menu ***Data*** will vary depending on the data type and (for windows containing a set of data) on the properties of the data set.

A summary of the data operation tools and data analysis options for spectral and time resolved data is given in section 3.7. “Data Operation and Analysis with F900” on page 42 of this User Guide.

This section describes in detail how to use the operations and analysis tools that are provided for time resolved data.

Some analysis routines are numerically complex. For more details on the lifetime data analysis used in the F900 see the ***FLSP920 Series Reference Guide***.

Edinburgh Instruments also offers a software package for advanced lifetime data analysis: *FAST*. This software is sold separately.

7.1. Add, Subtract, Multiply, Divide

These operations are similar to those described for spectral data (refer to section 6.1 on page 85).

However, pay attention that numerical operations like these may have an impact on the noise characteristics of the time resolved data. For instance, adding one time resolved measurement to another independently measured time course will maintain the Poissonian noise distribution, whereas adding an identical measurement to the original data will corrupt the Poissonian noise. A correct Poissonian noise distribution is important for fitting time resolved data.

An ***Append*** function is not available for time resolved measurements.

7.2. Scale, Normalise, Subtract Baseline, Crop Range

These operations are similar to those described for spectral data (refer to section 6.3 on page 86).

When using these function pay attention that this will adversely effect the noise distribution of the time resolved data. For instance, a non-manipulated time course might be fitted with an excellent fit and a χ^2 value of 1.00. The same measurement, multiplied by a factor 10, can only ever have a minimum χ^2 of 3.16 (=√10)!

Another difference to spectral data is the graphical demonstration: Time resolved measurements are often demonstrated in a semi-logarithmic plot. This plot might not be anymore appropriate after the data operation has been applied – the resulting data window might appear to be empty. Change over to linear scale and/or ***Scale from Zero*** (via ***Plot Options***) to retrieve the measurement.

7.3. Reverse

In some cases it might be necessary to reverse the time resolved measurements in respect to the time axis. For instance, reversing the time axis is desirable for lifetime measurements that were recorded in reverse mode with the memory not automatically taking care of the reverse operation.

For reversing data, simply select the ***Reverse*** operation and press ***Apply*** or ***OK***.

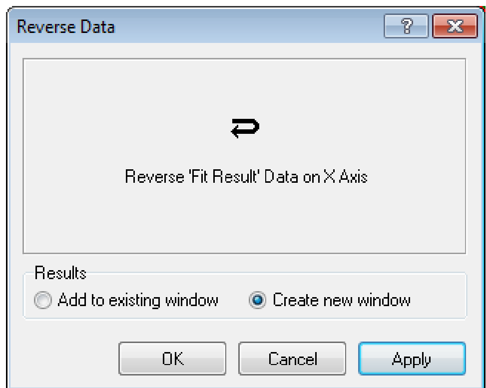


Figure 7-1: Reverse data dialogue box

7.4. Anisotropy

This data operation enables you to re-calculate the time resolved fluorescence anisotropy function from polarised time resolved emission measurements.

The Anisotropy option is only available if the active data window contains either two (I_{VV}(t) and I_{VH}(t)), or four (I_{VV}(t), I_{VH}(t), I_{HV}(t) and I_{HH}(t)) polarised measurements. If only I_{VV}(t) and I_{VH}(t) are present in the active window, the dialogue box for calculating the anisotropy (see Figure 7-2) will contain a default G-factor of 1.000. You may wish to edit this. If all four polarised measurements are present, then the G-factor will be calculated (using I_{HV}(t) and I_{HH}(t)) and displayed as default.

It is possible to calculate the time resolved fluorescence anisotropy curve, even if the active data window is not an original measurement container. This way manually acquired polarised lifetime traces can be used to generate the time resolved anisotropy curve. To do this you need to enter the correct polariser orientations into the properties of the raw measured data. Access the measurement properties (***right mouse click >> Properties***), tick the ***Edit values*** box (on the bottom right of the file properties window) and then set the ***Polariser*** values to 0° and 90°, respectively.

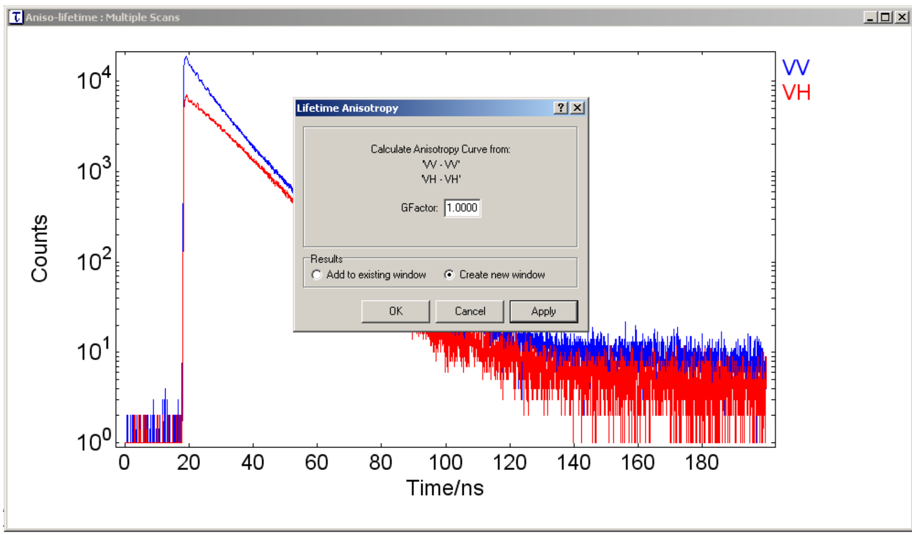


Figure 7-2: Calculation of the time resolved anisotropy function, using $I_{VV}(t)$ and $I_{VH}(t)$

7.5. Tail Fit Analysis

Use ***Exponential Tail Fit Analysis*** to fit a sample decay to a model function with one, two, three or four exponential terms. The fit will produce lifetimes, pre-exponential factors, a numerical value for a background level, and other derived parameters. To learn more about the numerical fitting algorithm and about the meaning of the fit parameters, refer to the ***FLSP920 Series Reference Guide***.

Use the Exponential Tail Fit Analysis only to analyse sample decay measurements that are not (or not significantly) affected by the instrumental response function. Tail fit analysis will only produce meaningful results, if the sample is allowed to decay without further “disturbance” by excitation.

Figure 7-3 below shows – on the example of two different measurements “A” and “B” – the selection of a suitable tail fit region (region with white background). The tail fit region excludes the time period for which the IRF is still present. For obtaining proper fit results, the far end of the tail that only contains detector background and noise, but no valuable information on the decay kinetics, should also be excluded.

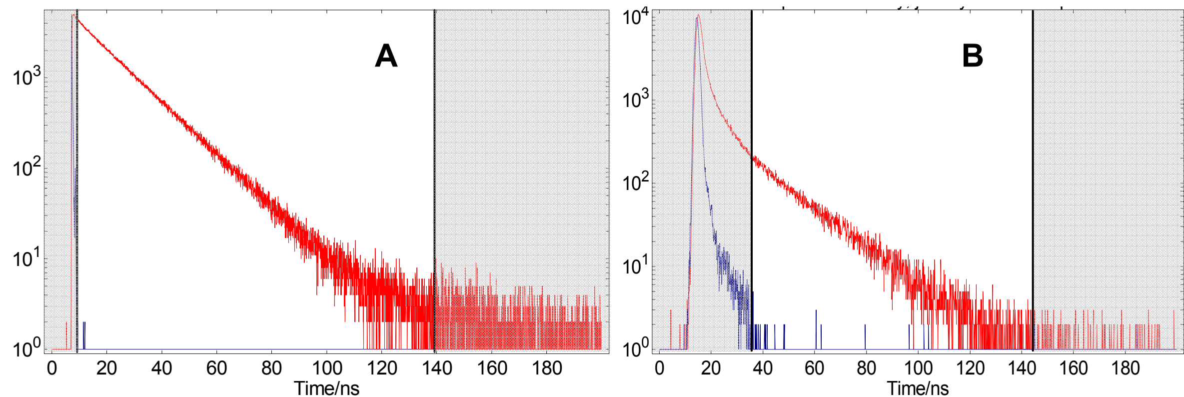


Figure 7-3: Tail fit regions, two different examples

To perform a tail fit...

1. Ensure the active window contains only one sample decay measurement, or a sample decay measurement with additional IRF.

2. Inspect your sample decay measurement and decide whether a good model function to fit to would be a single exponential decay, or a function that contains more than a single exponential term. Decay processes that are likely to be of single exponential nature are recognised by a sloping straight line in a semi-logarithmic plot (see tail fit region of decay “A” in Figure 7-3). Decays that deviate from a straight line (see tail fit region of decay “B” Figure 7-3) will not be satisfactory fitted with a single exponential term.

3. Use the ***Zoom*** function to select the tail fit region.

4. Select ***Data >> Exp.Tail Fit.*** This will open up a the dialogue box as shown in Figure 7-4. The fitting range shown on top of the dialogue box is inherited from the zoom in step 3 above, provided the zoomed region starts at, or beyond, the peak of the decay. If the zoomed region starts before the peak of the curve, then the ***Fitting Range From*** value will automatically be set to the peak of the curve. Remember, the peak is not necessarily the most appropriate start of the fitting range. The fit range for a tail fit should start when the IRF is completely finished.

5. Enter your estimate for the first lifetime, τ_1. This value will serve as a starting value in the following fitting process. A sufficiently good estimate for τ_1 can be made by looking at the original data: Remember that for a single exponential decay the lifetime τ is the time it takes to decay from 100% to 37% (0.367=1/e). Even if your judgment of the decay was that the decay is more complex than single exponential (see step 2 above) it is probably a good idea to start with a single exponential fit. The fit result of the single exponential trial will provide you with information about the estimate of a potential second lifetime component.

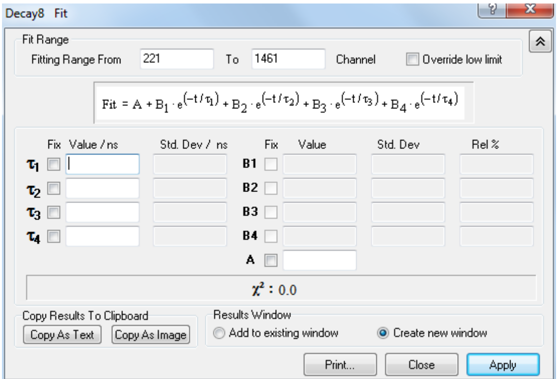


Figure 7-4: Tail fit dialogue box

6. You do not need to enter an estimated for the background value ***A***. If this box is left blank the fit will be started with an initial background of Zero. The background A is a numerical value that accounts for a constant detector background, or for a very long underlying photoluminescence signal that has built-up as a consequence of the repetition rate of the light source.

7. Click ***Apply*** to start the fit.

8. The fit result will be presented almost instantly. The fit result will comprise:
   - an updated fit-plot which contains the raw data, the fitted function (file suffix ***F1***), and the residual function (file suffix ***F1R***). The plot also contains a display of the fitted lifetime τ_1 and of the fit quality parameter χ^2.
   - An updated fit dialogue box, containing the calculated lifetime, τ_1, the calculated pre-exponential factor, B_1, the asymptotic standard errors for these two parameters, the calculated background, A, and the goodness of fit (or chi-squared), χ^2.

9. You now need to make an evaluation of the quality of the fitted result, based on two parameters;

   (1) the value of the χ^2: For a good fit this should be close to Unity. (*)
   (2) the residuals (shown in the fit plot): For a good fit they should are randomly distributed around Zero.

   Figure 7-5 shows as satisfactory single exponential fit result of decay “A” of Figure 7-3. In contrast, Figure 7-6 (showing a tail fit result of decay “B” of Figure 7-3) is an example for an unsatisfactory fit result.

10. Repeat the fitting process, if the fit result was not satisfactory. Add a second exponential lifetime, τ_2 , this will test for a double exponential model. Repeat with three and four exponential terms, if necessary.

    Fit results of models with 2, 3 and 4 exponential components will show a Rel% figure that is different from 100%, provided none of the pre-exponential factors is negative. The Rel% figure states the intensity contribution of each of the exponential components to the overall fluorescence intensity.

11. When you are happy with the fit you can print the results directly from within the dialogue box (Print), or you can send the data to the clipboard, either as text or as image (Copy as Text, Copy as Image), for use in other software applications. To copy the fit plot, close the tail fit dialogue box, then press Ctrl+C on the graph.

Fit results (file suffix ***F1***) and residuals (file suffix ***F1R***) may be reviewed any time later by looking up the properties of the files with suffices ***F1*** and ***F1R***, respectively. The properties of the residuals file also contain extended fit parameters, such as the Durbin-Watson parameter.

(*) The theoretical value is Unity, provided the data have pure Poissonian noise, the fitting range has been appropriately selected, and the chosen model is a correct description of the measured decay.

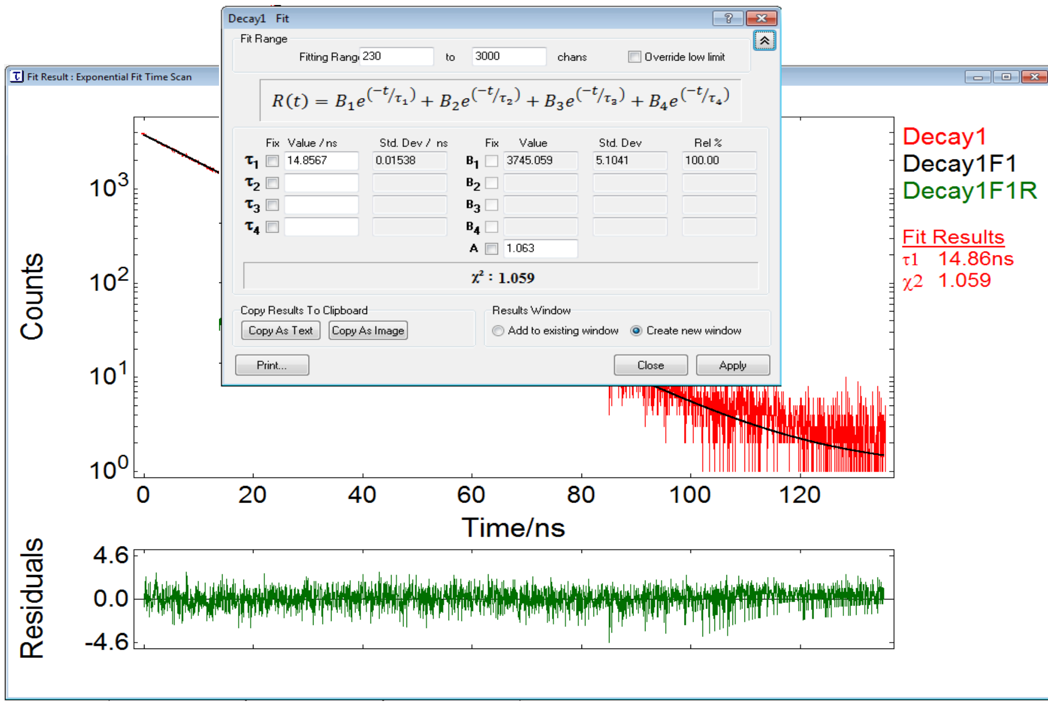


Figure 7-5: satisfactory tail fit

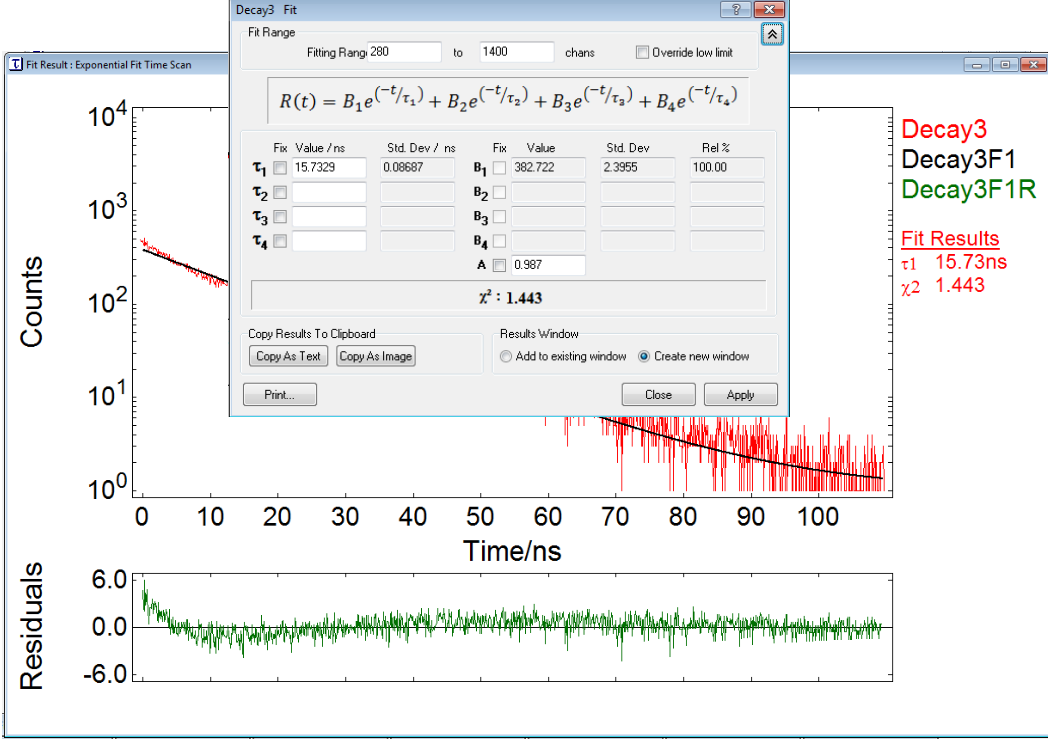


Figure 7-6: unsatisfactory fit

7.6. Reconvolution Fit Analysis

Use ***Reconvolution Fit Analysis*** to fit a measured sample decay to a model function with one, two, three or four exponential terms. The fit will produce lifetimes, pre-exponential factors, a numerical value for a background level, and other derived parameters. In contrast to the Tail Fit Analysis described in the previous section, where only the decay was analysed, Reconvolution Fit Analysis extract the lifetime parameters from the whole time resolved sample measurement, including the rising edge of the sample response. This way shorter lifetimes, that may even be shorter than the pulse width of excitation, may be reliably extracted.

In order to do numerical reconvolution, not only a measurement of the sample decay is needed, but also an accurate measurement of the IRF. For a more detailed description of the numerical reconvolution, refer to the ***FLSP920 Series Reference Guide***.

Figure 7-7 below shows – on the example of two different measurements “A” and “B” – the selection of a suitable fit region suitable for Reconvolution Analysis (region with white background). Note that – in contrast to Tail Fit Analysis – the region of the IRF is included. This way all information of the sample decay can be processed during the analysis. This is clearly beneficial for the analysis of a decay as shown in the example “B”, but even can reveal a short lifetime component of the measurement example “A”.

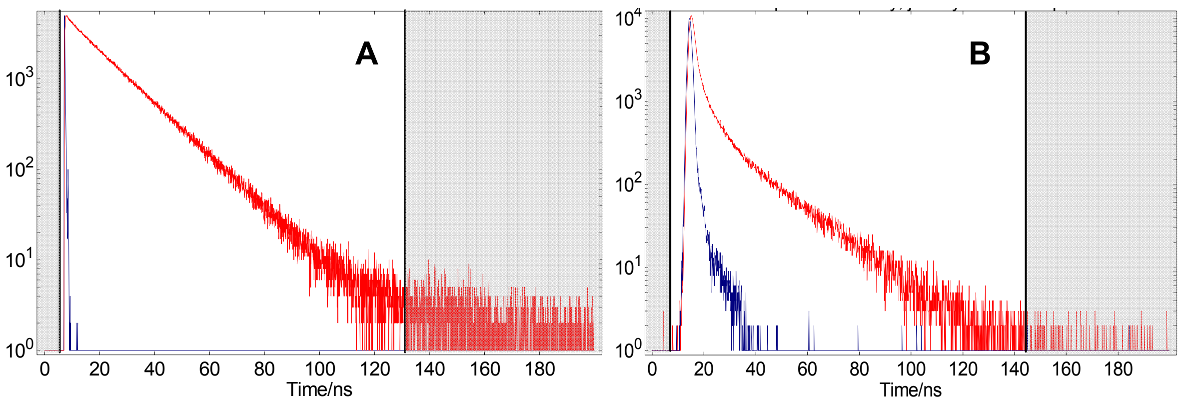


Figure 7-7: Fit regions for Reconvolution Fit Analysis

To perform a tail fit...

1. Ensure the active window contains one sample decay measurement and the corresponding IRF.

2. Inspect your sample decay measurement and decide whether a good model function to fit to would be a single exponential decay, or a function that contains more than a single exponential term. Decay processes that are likely to be of single exponential nature are recognised by a sloping straight line in a semi-logarithmic plot (see decay “A” in Figure 7-3). Decays that deviate from a straight line (see decay “B” Figure 7-3) will not be satisfactory fitted with a single exponential term.

3. Use the ***Zoom*** function to select the tail fit region. This should include the region of the IRF, otherwise refer to the previous section for Tail Fit Analysis.

4. Select ***Data >> Exp. Reconvolution Fit...*** This will open up a the dialogue box as shown in Figure 7-8. The fitting range shown on top of the dialogue box is inherited from the zoom in step 3 above.

5. Enter your estimate for the first lifetime, τ_1. This value will serve as a starting value in the following fitting process. A sufficiently good estimate for the start value (for a single exponential fit) is 1/10 of the full scale of the fitting range. You will get experience in finding a suitable start value for τ_1. It is also good to know that any lifetime estimate that is between 1/10 to 10 times of the fit results will be sufficiently good for a successful single exponential fit. For fits with 2, 3 and 4 exponential terms, however, the estimates have to become more and more accurate.

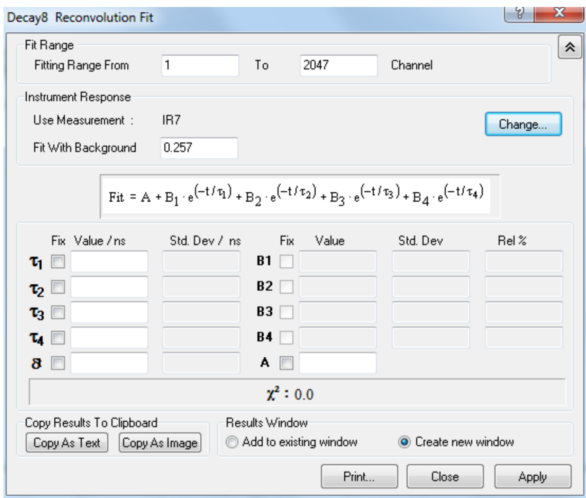


Figure 7-8: Reconvolution Fit dialogue box

12. You do not need to enter an estimated for the background value ***A***. If this box is left blank the fit will be started with an initial background of Zero. The background A is a numerical value that accounts for a constant detector background, or for a very long underlying photoluminescence signal that has built-up as a consequence of the repetition rate of the light source.

13. Click ***Apply*** to start the fit.

14. The fit result will be presented almost instantly. The fit result will comprise:
    - an updated fit-plot which contains the raw data, the fitted function (file suffix ***F1***), and the residual function (file suffix ***F1R***). The plot also contains a display of the fitted lifetime τ_1 and of the fit quality parameter χ^2.
    - An updated fit dialogue box, containing the calculated lifetime, τ_1, the calculated pre-exponential factor, B_1, the asymptotic standard errors for these two parameters, the calculated background, A, and the goodness of fit (or chi-squared), χ^2.

15. You now need to make an evaluation of the quality of the fitted result, based on two parameters;

    (1) the value of the χ^2: For a good fit this should be close to Unity. (*)
    (2) the residuals (shown in the fit plot): For a good fit they should are randomly distributed around Zero.

    Figure 7-9a and Figure 7-10a show unsatisfactory single exponential fit results for decay “A” and “B”, respectively. It is clear from the look of the residuals that a fit with more than a single exponential model function would give better results.

16. If you are not satisfied with a single exponential fit, repeat the fitting process with an added second, third, or even fourth exponential lifetime.

    Figure 7-9b and Figure 7-10c show satisfactory fits for the decays “A” and “B”.

17. When you are happy with the fit you can print the results directly from within the dialogue box (Print), or you can send the data to the clipboard, either as text or as image (***Copy as Text***, ***Copy as Image***), for use in other software applications. To copy the fit plot, close the tail fit dialogue box, then press ***Ctrl+C*** on the graph.

Fit results (file suffix ***F1***) and residuals (file suffix ***F1R***) may be reviewed any time later by looking up the properties of the files with suffices ***F1*** and ***F1R***, respectively. The properties of the residuals file also contain extended fit parameters, such as the Durbin-Watson parameter.

During fitting, two potential error messages may be displayed:
(1) “A matrix singularity occurred during fitting”: One of your estimated lifetime guesses was two small, enter a value that is bigger and repeat the fit.
(2) “A suitable fit was not found after 500 iterations”: The fitting process was not convergent and was stopped after 500 iterations. This error occurs typically when trying to fit complex decays and the initial guesses are not sufficiently close to the “true” values. Another problem may be a mismatch between the decay and the IRF, i.e. the measured IRF does not represent the real function that was responsible for generating the measured ssample decay.

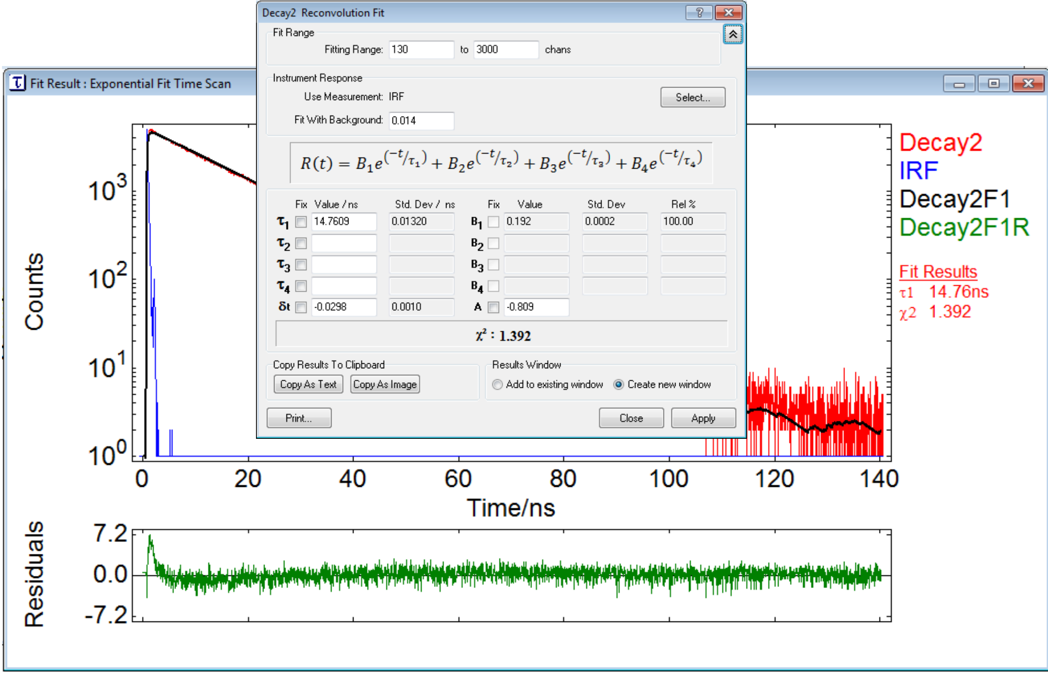


Figure 7-9a: Single exponential reconvolution fit of example decay "A"

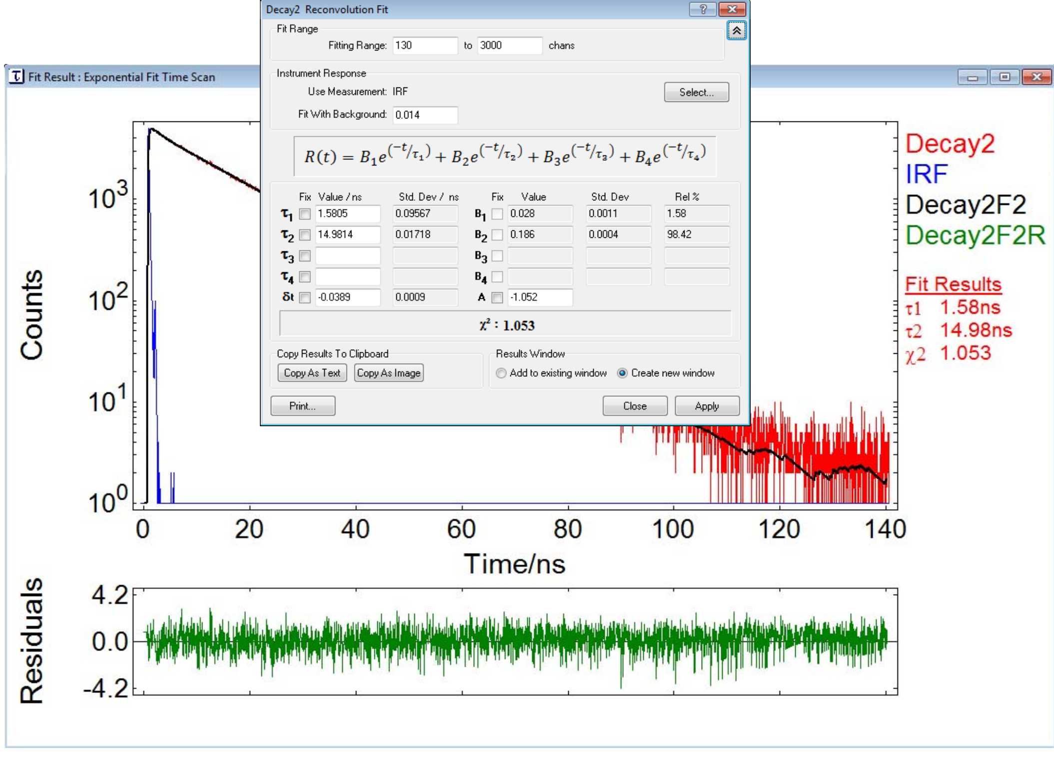


Figure 7-9b: Double exponential reconvolution fit of example decay "A"

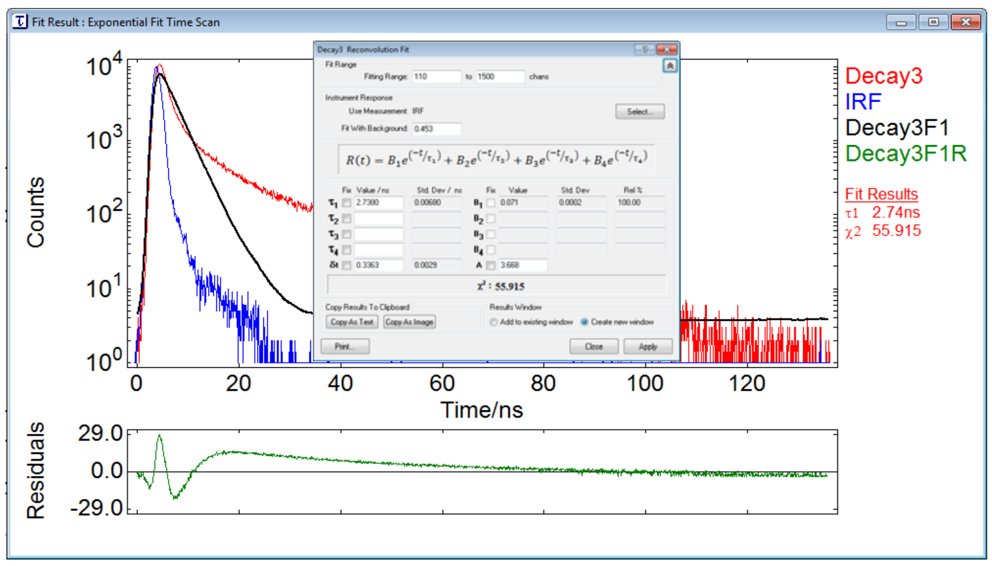


Figure 7-10a: Single exponential reconvolution fit of sample decay "B"

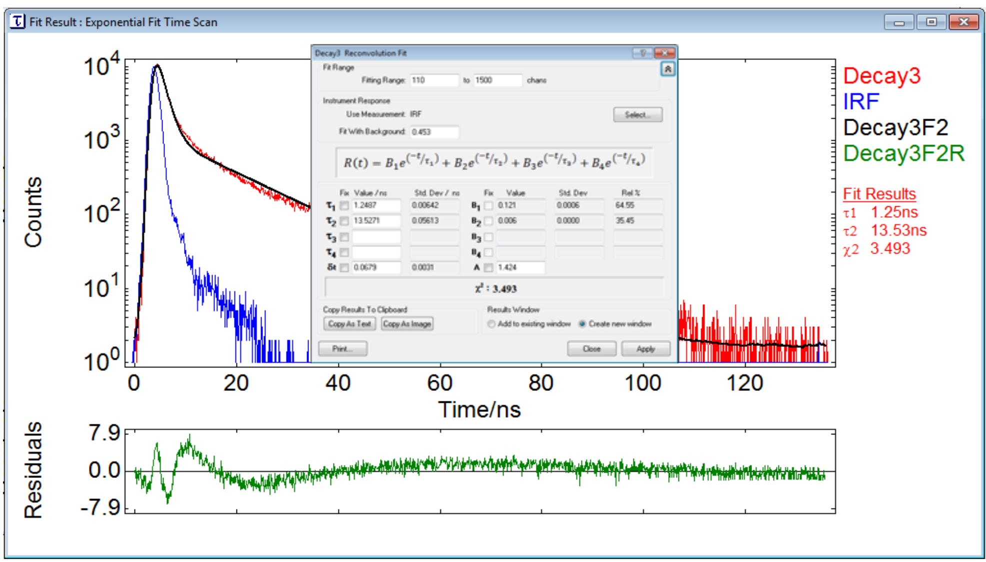


Figure 7-10b: 2-exponential reconvolution fit of sample decay "B"

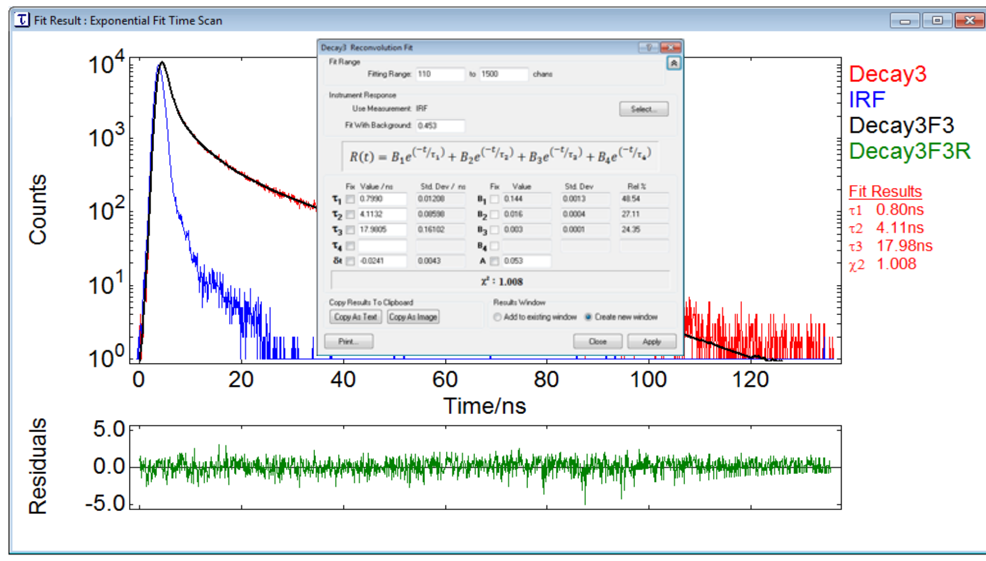


Figure 7-10a: 3-exponential reconvolution fit of sample decay "B"

7.7. Autocorrelation

The facility to calculate the autocorrelation function of the residuals will be available, if either a fit plot, or a 2D display of a residual curve is in focus. If this is given, the autocorrelation function may be calculated via ***Data >> Autocorrelation...*** .

Upon opening of the dialogue box, simply click ***APPLY*** to calculate the autocorrelation function. The function will be displayed in a new window and the file will carry the extension suffix ***1RA***, ***2RA***, etc, for single, double, etc fits.

7.8. TRES Data Slicing

TRES-Map measurements (refer to section 5.5 on page 75) can be converted into time resolved emission (or excitation) spectra by “slicing”. The slices represent spectra of the sample’s emission for given time windows. These spectra can provide valuable information as they show the dynamics of the emission during the sample’s decay process.

To convert a TRES-Map measurement into TRES ...

1. Click ***Data >> TRES data slicing ...***. This will open up a dialogue box as shown in Figure 7-11.
2. Define ***Start time***, ***Stop time***, and ***No. of slices.*** Update the ***Current Slice Setting Information*** by using the ***Apply*** button. This will not yet generate the data slices. You can control the width of the data slice by changing one of the three input parameters.
3. You may wish to set up each slice individually. You can do this by accessing the ***Advanced*** setup.
4. Press ***OK*** to process the data slicing. A new window will be created, and the spectra within the new graph will be labelled with the starting time of each data slice.

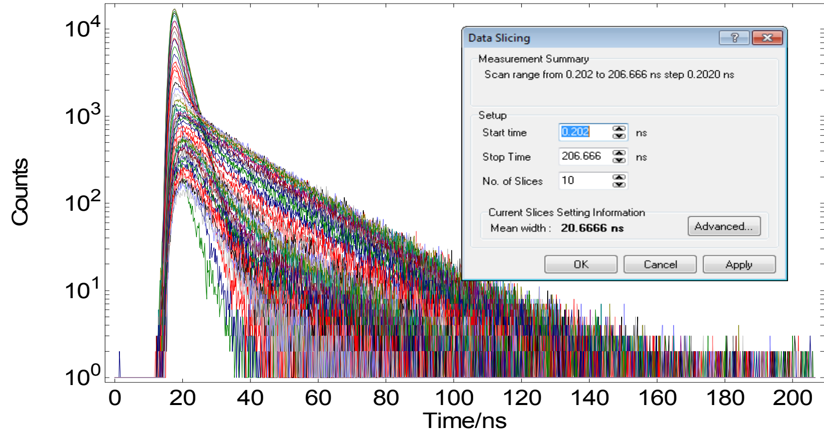


Figure 7-11: TRES-Map with dialogue for data slicing

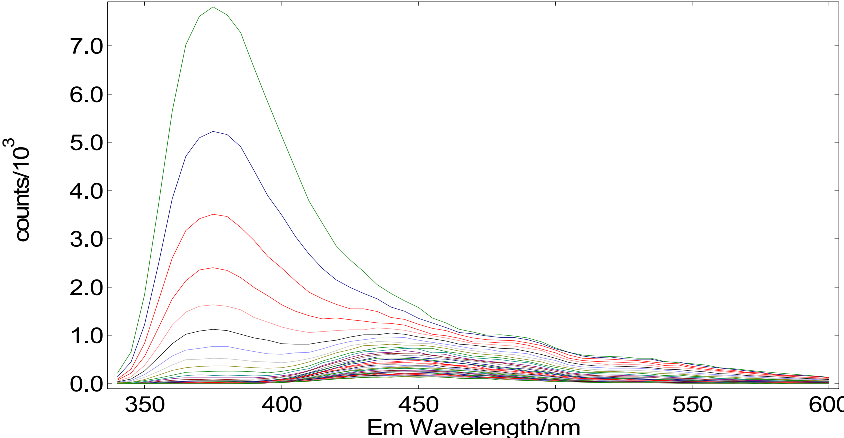


Figure 7-12: Time Resolved Emission Spectra (TRES)

Formulas and Definitions

Definitions of Steady State Excitation Anisotropy		
Excitation anisotropy	$r(\lambda_{exc}) = \frac{G\, I_{VV}(\lambda_{exc}) - I_{VH}(\lambda_{exc})}{G\, I_{VV}(\lambda_{exc}) + 2 I_{VH}(\lambda_{exc})}$	**(1)**
Total fluorescence excitation spectrum	$I_T(\lambda_{exc}) = I_{VV}(\lambda_{exc}) + 2 I_{VH}(\lambda_{exc})$	**(2)**
G-factor	$G = \frac{I_{HH}(\lambda_{em})}{I_{HV}(\lambda_{em})}$	**(3)**

Definitions of Steady State Emission Anisotropy		
Emission anisotropy	$r(\lambda_{em}) = \frac{I_{VV}(\lambda_{em}) - I_{VH}(\lambda_{em})}{I_{VV}(\lambda_{em}) + 2 I_{VH}(\lambda_{em})}$	**(4)**
Total fluorescence emission spectrum	$I_T(\lambda_{em}) = I_{VV}(\lambda_{em}) + 2 I_{VH}(\lambda_{em})$	**(5)**
G-factor curve	$G(\lambda_{em}) = \frac{I_{HH}(\lambda_{em})}{I_{HV}(\lambda_{em})}$	**(6)**

Definitions of Time Resolved Fluorescence Anisotropy		
Time resolved fluorescence anisotropy	$r(t) = \frac{I_{VV}(t) - I_{VH}(t)}{I_{VV}(t) + 2 I_{VH}(t)}$	**(7)**
Total fluorescence decay	$I_T(t) = I_{VV}(t) + 2 I_{VH}(t) = 3\, I_{ma}(t)$ *ma = magic angle*	**(8)**
G-factor	$G = \frac{\sum I_{HH}(t)}{\sum I_{HV}(t)}$	**(9)**

Exponential Decay Kinetics (example: 3-exponential model function)		
Exponential model function	$R(t) = B_1 \exp\left\{-\frac{t}{\tau_1}\right\} + B_2 \exp\left\{-\frac{t}{\tau_2}\right\} + B_3 \exp\left\{-\frac{t}{\tau_3}\right\}$	**(10)**
Relative concentration of the second component	$c_2 = \frac{B_2}{B_1 + B_2 + B_3}$	**(11)**
Relative fluorescence intensity of the second component, as a percentage	$\phi_2 = \frac{B_2 \tau_2}{B_1 \tau_2 + B_2 \tau_2 + B_3 \tau_3} \cdot 100\%$	**(12)**
Average lifetime of the entire fluorescence decay process	$\langle \tau \rangle = \frac{B_1 \tau_1^2 + B_2 \tau_2^2 + B_3 \tau_3^2}{B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3}$	**(13)**

Reconvolution of the Fluorescence Decay Model	
Exponential model function	$$S(t) = \int_0^t E(t')\, R(t - t')\, dt' \qquad \textbf{(14)}$$ *S(t) – measured fluorescence decay* *E(t) – measured instrumental response function* *R(t) – theoretical sample decay model function*

Glossary

Acronym	Definition
CCD	Charge-Coupled Device
EHT	extra-high tension
EPL	Picosecond pulsed diode laser
EPLED	Picosecond pulsed LED
IRF	Instrument response function
MCP-PMT	Multi-Channel Plate Photomultiplier
MCS	Multi-Channel Scaling
NIR	Near Infrared
NIR-PMT	Near Infrared Photomultiplier
PMT	Photomultiplier
TCSPC	Time-Correlated Single Photon Counting
TRES	Time-Resolved Emission Spectra
TRES-Map	Map of Time Resolved Measurements for the purpose of generating TRES
UV	Ultraviolet
VUV	Vacuum UV. Ultraviolet radiation absorbed by air

Index

Absorption 10, 43, 53, 59
Analogue detector 25
Analysis Options 43
Anisotropy 10, 42, 52, 53, 54, 55, 56, 77, 88
Anisotropy Analysis 42, 88
Attenuator 21, 22
Bandwidth 29
CCD 20
Combine 42
Correction File 43
Correction Scan 49
Crop Range 42
Cryostat 10
Cuvette 10, 23, 24
Czerny-Turner 17
Dark Count Rate 25, 26
Data
- Anisotropy Analysis 42, 88
- Combine 42
- Crop Range 42
- Exp. Reconvolution Fit 42
- Exp. Tail Fit 42
- Normalise 42
- Reverse 42
- Scale 28, 42
- Subtract Baseline 42

Data Acquisition Card 4, 5, 6, 7, 8, 10, 43
Detector 4, 5, 6, 7, 8, 10, 17, 19, 20, 21, 22, 25, 26, 29
Detector Gating 31, 39, 40, 41, 45, 51, 66, 101
Deuterium 16
Differentiate 42
Diode 10
Display Ref Signal 29
Dwell Time 13, 47
Electrode 16, 30
Electrode Gap 30
Em Detector 29
Em Polariser 29
Em Wavelength 29
Emission Correction 51
Emission Map 56
Emission Scan 45, 46, 47, 56, 67
Entrance Port 18
EPL 10
EPR Dewar 10
Ex Polariser 29
Excitation Correction 50
Excitation Scan 47
Excitation Wavelength 29
Exp. Reconvolution Fit 42
Exp. Tail Fit 42
F900 3, 4, 15, 27, 29, 30, 32, 42, 43, 45, 65, 66, 83, 91
FAST 91
File Icons 28
Filler Gas 16
Filter 21, 22
FL920 6, 8
FLP920 7
FLS920 8, 11
FLSP920 8
Fluorescence 3, 4, 8, 10, 15, 17, 27, 45, 52, 53, 54, 55, 56, 77, 101
Fluorophores 52
Focal Length 17
FP920 6
FS920 5, 8
FSP920 7
Gas Discharge 15
G-Factor 56
Glycerol 53, 56
Grating 12, 17, 18, 19, 20, 22, 43
Grating Change 43
High Repetition Rate 15
Hydrogen 16
InGaAs Detector 26
Instrument Response Function 42
Integrate 42
Integrating Sphere 24, 43
Iris Setting 29
Kinetic Scan 65, 67
Lamp Frequency 31, 39, 40, 41, 45, 51, 66
Lanthanide 7
Lens Assembly 13, 22, 24, 54, 55
Lens Optics 22
Lifetime Measurement 3, 7, 10, 12, 13, 15, 16, 28, 42, 43, 65, 79
- Kinetic 65, 67
- Manual 68, 69, 72
- Multiple 74

Light Source 4, 5, 6, 7, 8, 11, 15, 17, 26, 29, 30
Liquid Nitrogen 10, 26
Logarithmic Scale..28
Magnetic Stirrer 10, 24
Manual Lifetime Measurement..........68, 69, 72
Measured Parameters..................................30
Measurement Container..........................28, 42
Measurement Container Icons 28
Measurement Mode 27
Measurement Options 43
Measurement Plot Icons...............................28
Monochromator4, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23
  Double Monochromator............................10
  Emission Monochromator..10, 17, 19, 20, 43
  Excitation Monochromator11, 12, 13, 16, 17, 18, 19, 22
Multi-channel Scaling 4, 13
Multiple Lifetime Measurement 74
Multiple Scans 28, 29
Nanosecond4, 6, 7, 8, 10, 11, 15, 16, 18, 30, 71
Nanosecond Flashlamp4, 6, 7, 8, 15, 16, 18, 30, 71
Near Infrared Detector..................................10
nF Lamp Setup 30
Nitrogen 10, 16
Normalise 42
Options
  Analysis Options.......................................43
  Grating Change 43
  Measurement Options 43
  Oscilloscope Options................................43
  PCS900 Configuration...............................43
  Plot Defaults 28, 43
  Sample Holder Options 24, 43
Oscilloscope 43
Oscilloscope Options....................................43
Ozone 12
PCS900 ....5, 6, 7, 8, 43
PCS900 Configuration...................................43
Peak Value 85
Phosphorescence.............................3, 4, 7, 10
Photomultiplier..................5, 6, 7, 8, 16, 25, 26
  Gated 10
  Microchannel Plate....................................26
  Red Sensitive 25
Photon 4, 6, 11, 15, 17, 25, 47, 65
Picosecond 10
Picosecond Pulsed Diode Laser.................. 10
Plate Reader... 10, 24
Plot Defaults 43
Polarisation 20
Polariser... 10, 29
Power Supply....................... 11, 15, 25, 45, 66
Pressure 15, 16, 71
Properties 28, 29, 47
Pulse Repetition Rate 10, 13
Pulsed Light Emitting Diode 10
Quantum Yield 25
Reconvolution Fit 96
Reference Detector...................................... 22
Reflector 11, 16
Repetition Rate 11, 13, 15
Reverse 42
Rhodamin 56
Sample Chamber........ 4, 18, 19, 21, 22, 24, 59
Sample Cooling 10, 24
Sample Holder 10, 22, 23, 24, 29, 43
Sample Holder Options.......................... 24, 43
Sample Position 22
Sample Turret 24
Samples4, 10, 11, 13, 18, 19, 21, 22, 23, 24, 29, 42, 43, 45, 52, 53, 59, 66, 77, 93
Scale 28, 42
Scale Icons 28
Scan Display Area 29
Scan Icons 28
Scan View Icons 28
Setup
  nF Lamp Setup 30
  Signal Rate 27, 28, 29, 45, 59, 66
  μF Lamp Setup 31
Signal Rate 27, 28, 29, 45, 59, 66
Silica 22
Single Photon Counting..................... 4, 13, 45
Smooth.. 42
Source... 29
Spark Gap.. 15, 16
Spectral Measurement................ 28, 46, 59, 79
  Anisotropy Scan........................... 52, 54, 55
  Correction Scan 49
  Emission Map 56
  Emission Scan 46, 47, 56, 67
  Excitation Scan 47
  Synchronous Map.................................... 58
Spectral Output.................................... 12, 16

START 16
Status Bar 29
Steady State 7, 11, 25
Stored Parameters.. 30
Stray Light... 10, 20, 21
Subtract Baseline.. 42
Swing Mirror...................................... 17, 19, 20
Switch Lamp On/Off...................................... 30
Synchronous Map.. 58
Tail Fit 93, 96
TCC900 .... 6, 7, 8
Temperature 10, 24, 26, 43
Temporal Resolution..................................... 10
Thyratron.. 15, 71
Time Correlated Single Photon Counting (TCSPC)...................4, 6, 11, 13, 16, 26, 45
Time Range.. 65
Time Resolved 4, 7, 25, 26, 65
Tool Bar.. 28
Ultraviolet 12, 14, 20, 22, 45
X-axis 42
Xenon Flash Lamp................................. 13, 14
Xenon Lamp................4, 5, 7, 8, 11, 12, 14, 18
Y-axis 28, 47
Zoom 28
μF Lamp Setup 31