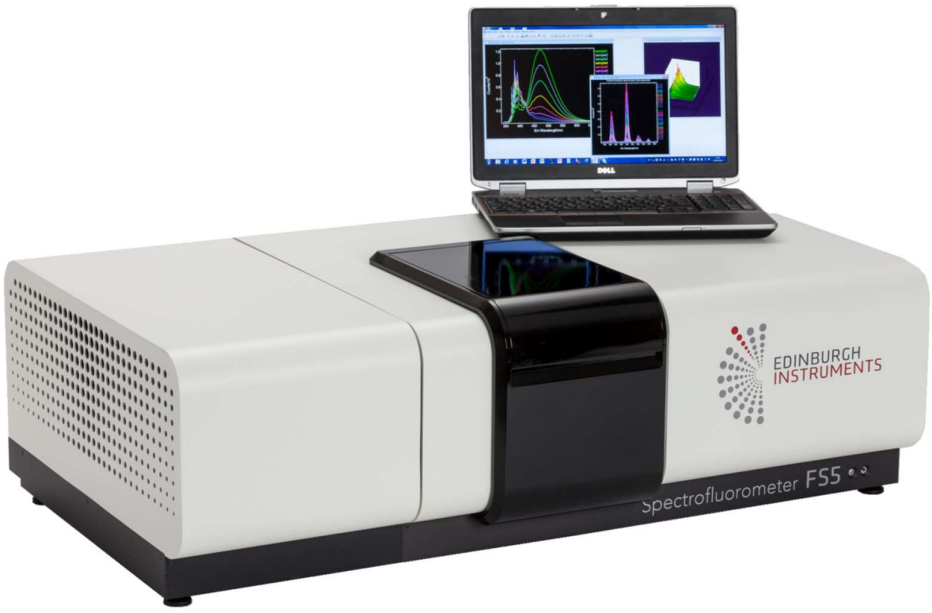
EDINBURGH INSTRUMENTS

FS5

User Guide



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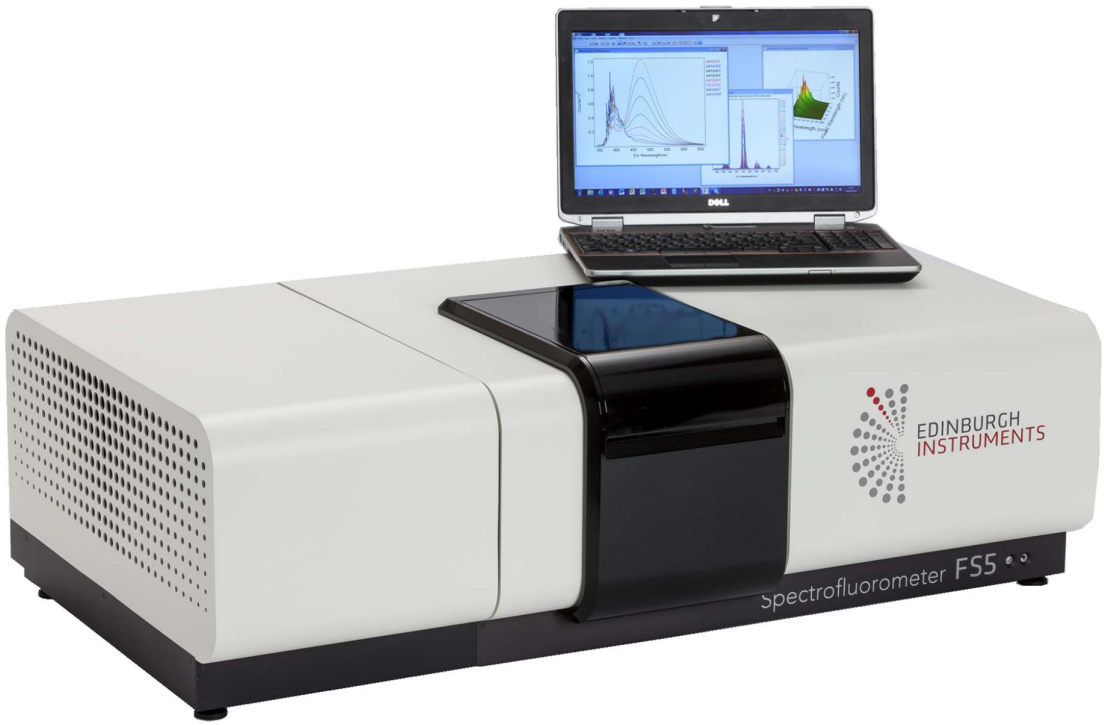
1. Introduction

The FS5 spectrofluorometer is designed to provide a complete measurement system for photoluminescence spectra and kinetics.

This user guide contains general information for the preparation of the installation site, details the requirements that your facility must provide to ensure that the system can be properly and safely operated, and gives instructions for the installation of your FS5 instrument.

Please read this user guide if you plan to use the spectrometer to make measurements or use the software to present or analyse your measurement results. The document describes the spectrometer hardware components, the software you use to control the instrument, explains how to make spectral, kinetic and phosphorescence lifetime measurements, and gives details on how to service the instrument.

The software features and dialogue boxes described in this manual refer versions 2.0 and above of the spectrometer operation software FLUORACLE.



1.1. Safety Information

The FS5 has been designed for routine use in a laboratory environment, where standard safety regulations will apply.

During routine operation of the FS5 there is a potential safety hazard of UV exposure!

 **Caution** **UV Radiation**	FS5 can irradiate the sample and the sample compartment with UV light between 230 nm and 450 nm. Do not look into the sample compartment during measurements or while the signal rate display is open. Avoid direct or indirect exposure to the eyes. Use UV eliminating safety goggles.

Safe operation can only be guaranteed for the intended operation conditions. They DO NOT include the use of tools, such as screw drivers and Allen keys.

 **Caution**	The use of tools, such as screw drivers or Allen keys, for the removal of panels or other parts of the instrument is not part of routine operating procedures. Additional risks will apply if service panels of the instrument have been removed.

The exchange of the xenon lamp is part of the service routine, refer to section 11.2 on page 67. This service routine includes the use of screw drivers for the removal of panels and xenon lamp adjustments. This lamp replacement routine contains a number of additional hazards:


Risk of Electrical Shock by High Voltage


Risk of Explosion


Risk of Burns on Hot Surfaces


Risk of Exposure by Intense Visible or Invisible Radiation

For safe operation during servicing, follow the safety instructions given in the service section of this User Guide.

1.2. Disclaimer

By setting up and starting to use the FS5 you are accepting the following terms:

You are responsible for understanding the information contained in this document. You do not rely on this information as absolute or all-encompassing. You may have to address local issues and procedures that are not covered in this document. You accept that there may be issues and procedures contained in this document that may not apply.

If you do not follow the instructions and procedures contained in this document Edinburgh Instruments takes no responsibility for any consequences. If you accept the terms in this document, you are still responsible for:

- Adhering to safety procedures
- Following all precautions
- Referring to additional safety documentations

You agree to safe operating procedures in the use of all products supplied by Edinburgh Instruments. You agree to an indemnity that Edinburgh Instruments will be free from any liability or obligation arising from the use or misuse of the FS5, including, and without limitation, any persons injured directly or indirectly in connection with the instrument. This indemnity shall in no way be deemed to have expanded Edinburgh Instrument's liability for their products.

The FS5 may only be used for analytical measurements and research.

Only qualified individuals who are trained and familiar with procedures may operate the FS5.

Edinburgh Instruments are not liable for any special, individual, indirect or consequential damage of any kind, or any damages whatsoever resulting from loss of use, loss of data, loss of profit, arising out of, or in connection with the FS5 or out of, or in connection with, the use or performance of our hardware or software.

Due to Edinburgh Instruments' effort to continuously improve their hardware and software products, all specifications, dimensions, internal workings, and operating procedures are subject to change without notice. All specifications and measurements are approximate, based on standard configuration and standard environmental conditions; results may vary with the application and the environment.

Any warranties or remedies with respect to the FS5 are limited to those provided in writing.

1.3. CE Compliance

The FS5 complies with the following European Directives:

2006/95/EC	Safety objectives of the Low Voltage Directive and its amending directives
2004/108/EC	Essential requirements of the Electromagnetic Compatibility Directive and its amending directives.

The FS5 has been designed and manufactured to the following specifications:

BS EN 61000-6-1:2007	EMC immunity for residential, commercial and light-industrial environments
BS EN 61000-6-3:2007	EMC emission standard for residential, commercial and light-industrial environments
BS EN 61010-1:2010	Safety requirements for electrical equipment for measurement, control and laboratory use

2. Requirements

2.1. Safety Training Requirements

As the user, you must ensure that all safety standards are met in your facility or laboratory that are required to operate the FS5.

The scientist or laboratory technician who performs measurements using the FS5 must be familiar with potential safety hazards that are associated with the operation of the system in your laboratory environment. Potential safety hazards are highlighted in the User Guide.

This User Guide provides instructions on how to use the system safely. If further training is required, both for the installation and for the operation, then this can be offered by Edinburgh Instruments or their distribution channels.

The exchange of the xenon bulb is a part of the service routines; this contains additional safety hazards which are highlighted in this User Guide. The person doing the service must obey the safety procedures and your facility or laboratory must be compliant and suitable.

2.2. Space and Environmental Requirements

The location of the FS5 spectrometer must meet the following requirements:

- It must be clean and dust free.
- It must be suitable for operation of optical and electrical equipment.
- It must have ambient temperature between 15°C and 30°C.
- It must be dry and the ambient humidity must be below 80%.

The location should have sufficient ventilation and should be separate from where you store chemical and biological substances.

The FS5 requires a stable laboratory table or optical bench suitable for the size and the weight of the FS5 spectrometer. The footprint of the FS5 spectrometer is 1 m (width) by 60 cm (depth). The weight of the standard FS5 (without computer and additional hardware options) is 55 kg.

The routine access to the sample compartment is from the front. The instrument can be positioned close to the back wall, access to electrical connections and ventilation is from the sides.

You must also plan for the space of the computer controlling the instrument. The best place for the computer is to the right of the FS5 spectrometer, the USB connection is also at the right side of the unit. However, a laptop can be positioned on top of the instrument, or alternatively a monitor and keyboard of your desktop computer.

Some special sample holder accessories may require access to the sample compartment, such as water pipes, tubes, etc.

2.3. Electrical Requirements

The FS5 has a universal mains input (100V ac – 240V ac, 50/60Hz).

The average power consumption is 500W.

The fuse rating of the instrument is 5A A/S.

2.4. Computer Requirement

The FS5 requires a computer for operation.

Consult the order details on whether the computer was part of the order. If the computer is provided locally, you must ensure that:

- The computer is either a desktop PC or a Laptop.
- The computer has a modern display, keyboard and mouse.
- The computer has at least two free USB2 connections (in addition to keyboard and mouse)
- The computer's operating system is Windows 7, 8 or 10

Edinburgh Instruments cannot guarantee that all features of the FLUORACLE software will be fully operational with all languages supported by Windows. It is advisable to have the computer set to standard English settings. If another language is used, some specific settings may have to be varied, such as punctuation by '.' or ','.

3. Installation

3.1. Inspecting the Shipping Crate

On receipt of the FS5 shipping crate, do not open the crate right away! Inspect it first!

Inspect the crate for any transit damage. If any signs of damage have been detected, contact your Edinburgh Instruments representative immediately. You may be asked to provide photographic evidence of the damage.

If further storage is required, be sure the crate is stored the right way up and in a dry location with a temperature range between 15°C and 30°C.

3.2. Unpacking the Shipping Crate

Procedure 1: Unpacking the shipping crate:

1-1 Remove all cross-headed screws on the top lid of the crate, ideally use an electrical screw driver. Then remove the lid of the crate.

You should now see the FS5 and a document box as shown in the picture below. The document box contains a flash memory with FLUORACLE software and hardware configuration files, a software key (HASP key-software protection device), a USB cable and this User Guide.

The consignment may also include additional sample holder modules. They are located in separate partitions of the crate.

1-2 Remove the upper packing material.
Using sufficient man-power, carefully lift the instrument out of the shipping crate.

Caution **Heavy**	The FS5 has a weight of approximately 55 kg. Four people are required to lift the FS5 out of the shipping crate.

1-3 Remove all packaging. Move the instrument to the final location

3.3. Installation of the FS5 Instrument

Install the FS5 at a place that meets the safety, space, environmental, and electrical requirements as described in section 2 of the User Guide.

Procedure 2: Installation of the FS5:

2-1 Positioning

Position the FS5 on a stable laboratory table. The instrument may be pushed right back against the wall. Note that mains and USB connector are on the right side of the instrument.

2-2 Ventilation

The instrument has ventilation holes (air outlet) on the left side. Air inlets are on the rear side, beneath the instrument cover.

Do not block these ventilation holes. Overheating can cause instrument failure. Heat sensors are fitted. If overheating occurs the instrument will turn off automatically and the software will display an error message.

2-3 Height adjustment and levelling

The FS5 comes with adjustable feet. To gain access to the feet adjusters the left and right trim strips on the bottom of the instrument must be removed. The feet can be adjusted by means of an M10 spanner.

Refit the trims when the levelling of the instrument is complete.

2-4 Electrical connections

Connect the mains power to the instrument using a mains cable with standard IEC plug.

Do NOT connect the USB cable or the software protection device to the computer at this stage. You must install the software first.

3.4. Installing the FLUORACLE Software

Do not connect the instrument or the USB software protection device (SPD) to the computer until after the software has been installed.

The FLUORACLE software requires a PC with Microsoft Windows and at least two free USB2 ports in addition to keyboard and mouse.

Procedure 3: Installation of the FLUORACLE software:

3-1 Ensure you are logged in as a user with Administrator privileges for installation.

3-2 Insert the USB flash drive into your computer and locate it in an Explorer Window.

3-3 Double-click on the 'EI FLUORACLE Setup' program. The computer may ask if you want to allow the program to make changes to your computer and verify the publisher as Edinburgh Instruments Ltd. Click *Yes*.

3-4 Follow the instructions displayed in the FLUORACLE Setup Wizard. It is recommended to keep all options as defaults. On first installation this may spawn other wizards to install hardware drivers.

3-5 After the wizard is finished connect the instrument USB cable and connect the instrument to the mains. Then switch the instrument on.

3-6 Wait while the USB devices are detected and their drivers are installed. If necessary the instrument driver installation can be verified by looking in Device Manager. Two devices should be displayed under 'Edinburgh Instruments Devices'.

You are now ready to start the instrument (mains switch on the right of the instrument near the mains input) and operate it by means of the FLUORACLE software. To start the FLUORACLE software either double click the F icon or select FLUORACLE on the Start Screen.

You may now proceed straight to section 5: System Operation.
Alternatively, learn more about details of your spectrometer by continuing with section 4.

4. System Description

4.1. Principle of Operation

In fluorescence or photoluminescence spectroscopy, the sample is excited with light of a certain wavelength and the emission spectrum, typically at longer wavelengths, is recorded and displayed as the number of emitted photon versus the emission wavelength.

The fluorescence spectrometer (or spectrofluorometer) is the device to measure the fluorescence or photoluminescence spectra. It consists of a xenon lamp that provides a broad spectrum for a choice for excitation wavelengths, an excitation monochromator that selects a specific wavelength out of the broad xenon lamp spectrum, a sample compartment, and an emission monochromator that selects a specific emission wavelength and provides scanning through a spectral range, and a sensitive detector that is capable to detect single photons.

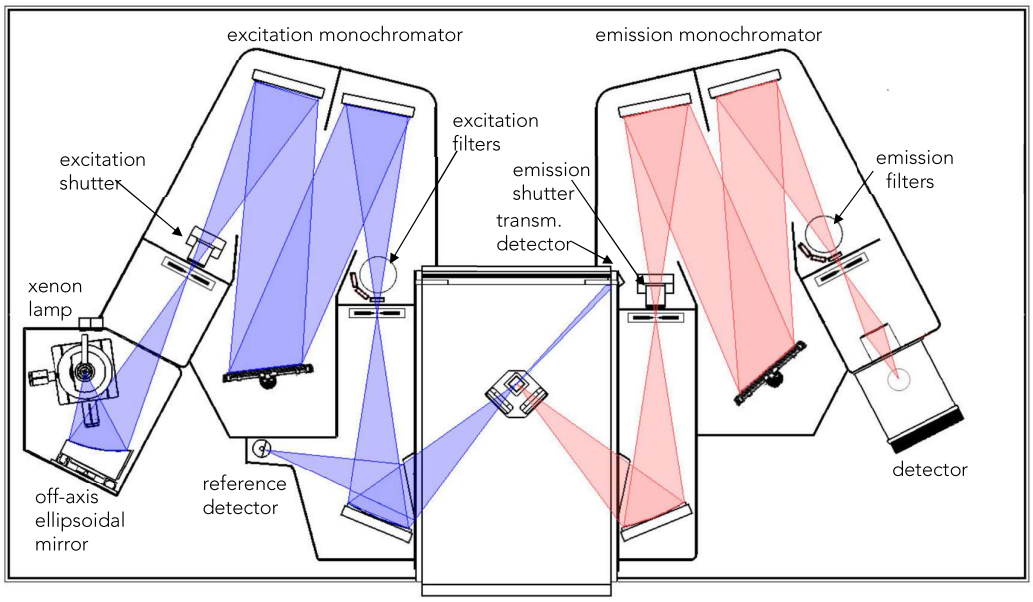
Within the FS5 practically all hardware functions are computer controlled or computer monitored. This includes the xenon lamp, excitation and emission wavelength and bandwidth, filter control, shutters, detector's high voltage, temperature control, gain, offset, threshold settings, etc.

The raw measured spectra will be distorted by spectrally dependent efficiency functions of xenon lamp, monochromators and detectors. The FS5 fully corrects for these instrumental functions and outputs "true" spectra, calibrated in number of emitted photons per unit time per unit bandwidth.

4.2. Principle Building Blocks

4.2.1. Optical Layout

The principle optical design is shown in the schematic below. The design is based on two Czerny turner monochromators, a continuous xenon light source and three detectors for detecting the sample emission, the reference light and light transmitted through the sample.



The whole optical design is based on reflective optics. The exception is the collecting lens in front of the detector where achromatic aberrations in the UV do not matter.

4.2.2. Xenon Lamp

The continuous light source is a 150W xenon arc lamp. This is computer-controlled and the hours used and number of ignitions are monitored. This lamp is primarily used for spectral measurements.

The xenon arc lamp has an enclosure of a special synthetic quartz material that transmits UV light from approximately 230 nm and absorbs practically all light below 200 nm. This way hazardous ozone cannot be generated.

The light from the continuous xenon lamp is collected and refocused on the entrance slit of the excitation monochromator by means of a diamond machined off-axis ellipsoidal mirror.

4.2.3. Monochromators

The FS5 uses Czerny-Turner monochromators for both excitation and emission arms. This type of monochromator uses plane gratings for the dispersion of the spectrum and concave mirrors for imaging the dispersed image from the entrance slit to the exit slit. The design guarantees high resolution and minimizes spherical aberrations and re-diffraction.

A unique feature of the FS5 is the large size of the monochromators and the large reflective surfaces of the optical elements. This results in a favourable linear dispersion (3.5 nm/mm), high light gathering capability (and therefore higher system light throughput), and better quality of the bright light spot at the sample.

Gratings:

The gratings are high quality plane ruled gratings. The gratings have a groove density of 1200 g/mm and are optimised at 300 nm (excitation) and 500 nm (emission).

The gratings are operated by a direct drive mechanism, with an accuracy better than ±0.5 nm and a repeatability of ±0.3 nm.

Slits:

Both entrance and exit slits of the two monochromators are continuously adjustable between 0 and 8.6 mm. With a linear dispersion of 3.5 nm/mm this translates to a spectral bandpass of up to 30 nm:

bandpass [nm] = slit width [mm] x dispersion [nm/mm]

For very small slit widths the above formula will be no longer applicable as the monochromator will operate at the resolution limit. At the resolution limit the nominal bandwidth (displayed in the software) may be further reduced. However, this will not further reduce the bandpass, instead it will only reduce the amount light passing through the system.

Shutters:

Both monochromators have built-in shutters. The excitation shutter is controlled by the software: The sample is exposed to the excitation light only when measurements are taken or the signal intensity is viewed by the user. At all other times the shutter is closed. This minimizes photobleaching and photodegradation of the sample.

The emission shutter is interlocked to the sample chamber lid. The shutter will block when the lid is open or when the sample module is removed from the instrument. This way the detector is protected against potential over-exposure with ambient light.

Filter Turrets:

Both excitation and emission monochromators are fitted with filter turrets. When the turrets are in use the software automatically selects the correct filter to remove potential unwanted higher orders. The nominal wavelength that is selected in the software is only strictly correct if the automated filters are in use. If they are not in use a second wavelength half of the nominal wavelength may also pass through the system and may cause scatter or unwanted sample excitation (and confusion in the interpretation of spectra).

The operation of the filter turrets may be disabled from within the spectrometer operating software.

4.2.4. Detectors

The FS5 contains three detectors:

Signal Detector:

This primary detector detects the sample's photoluminescence signal and is mounted at the exit of the emission monochromator.

This detector is a temperature stabilised photomultiplier of the type R928P (Hamamatsu). The detector output are electrical pulses that are generated from individual photons incident at the photocathode of the detector. The spectral coverage of the detector is 200 nm to 870 nm, with a dark count of <300 counts per second (cps).

Reference Detector:

The reference detector detects a fraction of the light that is directed to the sample causing the sample excitation. This detector is used to correct for different excitation intensities.

This detector is a UV enhanced silicon photodiode with a spectral coverage of 200 nm to 1000 nm. The silicon photodiode is an analogue detector with a current output corresponding to the amount of sample excitation. The current output is converted into a pulse train. This results in a count rate that is processed similarly to that of a signal detector photomultiplier.

Transmission Detector:

The transmission detector detects a fraction of the light that is passing through the sample, i.e. a fraction of the light that has not been absorbed by the sample.

The detector and the processing of the detector signals is similar to that of the reference detector.

4.2.5. Electronics

The electronics compartment is in the lower section of the FS5. The compartment contains a power supply module, a controller module, and a data processing module.

The power supply module delivers power to all electrical units, apart from the continuous xenon lamp, which has its own power module, located near the xenon lamp.

The controller module drives numerous stepper motors and solenoids, delivers high voltage to the detector and operates the temperature stabilisation of the detector.

The data processing module contains three independent 100 MHz counters for synchronous and fast processing of the photomultiplier signal and the signals of Reference and Transmission detector.

4.3. Sample Modules

The FS5 sample modules are 'plug and play' modules that can be exchanged according to the type of sample and the experimental conditions required.

The software will recognise the particular module and will offer special measurement options that are associated with the module.

The sample modules are large and have various sample access options, depending on the type of sample and the way samples are handled and exchanged.

The following sample modules are available:

SC-05

Standard sample module with cuvette holder and filter slots

SC-10

Sample module with front-face sample holder on adjustable mount, with filter slots

SC-15

Sample module with powder tray.

SC-20

Sample module with cuvette holder for coolant circulation and temperature probe.

SC-25
Sample module with thermoelectrically cooled/heated cuvette holder temperature probe – standard temperature range.

SC-26
Sample module with thermoelectrically cooled/heated cuvette holder temperature probe – extended temperature range.

SC-28
Heated Sample Holder

SC-30
Integrating Sphere

SC-40
Microwell plate reader

SC-50
Remote Fibre interface

SC-60
Calibration Kit

SC-70
Low Cost Cryo Option - EPR Dewar

SC-80
Liquid Nitrogen Cryostat Module

4.4. FLUORACLE – the Spectrometer Operating Software

The spectrometer's operating software is FLUORACLE. FLUORACLE controls the hardware of the instrument and provides functions for data acquisition, data analysis and data presentation.

FLUORACLE requires Microsoft Windows environment to run and is connected to the FS5 spectrometer via USB interface.

FLUORACLE is protected by a USB HASP key (software protection device –SPD) when installed 'off-line' on a different computer for data analysis or presentation. When the FS5 is operated the SPD is not required; the FS5 (being switched on) is essentially the key to run FLUORACLE.

This section describes general features of the FLUORACLE software. Refer to sections 6-8 for detailed software functionality in the context of making measurements, data operations and data analysis.

4.4.1. Main Screen

The main screen contains menu bar and tool bar on the top, a large scan display area in the middle and the hardware status bar on the button.

Menu Bar:

The menu bar offers access to all software features of the FLUORACLE software.

Note that the list of different dialogues available depends on whether or not a data window is focus and on whether the data are spectral or lifetime data. The list of dialogues accessed through the menu bar also depends on which sample module is in operation. In addition, some features of the instruments (eg. use of filter turret, display of chromaticity diagrams and quantum yield wizards) can be disabled in the Options menu, and consequently the list of dialogues may be reduced.

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.

Note that not all menu options are covered by the displayed quick-access icons.

- Scan Icons

Opens the Signal Rate, New Spectral Measurement, New Lifetime Measurement, Re-run or Batch Measurement windows.

- 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 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.

Note that the scan display area can get cluttered when many scans are open. To obtain an overview select *Tile Horizontally* or *Tile Vertically* from the Windows menu. A large number of open scan displays can be best handled by minimizing displays that are not in focus.

Status Bar:

The Status Bar at the bottom displays information about the current scan and spectrometer settings, such as excitation and emission wavelength, bandwidth settings, which type of sample module is being used, emission count rate, etc.

An example of the status bar is shown in section 5.2 of this manual.

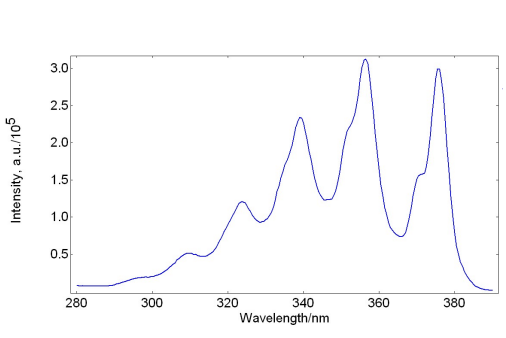
Both *Tool Bar* and *Status Bar* can be disabled using the *View* menu options.

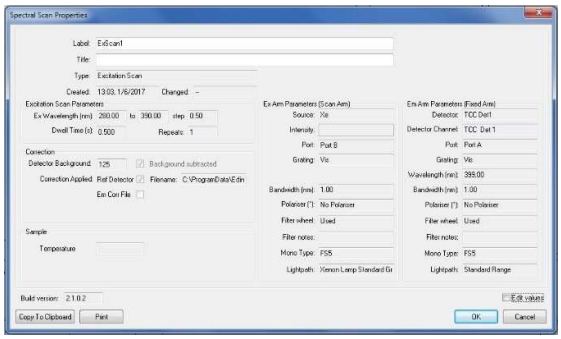
4.4.2. Scans and Scan Properties

Measurement data or analysed data are called *Scans* in the Fluoracle software. A variety of different scan plots are available and can be grouped into either spectral scan plots or time-resolved scan plots. Consequently they could be saved into two different file types, annotated with the file extensions .FS for spectral measurements and .FL for lifetime measurements.

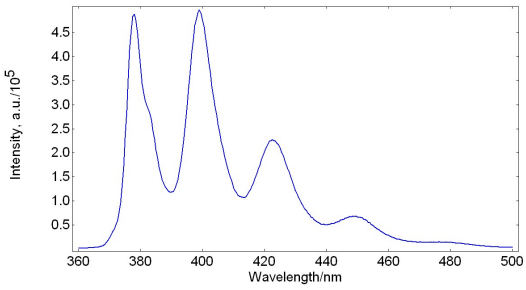
This section of the manual briefly describes the scan plot types and outlines the scan properties that are attached to each scan. The scan properties are available via *File* > *Properties*, or by right mouse click on the active data window and selection of *Properties*. If the active window contains 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.

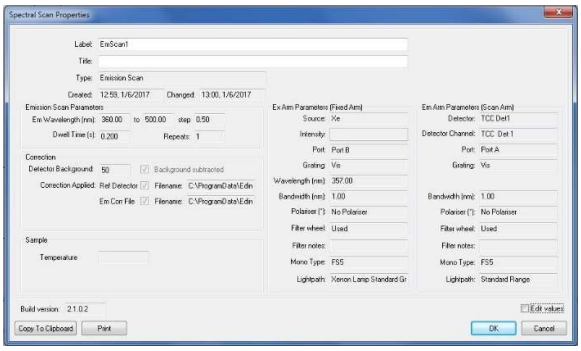
Excitation Scans, measured with fixed emission wavelength and scanning excitation wavelength.



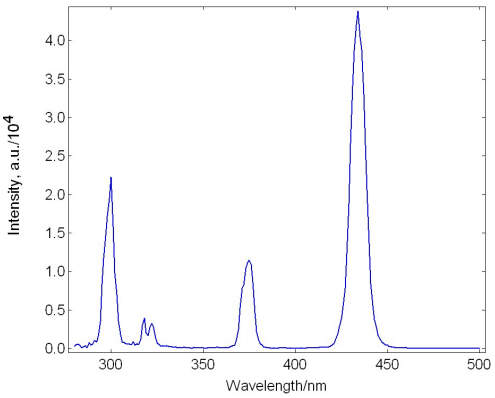


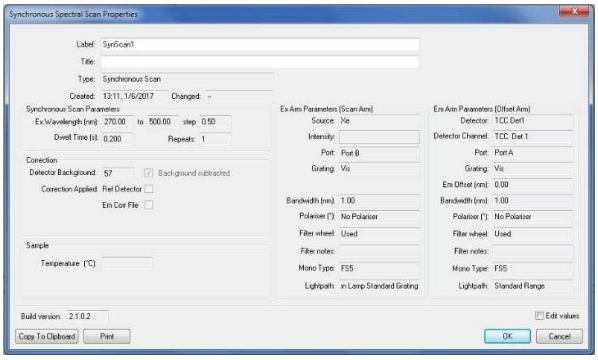
Emission Scans, measured with fixed excitation wavelength and scanning emission wavelength.



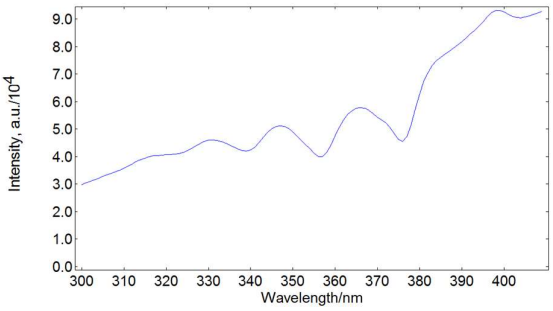


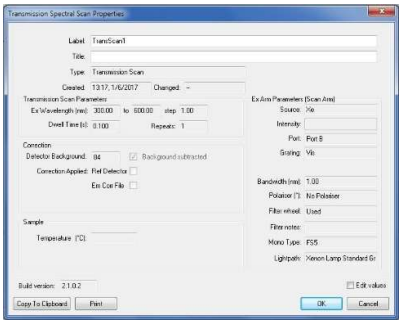
Synchronous Scans, measured with excitation and emission monochromators scanning simultaneously, using a fixed offset between excitation and emission wavelengths.



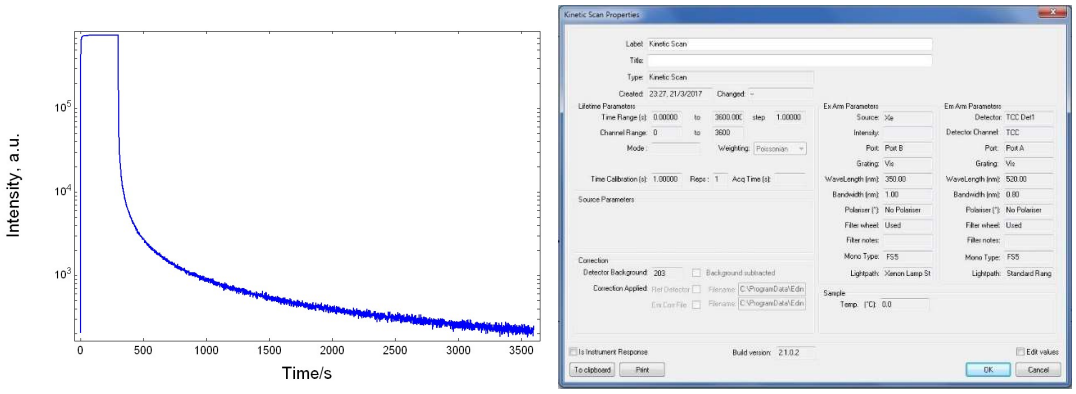


Transmission Scan, measured with scanning excitation wavelengh.





Kinetic Scan, measured with fixed excitation and emission wavelength, signal recorded over time.



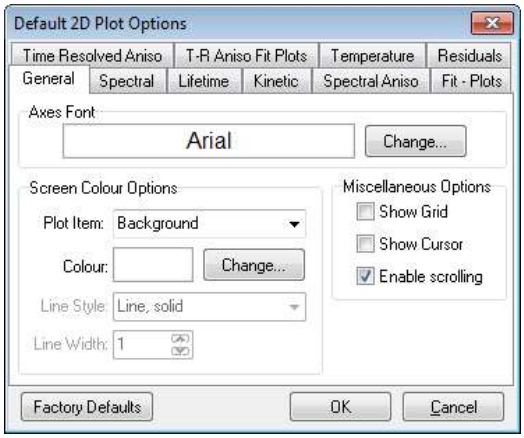
4.4.3. Data Presentation and Plot Options

FLUORACLE 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.

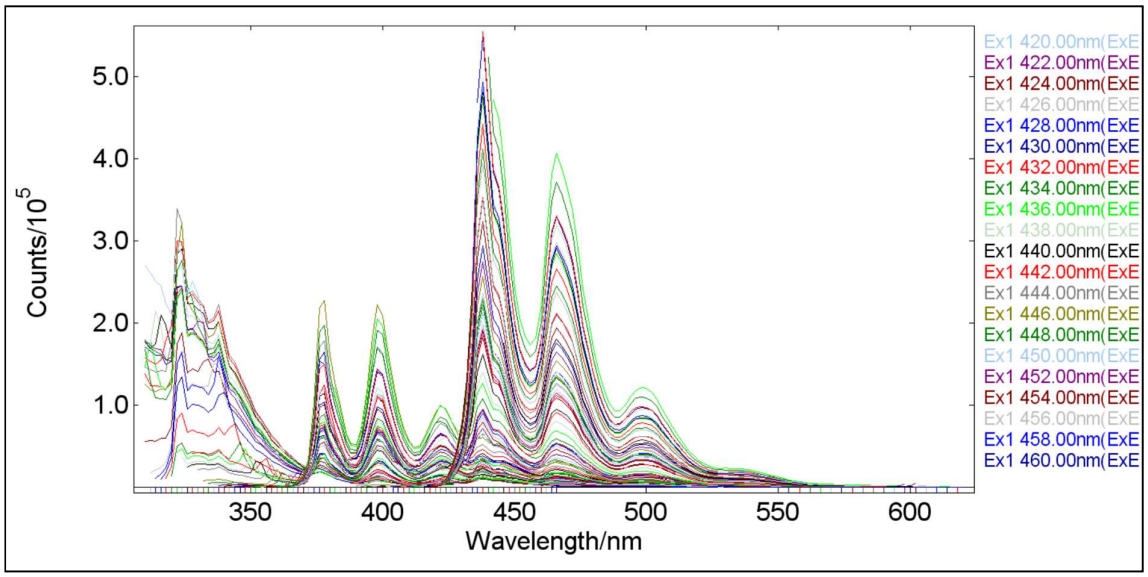
2D, 3D, and contour graphics have their own colour, font style and line style sets. These can be set up via the *Plot Options*. The three styles are used for the display of data within FLUORACLE and when the graph is copied into the clipboard for further use in different computer applications (*Ctrl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

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. It should be noted that in order to properly display data normalized to one in logarithmic scale 'scale from baseline' should be unchecked. This option can be found via *right mouse click > Plot options > Fit-Plots*.

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*.



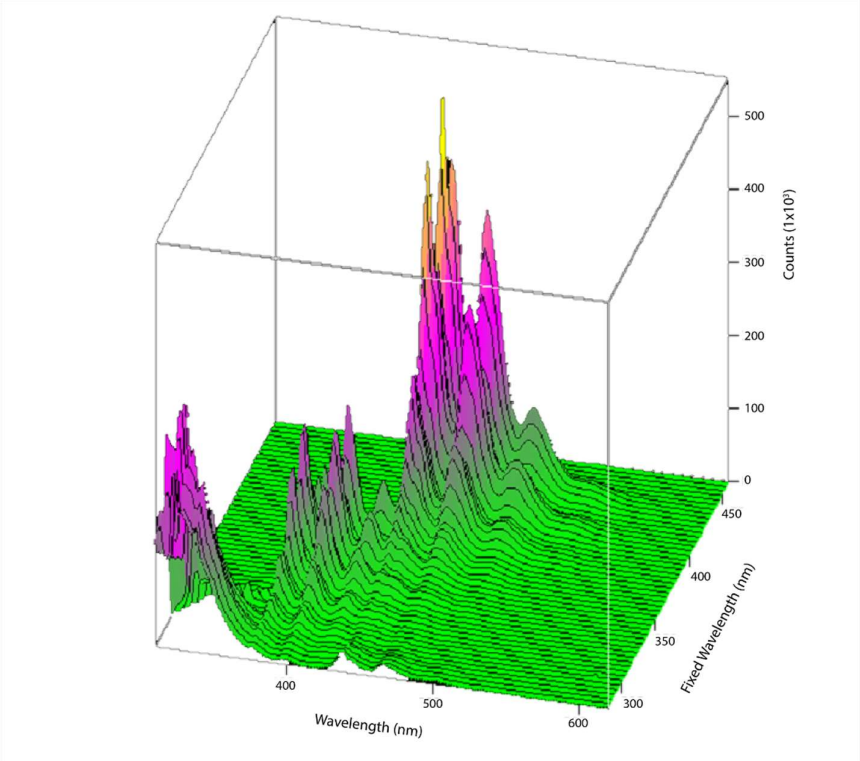
2D Graphs -



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 . When the curser is in use 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 /

3D Graphs -

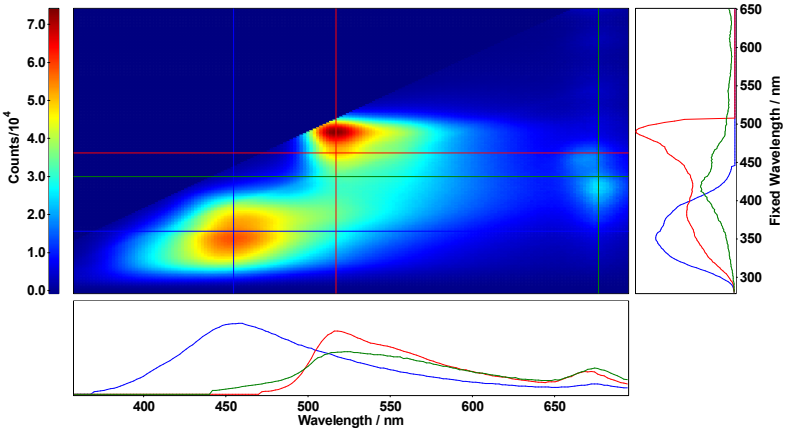
Data display in 3D is available if multiple scans or scan maps are contained in the same active window.



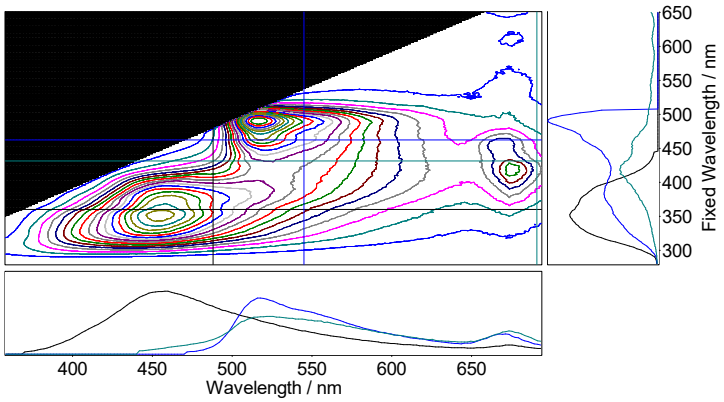
Task	Operation
Set up 3D defaults	*Options > Plot defaults > 3D...*
Change colours	*Plot options > Appearance*
Change the kind of mesh	*Plot options > Mesh*
Zoom on X, Y, and Z axes	*Plot options > Zoom*
Rotate 3D display	left mouse click and drag

Contour Graphs -

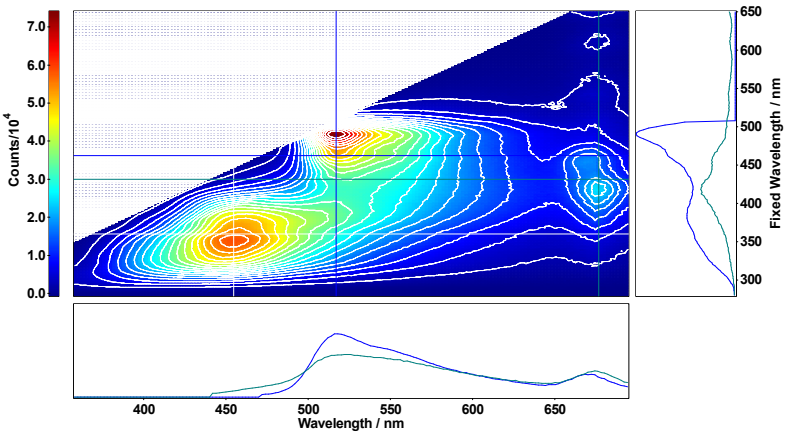
The contour plot option is available, if the active display contains more than one spectral scan or time resolved measurement.



filled colour contour graphic with three cross-hairs and intensity colour scale



standard contour graphic with three cross-hairs and coloured contour lines

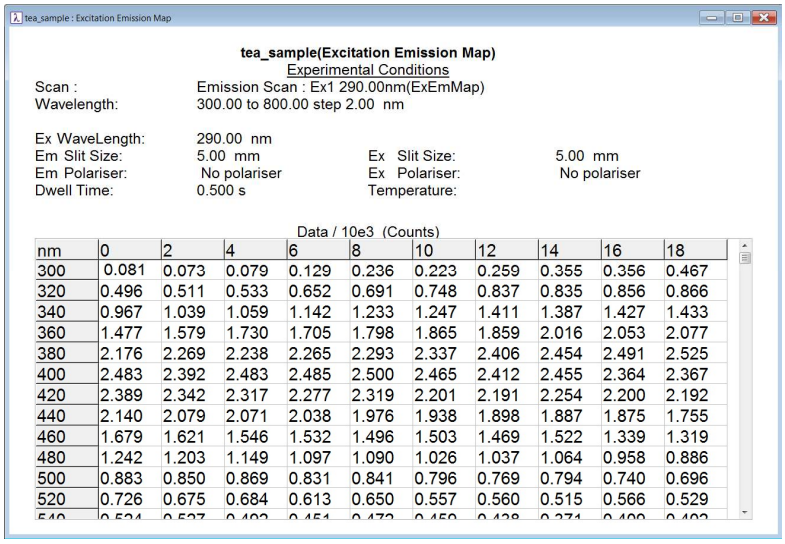


full contour graphic with filled colours and overlaid (white) contour lines.

Task	Operation
Set up Contour Graph defaults	*Options* > *Plot defaults* > *Contour...*
Change type of contour graph (see above three examples)	*Plot options* > *Colour Map* and *Countour* checkboxes
Change colours, add/ remove contour lines, make contour lines invisible	*Plot options* > *Colour Map* > toggle between *Jet, Hot* and *Greyscale* colour maps
Change resolution on wavelength axes (spectral data)	*Plot options* > use a slider to select between *LOW* an *HIGH* resolution
Change scale on intensity axis	*Plot options* > *Manual Height Scaler*
Zoom	use *View* > *Zoom In* / *Zoom Out* or  / 
Show live cross-hair and axes bars	*View* > *Show cursor*, or  . 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  in tool bar

View Data -

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



tea_sample : Excitation Emission Map

tea_sample(Excitation Emission Map)

Experimental Conditions

Scan : Emission Scan : Ex1 290.00nm(ExEmMap)
Wavelength: 300.00 to 800.00 step 2.00 nm

Ex WaveLength: 290.00 nm
Em Slit Size: 5.00 mm
Em Polariser: No polariser
Dwell Time: 0.500 s
Ex Slit Size: 5.00 mm
Ex Polariser: No polariser
Temperature:

Data / 10e3 (Counts)

nm	0	2	4	6	8	10	12	14	16	18
300	0.081	0.073	0.079	0.129	0.236	0.223	0.259	0.355	0.356	0.467
320	0.496	0.511	0.533	0.652	0.691	0.748	0.837	0.835	0.856	0.866
340	0.967	1.039	1.059	1.142	1.233	1.247	1.411	1.387	1.427	1.433
360	1.477	1.579	1.730	1.705	1.798	1.865	1.859	2.016	2.053	2.077
380	2.176	2.269	2.238	2.265	2.293	2.337	2.406	2.454	2.491	2.525
400	2.483	2.392	2.483	2.485	2.500	2.465	2.412	2.455	2.364	2.367
420	2.389	2.342	2.317	2.277	2.319	2.201	2.191	2.254	2.200	2.192
440	2.140	2.079	2.071	2.038	1.976	1.938	1.898	1.887	1.875	1.755
460	1.679	1.621	1.546	1.532	1.496	1.503	1.469	1.522	1.339	1.319
480	1.242	1.203	1.149	1.097	1.090	1.026	1.037	1.064	0.958	0.886
500	0.883	0.850	0.869	0.831	0.841	0.796	0.769	0.794	0.740	0.696
520	0.726	0.675	0.684	0.613	0.650	0.557	0.560	0.515	0.566	0.529

4.4.4. Optional Settings

Windows Options

Use this dialogue to configure your data window handling, whether or not you want to automatically update the file names and how many files you wish to have in your "recent" list.

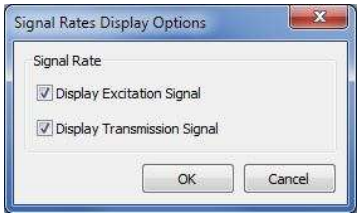
During your session you will notice that many data windows will be created. If you configure your system as shown in the above example then this will keep the number of data windows at a minimum.

It also has been found convenient to minimise data windows when not needed.

Signal Rate Display Options

You might prefer not to see signal rate bars of the reference detector or the transmission detector.

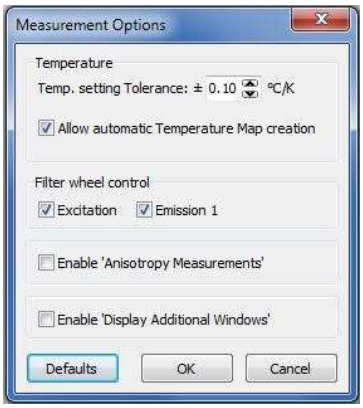
You can disable the bars here.



Measurement Options

Using this dialogue you can:

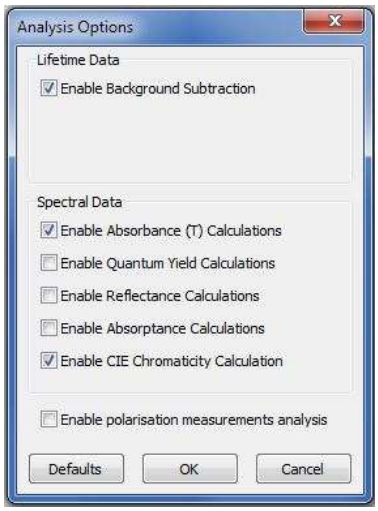
- enable or disable the automatic filter wheels. Note that the spectral correction of your system is only correct with the automatic filters enabled;
- enable or disable *Display Additional windows*. When the option is activated, additional windows will open during spectral scans, displaying the data of the reference and transmission detector detectors as well as raw (uncorrected) data of the emission detector;
- enable or disable *Anisotropy measurements* dialogues. This option is selected by default when computerised polarisers are present and could be selected for anisotropy measurements by using non-standard polarisers such as dichroic sheet polarisers (Refer to *FS5-Pol User Guide*).



Analysis Options

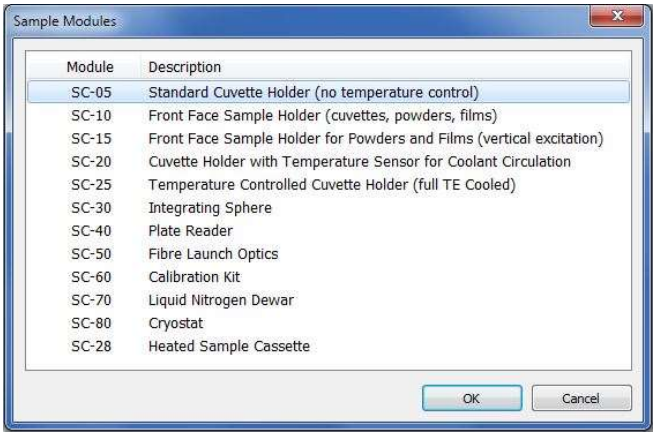
Using this dialogue you can enable or disable the analysis options that apply to your system.

- CIE Chromaticity Calculation
- Absolute quantum yield, Reflectance and Absorptance calculations will only be needed if an SC-30 Integrating Sphere module is available (refer to *FS5 Series Reference Guide – SC-30 Integrating Sphere*). When enabled these analysis options will appear in the Analysis menu.
- The option for polarisation measurements analysis should be selected for analysis anisotropy measurements performed using non-standard polarisers such as dichroic sheet polarisers (Refer to *FS5-POL User Guide*).



Sample Module Configuration

This display gives you an overview of which sample modules are available and which of them is currently active.



Sample modules are automatically detected by the system. You should only change your Sample Module when the *Sample Module Configuration* dialogue is open. If you change the sample module at other times, then the software will only recognise the new module when opening the signal rate screen or one of the scan dialogue boxes.

5. System Operation

5.1. Turning On the System

The procedure below assumes that the instrument hardware has been properly installed (section 3.3 on page 12) and the software has been installed (section 3.4 on page 13).

Procedure 4: Turning On the system:

4-1 Locate the mains switch near the mains inlet on the right hand side of the instrument.

Switch the mains on.

This will power up the instrument, including the USB interface. The green indicator on the front panel will light.

This will NOT start the xenon lamp and will NOT start the detector temperature stabilisation procedure. The detector shutter remains closed.

4-2 Locate the FLUORACLE icon on your computer screen.

Double-click the icon, or use an alternative way to start the FLUORACLE program.

At this point the computer will communicate with the FS5 via the USB interface and will initialise the FS5 hardware.

The first actions in the initialisation routine will be to start the xenon lamp and the detector temperature stabilisation. After this, all stepper motors will initialise, the sample module number will be read and the shutters will be enabled for operation.

The orange indicator on the front panel of the FS5 will be illuminated when the xenon lamp is on.

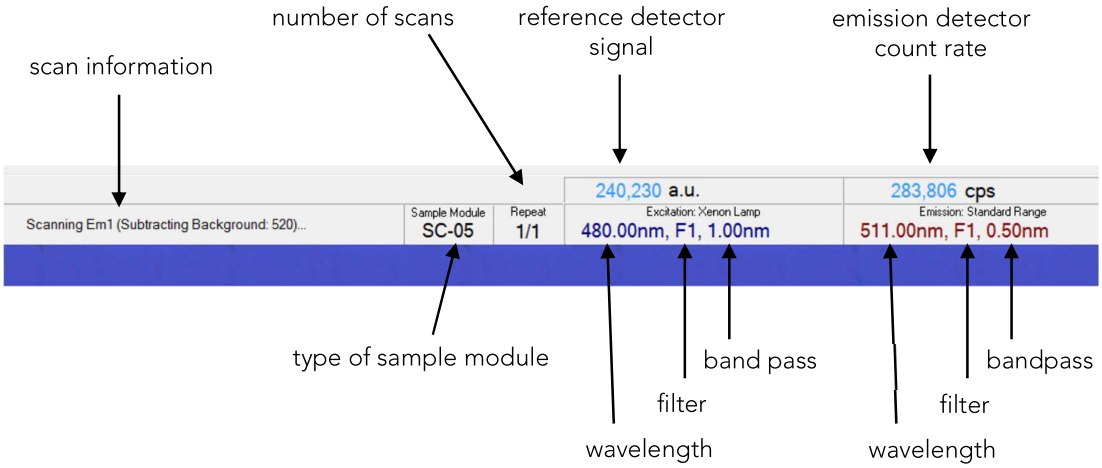
It will take about 10 minutes for the xenon lamp and the detector to stabilise.

Nevertheless, the instrument is now ready for measurements.

Note: If the FLUORACLE software has been in operation for some time (so that the lamp is hot) the lamp might not ignite when the software has been closed and immediately thereafter re-started. Wait about 5 minutes before re-starting the software.

5.2. System Status Indicators

During operation the status bar will inform about important actual settings. These includes:

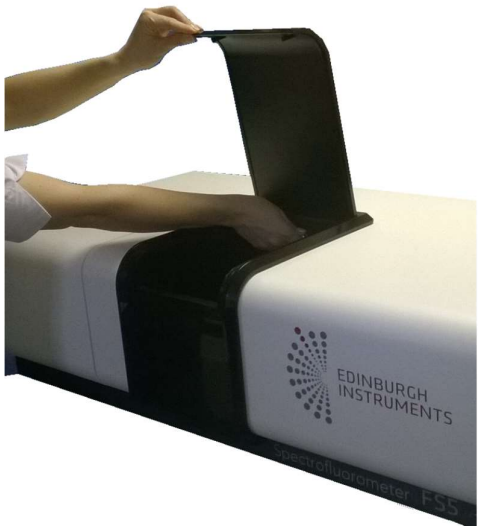


5.3. Exchanging a Sample Module

The sample module can be changed any time, no matter whether the instrument is turned on or not. However, the software will only 'look' for the sample module during initialisation and at display of the ***Sample Holder Options*** dialogue box. If a Sample Module is changed at other time, the software will still display the old module and module related measurement options will stay unchanged until either ***Signal Rate*** or ***Sample Holder Options*** dialogues have been opened.

Procedure 5: To replace the sample module:

5-1 Remove the original Sample Module from the instrument.



Release the Sample Module by undoing the screw at the back wall of the Sample Module

Pull the sample module out to the front, using the gap on the bottom between the sample module and the FS5 enclosure.

5-2 Installation of the replacement Sample Module

Fit the replacement sample module by ensuring that it is securely located on the rails on either side of the sample module space.

Push the module firmly into place, then tighten the screw on the back wall of the new module.

Never use force! If a sample module cannot be fitted easily check the correct height and for possible obstructions.

5.4. Turning Off the System

Procedure 6: Turning Off the system:

6-1 Quit your software session first.

This will stop the lamp operation and then will terminate the communication to the instrument.

Note that all settings (wavelengths, bandwidth, scan range, etc.) will be remembered for the next session.

6-2 Switch the instrument off.

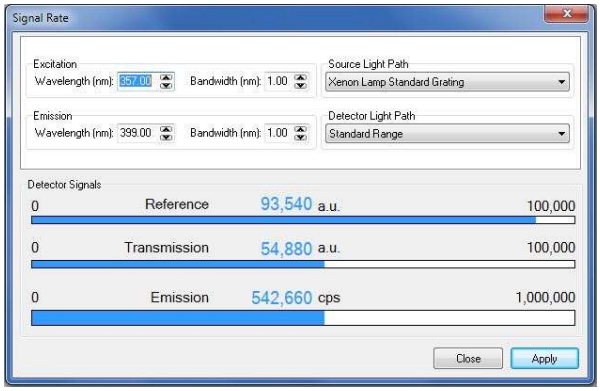
6. Making Spectral Measurements

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, refer to the previous section.

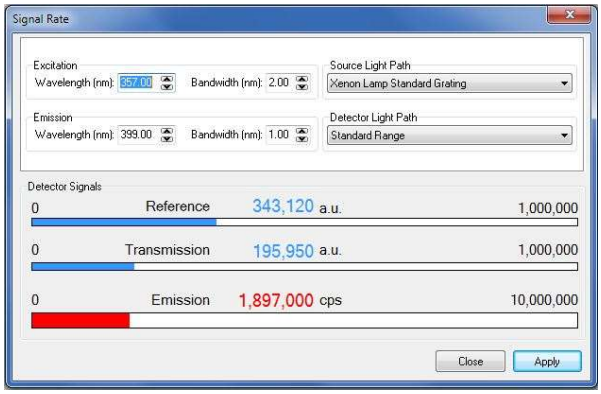
6.1. Preparing for a Measurement – the Signal Rate Dialogue

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 set there and not in any other spectral scan dialogue box. This applies in particular to the bandwidth settings.



During signal rate display and during recording of spectral scans the signal intensity should not exceed 1.5×10^6 cps. Bigger signals will cause saturation effects and non-linearity and will be shown in red.



The detector will not break, even with a reading of 5×10^6 cps (which corresponds to a true photon count rate that is much higher), but it is good practice not to exceed 1.5×10^6 cps.

During spectral acquisitions the status bar on the bottom of the screen informs the user about the running activities, such as the number of the current scan, the sample temperature, light path, spectral bandwidth, angle of polarisation, filter in use etc.

To assist the user to stay in the linear range of the signal, the signal bars on the *Signal Rate* screen, as well as the numerical values of the rates in both *Signal Rate* dialogue and status bar will turn from blue to red as soon as the linear range is exceeded.

The *Signal Rate* dialogue may contain only one or two bars, not three as shown in the above example. This depends on what Sample Module is in use and can also be configured in the Signal Rate display options.

The up and down arrows adjacent to the wavelength display may be used to manually scan through the spectrum. If you require a different scan step than the default of 1 nm, use the right mouse click to change the scan step parameter.

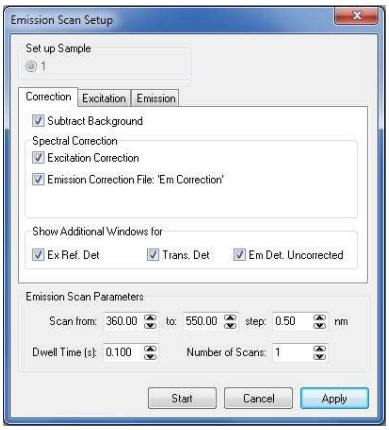
6.2. Emission Scans

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

Procedure 7: To set-up and perform an emission scan:

7-1 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. The photon count rate can be controlled by means of the spectral bandwidth. Often there is a compromise required between spectral bandpass and signal intensity. For emission scans most users prefer a defined bandpass, for instance 1 nm, whereas the bandpass in the excitation is used to control the signal intensity.

7-2 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.



- Change the *Excitation Scan Parameters* in the lower section of the dialogue box.
- The tab *Correction* offers correction options for this spectral scan. Typically all correction options will be ticked, so that the resulting measurement is the true spectrum, undistorted by instrumental effects. It is however strongly recommended that you measure at some point your sample fully uncorrected, so that you can see impact of the correction on the data.
- 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 will then be subtracted from the raw scan data..

- The field *Display Additional Windows* are only present if this option has been chosen in *Options > Measurement Options*. If you wish to record reference detector and/or transmission detector and/or raw (uncorrected) data from emission detector tick the corresponding checkbox. The data will be displayed in separate window/s. This might be useful for subsequent correction.
- The *Excitation* tab confirmes the excitation wavelength and spectral bandpass.
- The *Emission* tab confirmes the spectral bandpass in the emission.

7-3 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 displayed scan parameters will be saved, the dialogue box will close and the spectral scan will start.

7-4 The Excitation Scan graph is displayed with points plotted in real-time. The auto-scaling Y-axis shows the emission intensity for the total dwell time measured. If more than one repeat have been chosen, then the data of a new repeat scan will be added to the previous scan. The final scan will therefore display the data for the total dwell time. Note that the count rate display in the status bar at the bottom of the screen is different: This shows the (uncorrected) number of counts per second.

7-5 Active spectral scans can be aborted by either pushing the *Esc* button or by clicking the  tool button on the top right of the FLUORACLE screen. Note that the *Esc* button will only be active if the window containing the running scan is in focus.

7-6 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.

7-7 Select *File > Save* to save the scan.

7-8 Details of the parameter settings of a completed scan can be viewed in the *Spectral Scan Properties*. The properties can be accessed via *right click > Properties* if the active window contains only one measurement or via *right click > Properties > Measurement List* if more than one scan is present in the active data window.

6.3. Excitation Scans

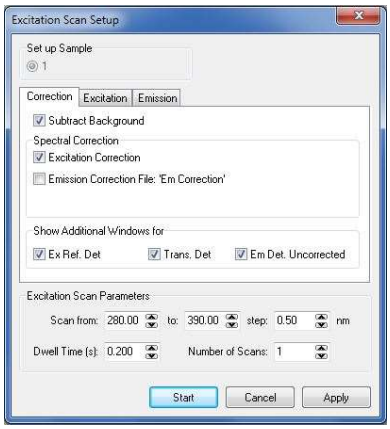
Excitation scans reveal the absorption properties of a sample. They are useful in particular for the investigation of sample mixtures, as by means of the fixed emission wavelength species can be selectively measured.

Excitation scans are scans with the emission wavelength fixed and the excitation wavelength scanning over a pre-defined range. The scan range is typically at shorter wavelengths than the fixed emission.

Procedure 8: To set-up and perform an excitation scan:

8-1 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. The photon count rate can be controlled by means of the spectral bandwidth. Often there is a compromise required between spectral bandpass and signal intensity. If the absorption spectrum does not have a thin structure it is recommended to set an excitation bandpass no less than 1 nm and to control the signal intensity via the emission bandpass.

8-2 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 scan.



- Change the *Excitation Scan Parameters* in the lower section of the dialogue box.
- The tab *Correction* offers correction options for this spectral scan. Typically the excitation correction option will be ticked, so that the resulting measurement is the true spectrum, undistorted by instrumental effects. As for excitation scans the emission wavelength is a fixed value, the *Emission Correction* will only work like a scaling factor for the entire measurement.
- 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.
- The field *Display Additional Windows* are only present if this option has been chosen in *Options > Measurement Options*. If you wish to record reference detector and/or transmission detector and/or raw (uncorrected) data from emission detector tick the corresponding checkbox. The data will be displayed in separate window(s). This might be useful for subsequent correction.
- The *Excitation* tab confirmes the spectral bandpass in the excitation.
- The *Emission* tab confirmes the emission wavelength and spectral bandpass.

8-3 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 displayed scan parameters will be saved, the dialogue box will close and the spectral scan will start.

8-4 The Excitation 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. If more than one repeat has been chosen, then the data of a new repeat scan will be added to the previous scan. The final scan will therefore display the data for the total dwell time. Note that the count rate display in the status bar at the bottom of the screen is different: This shows the (uncorrected) number of counts per second.

8-5 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.

8-6 Select *File > Save* to save the scan.

8-7 Details of the parameter settings of a completed scan can be viewed in the *Spectral Scan Properties*. The properties can be accessed via *right click > Properties* if the active window

contains only one measurement or via *right click* > *Properties* > *Measurement List* if more than one scan is present in the active data window.

6.4. Transmission Scans

Transmission scan are scans of the excitation monochromator, while the signal is measured immediatelty after the sample, using the dedicated transmission detector.

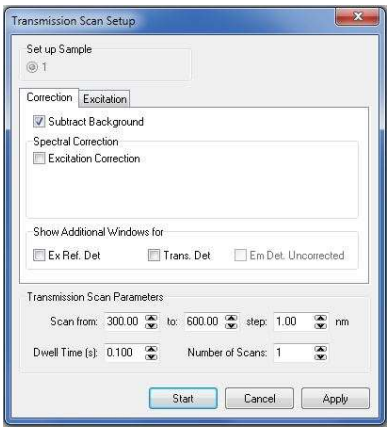
Transmission scans can be used to calculate the absorption spectrum of your sample, by recording the scans twice, once with your sample in place, the second time with your reference sample. A wizard is provided to calculate the absorption spectrum.

Procedure 9: To set-up and perform a transmission measurement:

9-1 Open the *Signal Rate* screen and enter the excitation wavelength for which you expect the maximum signal at the transmission detector. Then select the spectral bandwidths in the excitation path. The signal level on the transmission detector should not exceed 4.7×10^6 a.u.

Note that the highest signal might not be the wavelength at which the sample's transmission is at a maximum (or the sample's absorption at a minimum). If the spectral range of interest contains the region where the xenon lamp has strong spikes, then it is likely that the maximum signal will be at one of the spikes (main xenon spike at 468 nm).

9-2 Close the signal rate screen and open the *Transmission Scan Setup* dialogue box.



- Change the *Transmission Scan Parameters* in the lower section of the dialogue box.
- The tab *Correction* offers correction options for this spectral scan. Typically the excitation correction option will be ticked, to account for xenon lamp fluctuations during the measurements.
- The function *Subtract Background* is switched on by default as soon as the excitation correction method 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.
- The field *Display Additional Windows* are only present if this option has been chosen in *Options* > *Measurement Options*. If you wish to record reference detector and/or transmission detector tick the corresponding checkbox. The data will be displayed in separate window/s. This might be useful for subsequent correction.
- The *Excitation* tab confirmes the spectral bandpass in the excitation.

9-3 The scan parameters can be saved by checking the *Apply* button. By checking the *Start* button the demonstrated scan parameters will be saved, the dialogue box will close and the spectral scan will begin.

9-4 Active spectral scans can be aborted by either pushing the *Esc* button or by clicking the  tool button on the top right of the FLUORACLE screen. Note that the *Esc* button will only be active if the window containing the running scan is in focus.

9-5 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.

9-6 Select *File* > *Save* to save the scan.

9-7 Details of the parameter settings of a completed scan can be viewed in the *Spectral Scan Properties*. The properties can be accessed via *right click* > *Properties* if the active window contains only one measurement or via *right click* > *Properties* > *Measurement List* if more than one scan is present in the active data window.

6.5. Synchronous Scans

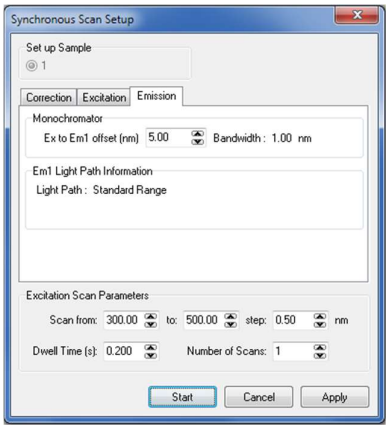
Synchronous scans are scans where both the excitation and the emission wavelengths are scanned simultaneously, with the offset between excitation and emission being either zero or with the emission wavelength at a fixed offset above the excitation wavelength.

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 these 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.

Procedure 10: To set-up and perform a synchronous scan:

10-1 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.

10-2 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.



➢ Change the *Synchronous Scan Parameters* in the lower section of the dialogue box.

- Set *Offset* parameter on the *Emission* tab.

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 bandwidth of both excitation and emission smaller than the offset.

- As for excitation and emission scans, the tab *Correction* provides options for the spectral correction for this spectral scan. Typically all correction options will be selected to obtain the true spectrum, undistorted by instrumental artefacts. However, it is recommended to study the effect of spectral correction by disabling one or the other correction option at a convenient time.
- 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. Thisd will then be subtracted from the raw scan data.
- The field *Display Additional Windows* are only present if this option has been chosen in *Options > Measurement Options*. If you wish to record reference detector and/or transmission detector tick the corresponding checkbox. The data will be displayed in separate window/s. This might be useful for subsequent correction.
- The *Excitation* tab confirmes the spectral bandpass in the excitation.

10-3 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.

10-4 The *Synchronous 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. If more than one repeats have been chosen, then the data of a new repeat scan will be added to the previous scan. The final scan will therefore display the data for the total dwell time. Note that the count rate display in the status bar at the bottom of the screen is different: This shows the (uncorrected) number of counts per second.

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.

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.

10-5 Select *File > Save* to save the scan.

10-6 Details of the parameter settings of a completed scan can be viewed in the *Spectral Scan Properties*. The properties can be accessed via *right click > Properties* if the active window contains only one measurement or via *right click > Properties > Measurement List* if more than one scan is present in the active data window.

6.6. Emission Maps

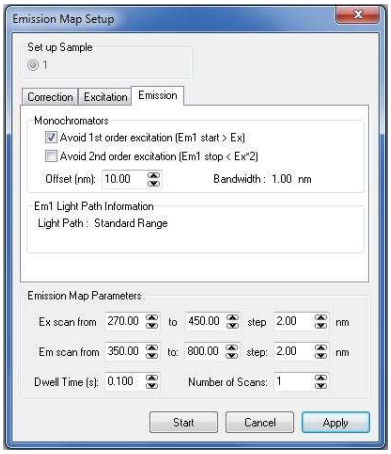
Emission Maps are a series of emission scans (see Section 6.2 on page 38) with excitation wavelengths being systematically changed. Emission maps are often displayed by means of a colour map graphic; this view is often referred to as EEM (Excitation-Emission-Map)

Procedure 11: To set-up and perform an emission map measurement:

One must have sufficient background knowledge about the sample's emission properties. It is useful to perform one or more emission scans (refer to section 6.2 on page 38) with the aim to find the set of excitation and emission wavelengths at which the signal is at the maximum.

11-1 Open the *Signal Rate* screen; enter the set of excitation and emission wavelength for the expected maximum signal. Then select the spectral bandwidths so that the detector signal is appropriate, i.e. < 1.5×10^6 cps. It might be appropriate to choose identical bandwidths for both excitation and emission.

11-2 Close the signal rate screen and open the *Emission Map Setup* dialogue box.



- 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 colour map 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. The time for a measurement can be estimated by

$$t = \frac{\Delta\lambda_{exc}}{S_{exc}} \cdot \frac{\Delta\lambda_{em}}{S_{em}} \cdot d$$

where $\Delta\lambda_{exc}$ – excitation range; $\Delta\lambda_{em}$ – emission range; S_{exc} – excitation step; S_{em} – emission step; d – dwell tim

- Select the *Correction* tab and choose the method of spectral correction.
- Select the *Excitation* tab and decide whether the excitation should be stepwise increased – unchecked tick box, or stepwise decreased – checked tick box *Reverse Excitation Order*. 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.

- Select the *Emission* 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. For scattering samples, the cross-scanning is not desirable, in this case tick the top box of the two. Once the box is ticked you have the option to decide how many nanometres away from the actual excitation wavelength the emission scan should start.

As your FS5 has been fitted with automatic filter wheels to eliminate second order effects, there should be no need to tick the second of the two boxes in the upper section. However, you might prefer to operate the FS5 without the filter wheel (*Options* > *Measurement Options*). In this case the option *Avoid 2nd order excitation* should be selected, and should then run the scans uncorrected as the correction files contain the filter responses.

11-3 Start the scan: *Start*.

11-4 Spectral map measurements can be aborted by either pushing the *Esc* button or by clicking the  tool button on the top right of the FLUORACLE 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. When aborting a map measurement the last active scan will be lost, all previous scans of the map will remain and can be further processed.

11-5 Select *File* > *Save* to save the map.

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6.7. Synchronous Maps

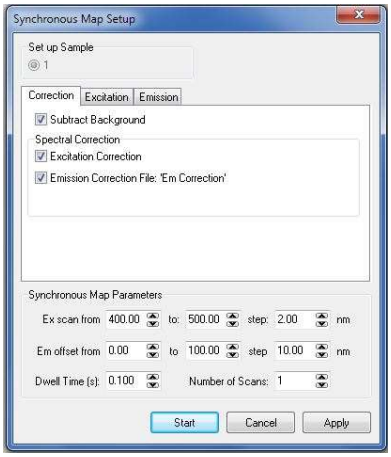
Synchronous Maps are a series of synchronous scans (see Section 6.5 on page 42) with the offset between excitation and emission being systematically increased.

Procedure 12: To set-up and perform a synchronous map measurement:

Firstly, one must gain sufficient background knowledge of the sample's emission properties. It is useful to perform one or more synchronous scans (refer to section 6.5 on page 42) with the aim to find the set of excitation wavelength and emission offset for which the signal is at the maximum.

12-1 Open the *Signal Rate* screen; enter the set of excitation and emission wavelength for the expected maximum signal. Then select the spectral bandwidths and the attenuator setting so that the detector signal is appropriate, i.e. < 1.5×10^6 cps). It might be appropriate to choose identical bandwidths for both excitation and emission.

12-2 Close the signal rate screen and open the *Synchronous Map Setup* dialogue box.



- Use the lower section of the dialogue box to enter the appropriate *excitation range* and the range for the *emission offset*, select the *step size*, 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. The time for a measurement can be estimated by

$$t = \frac{\Delta\lambda_{exc}}{S_{exc}} \cdot \frac{\Delta\lambda_{offset}}{S_{offset}} \cdot d$$

where $\Delta\lambda_{exc}$ – excitation range; $\Delta\lambda_{offset}$ – offset range; S_{exc} – excitation step; S_{offset} – offset step; d – dwell time.

- Select the *Correction* tab and choose the method of spectral correction.

12-3 Start the map measurement: *Start*.

12-4 Spectral map measurements can be aborted by either pushing the *Esc* button or by clicking the STOP tool button. When aborting a map measurement the last active scan will be lost, all previous scans of the map will remain and can be further processed.

12-5 Select *File* > *Save* to save the map.

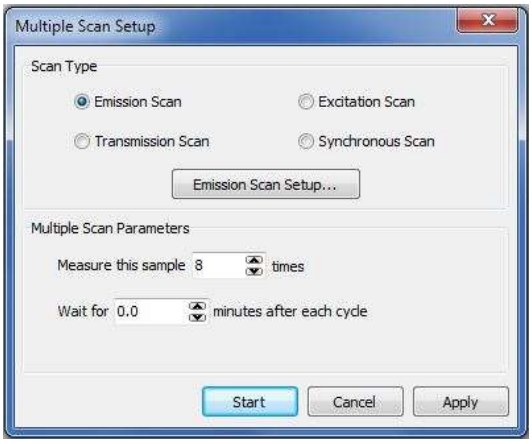
6.8. Multiple Spectral Measurements

Multiple spectral measurements allow for repeated measurements of either emission scans, excitation scans, transmission scans or synchronous scans. These measurements are ideal to follow slow kinetic processes or to study photobleaching and other long term stability effects of your sample.

Procedure 13: To set-up and perform a multiple spectral measurement:

13-1 Open the *Signal Rate* screen and enter the set of excitation and emission wavelengths for the expected maximum signal. Then select the spectral bandwidths and the attenuator setting so that the detector signal is appropriate, i.e. < 1.5×10^6 cps). Take into account that over the length of the full measurement the signal may be growing, or that peak excitation and emission wavelengths may be shifting.

13-2 Close the signal rate screen and open the *Multiple* scan setup dialogue box.



13-3 Decide whether you want to produce a series of emission scans, excitation scans, transmission scans or synchronous scans. Once selected by the radio button, you will be able to fine-tune the respective scan parameters through the button *Scan Setup…* . These settings are inherited from the previous single scans, but it is advisable to check them again before committing to the longer multiple scan.

A particular point to consider is the duration for a single scan. You may want to change the dwell time and/or the number of repeats to obtain an acceptable (short) measurement time.

13-4 Now select the parameters for the full series of your measurements: how often you wish to repeat the measurement (*Measure the sample …*) and the time you want to pause between individual measurements (*Wait for …*)

13-5 Start the map measurement: *Start*.

13-6 Multiple spectral measurements can be aborted by either pushing the *Esc* button or by clicking the  tool button on the top right of the FLUORACLE 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. When aborting a multiple spectral measurement the last active scan will be lost, all previous scans of the map will remain and can be further processed.

13-7 Select *File* > *Save* to save the measurements.

6.9. Service Scans

Service scans are special scans that enable you to quickly validate the performance of your spectrometer, or to measure reference scans that are characteristic for your spectrometer.

The service scan menu is not accessible through the  button, they are only accessible through the File menu.

There are three scans that are used for instrument validation:

- Excitation Wavelength Validation Scan
- Emission Wavelength Validation Scan
- Sensitivity Validation Scan

These spectral scans are pre-defined (you cannot set scan parameters) and you may have to use special samples, such as a Water Raman reference sample for the sensitivity validation scan. The detailed description of the scans is given in Section 12 of this User Guide.

The two reference scans are:

- Excitation Reference Scan
- Emission Reference Scan

The excitation reference scan is a record of your excitation path, measured with the reference detector at the exit of your excitation path. This file contains the spectral output of the xenon lamp and the throughput function of the excitation monochromator.

The emission reference scan is a record of your emission path. This is NOT the emission correction file. The measurement of the emission reference scan requires an external light source, such like a calibrated tungsten lamp or a calibrated deuterium lamp, or the Calibration Cassette SC-60.

If emission reference scans are made with calibrated light sources, then this will allow you to generate a new spectral correction file for your system.

6.10. 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 is not available, just looking at the sample can provide valuable information. For example, samples that are transparent will most likely absorb in the UV, and emit in the blue spectral range. Samples that have yellow colour will absorb in the 400 nm region and emit green or orange, samples that are blue will absorb at around 600 nm – 700 nm and 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), and 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, the sample's emission light might not reach the detector because of the sample concentration being too high.

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 490 nm.

Tips:

- Reduce the concentration of the fluorophore(s).
- Choose 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 module SC-10, this allows to detect the emission at the same surface that also faces the excitation beam.

2. Higher Order Effect

This effect will only show up, if the FS5 internal automated filters have been disabled. If the filters are enabled higher order effects should be completely removed.

With the FS5 operating in the a-typical configuration with the filters disabled, a large spike may appear at a wavelength that has the doubled value of the chosen 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 a third, and a 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 the integrated filters in your FS5 spectrometer. Per default, the filters are enabled and will automatically be selected. If this is the case you should not see the second order problem. Check the dialogue *Options > Measurement Options* and enable the filters for both excitation and emission.
- Use an additional wavelength cut-off filter (blocking at shorter, transmitting at longer wavelength) in the emission beam path (behind 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 nm – 800 nm) one might even see the second order of the emission (in this case the emission from 300 nm – 400 nm re-appearing between 600 nm – 800 nm). In this situation the only proper solution is to select the automated filter option. A more laborious way is to measure 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 most 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 temporarily changed to 500 nm 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.
- Be sure the sample concentration is not too high . Refer to Problem1-Inner Filter Effect above.
- Bulk or film samples might have to be re-positioned, ideally use Front Face sample holder module SC-10.
- Increase the spectral bandpass. 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 bandpasses in excitation and emission have to be increased.

Please note: With very wide spectral bandwidths 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 FS5 spectrometer will be unable to "count all photons" that impact on the detector's active area. 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 the data will be 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 100 MHz counter would be able to count 100 million photon pulses equi-spaced in time, but with random arrival times the narrowest spaced pulses may be missed due to the detector dead time, 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 0.01 s will be affected by saturation although the biggest signal is only 15,000 counts. Remember the typical saturation threshold is at 1.5×10^6 !

Tips:

- Use the Signal Rate screen prior to measurements to check for the maximum possible signal. If the count rate and the count rate bar turn red, then the signal exceeds the linear operating range and you should reduce the signal before starting the scan.
- Start with narrow spectral bandwidths before attempting bigger bandwidths.
- Investigate 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. 0.1 s or 0.2 s) and 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.

6. Spectral Correction

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

In the majority of applications the full automated correction is best. Nevertheless, it is unavoidable to study the correction and check out the various options.

Tips:

- Do a fully uncorrected measurements and use the manual correction (*Data > Correction*) to correct your data. Compare the uncorrected and the corrected scan to study the effects of correction.
- Study the impact of the background (high background, small signal levels).
- Data that are measured in spectral regions of reduced detector sensitivity (>850 nm) may be significantly modified (with an apparent positive and negative baseline) by the correction.

Spectral correction in this spectral region is particularly demanding. The best recipe is to achieve a maximum signal to background ratio.

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 10 nm to 50 nm before the peak. This increases the range over which the emission can be scanned without distortions by scattered light.
- Samples with strong absorption-emission overlap are also prone to re-absorption, i.e. photons that are emitted at the short wavelength edge of the emission spectrum are re-absorbed (by neighbouring molecules) at the long edge of the absorption spectrum. This process can distort both excitation and emission spectra. Use low sample concentrations to avoid this effect.

7. Making 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 second to seconds. Excitation and emission wavelengths remain fixed for the time of the measurement.

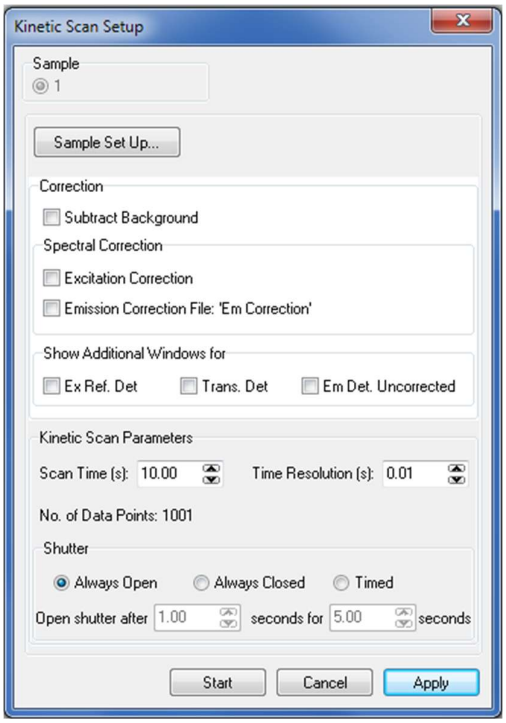
The light from the excitation source (which is controlled by the excitation shutter) may be permanently on, or off, or may be switched on and off during the kinetic scan.

Use the *Signal Rate* dialogue box to prepare for a kinetic scan, then use the button to access the Kinetic Scan dialogue.

Procedure 14: To set-up and perform a kinetic measurement:

14-1 Use the *Signal Rate* dialogue box to set up measurement parameters, i.e. excitation and emission wavelength and bandwidth. Ensure that the correct excitation light path and the emission light path have been selected.

14-2 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 cannot exceed 10,000.
- 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.
- To correct the Kinetic measurements for fluctuations of the excitation light select the option *Excitation Correction*. The reference signal may also be recorded and displayed by ticking the box *Show Additional Windows* for *Ex Ref.Det*. This option is only available, if activated in *Options > Masurements Options*.

14-3 Start the Kinetic Scan: *Start*.

14-4 An active kinetic measurement can be stopped by either pushing the *Esc* button or by clicking the  tool button. Note that the *Esc* button will only be active if the window containing the scan is in focus.

Kinetic scans can be analysed by exponential fitting. This is available via *Analysis* > *Exp. Tail Fit*. However, kinetic processes in the time regime of seconds or minutes often follow more complex models than standard exponentials, the tail fit option might only provide a "crude" (or average) time constant for the overall decay (or rise) process.

8. Re-Run and Batch Measurements

8.1. Re-run a spectral measurement

An excitation, emission or synchronous scan, as well as an excitation-emission map or a synchronous map can be repeated automatically (i.e. without manual setting up of the required parameters) using the *Re-run* command. The command is available via the Tool bar ( ) or via right-mouse click > *Re-Run Measurements*.

Note that only spectral measurements performed with standard continuous source (Xe lamp) can be Re-run. The command will be inactive in the following cases:

- If spectral measurement has been performed with any other light source than Xe lamp;
- If spectral measurement has been performed using unrecognised excitation/emission lightpath.

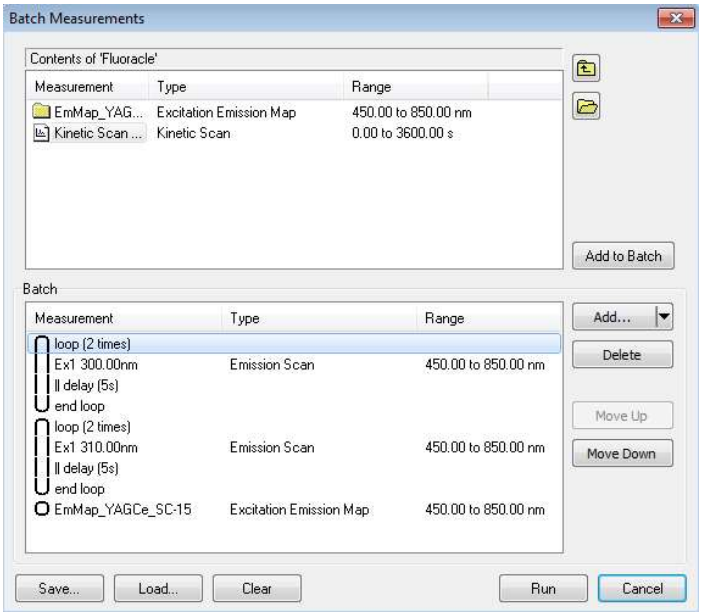
8.2. Batch measurements

Combinations of excitation, emission, synchronous scans, excitation-emission or synchronous maps can be run in *Batch Measurements*. This means that several scans can be set for a sample and measured automatically without the presence of the user. The scans can be set to repeat in loops as many times as required, with a fixed preset delay between each scan. The batch of measurements can be saved and loaded for future use.

Procedure 15: To set-up and perform Batch measurements...

15-1 Open the Batch measurements setup window via *File* > *Batch Measurements...* menu option or by pressing the Batch icon ( ) on the tool bar.

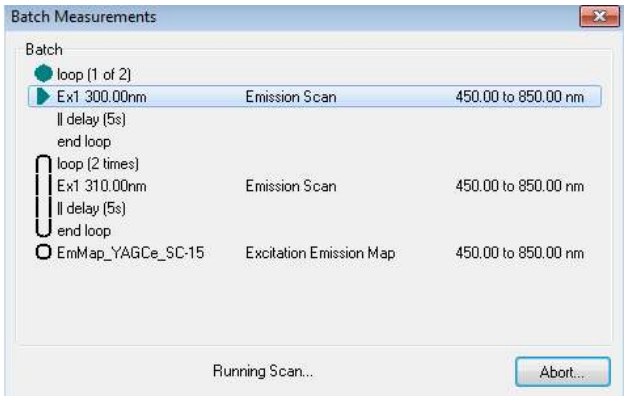
15-2 The upper panel of Batch Measurements setup dialogue shows all the current content of Fluoracle. The lower panel allows organising a batch protocol for multiple sample measurements.



To organise a protocol:

- Select a spectral measurement. You can either drag-and-drop it into Batch container or use *Add to Batch* button. Note that only spectral measurements performed with the standard continuous source (Xe lamp) could be added into batch protocol. *Add to Batch* button will be inactive (and drag-and-drop will be not possible) in the following cases:
    - If spectral measurement has been performed with any other than Xe lamp light source;
    - If spectral measurement has been performed using unrecognised excitation/emission lightpath.
- Using *Add* > *Delay* if you wish to set a delay in seconds between consecutive measurements.
- To repeat a series of measurements a desired number of times add *Loop* (*Add > Loop*) before the first one in the series and *End Loop* (*Add > End Loop*) after the last one.
- Change the content and order of measurements within a Batch protocol by using the *Delete*, *Move Up* and *Move down* buttons.
- You can *Save* a Batch protocol into a file of *.fb type. Later you can *Load* a previously saved Batch protocol.

15-3 Press *Run* to start Batch measurements. The Batch Progress window will appear and the currently performed stage of measurements will be indicated by green triangle. Every measurement will appear in a separate plot window.



9. Data Operations

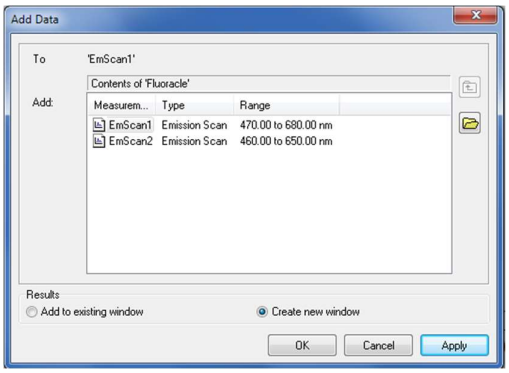
A variety of the data operation tools and data analysis options are available. Some data manipulation options are common to both spectral and kinetic data, others are specific. Therefore, the list of options that is available by clicking the pull down menu *Data* will vary depending on the data file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

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*. These advanced options require data that can only be measured with special hardware (integrating sphere, polarisers).

9.1. Add, Subtract, Multiply, Divide

With these data 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.*. The list that is provided within this dialogue box contains all data windows that are open or minimised within the FLUORACLE program. The sequence is in order of time accessed. In the example display below the spectral scan *EmScan1* will be added to the scan *EmScan2*. Instead of *EmScan2* another measurement may be picked that may also be in the data container *MultiScans*. Double click on the container to open it. In order to return to the original list of files and containers, it is necessary click on the  icon that will have opened up on the top right of the dialogue box.



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.

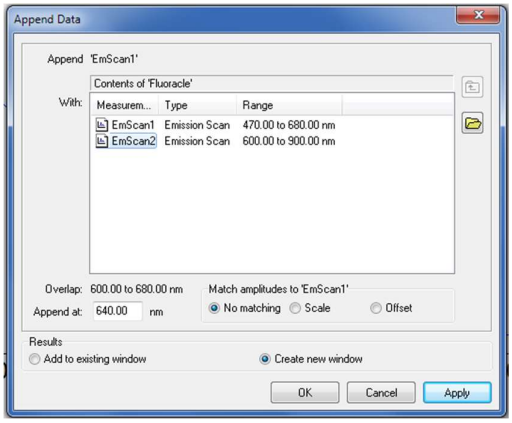
By clicking the *Apply* button the numerical operation will be processed and the new data will be displayed. The dialogue box will remain open for further data adding. Selecting *OK*, in contrast, will perform as with *Apply*, but will automatically close the dialogue box.

9.2. Append

The *Append* operation 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 9.1 to 9.2, 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 9.1. The difference is additional information and select options that are given beneath the list of data.

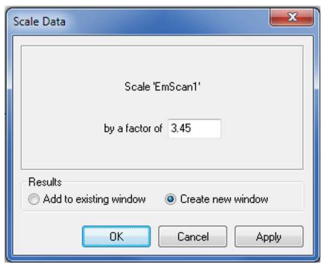


If the selected file fulfils the conditions that are required to append it to the original file the overlap range will be displayed and the option will be given to select a wavelength within the overlapping range (centre of the overlap as default). The selected wavelength in the *Append at* box has no 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.

9.3. 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.



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.

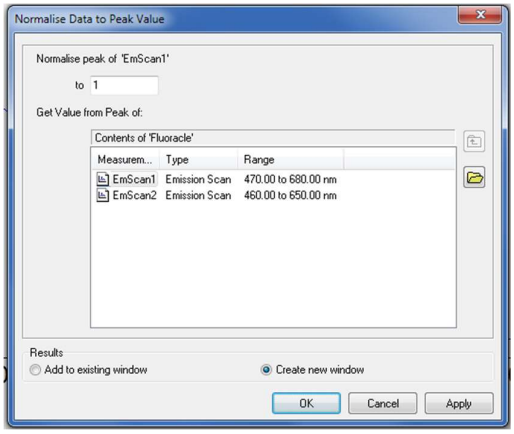
Note that a negative scaling factor is possible; this can be used to invert the data. Note that a scaling factor of zero is not possible.

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 to Peak Value

The *Normalise* > *to Peak Value…* 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. For a set of scans, the peak of the individual scans will not necessarily be at 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.



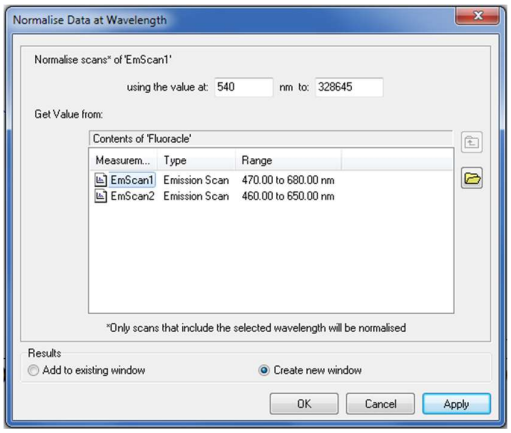
Normalisation to negative numbers will invert the data and all data will have a common minimum value. Normalisation to zero will set all curves to zero.

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 / …*)

Normalise at Wavelength

The ***Normalise*** > ***at Wavelength…*** operation scales a single measurement or a set of measurements contained in a single data window by factor/s, so that the resulting curve(s) have the same intensity value at a specified wavelength. This intensity value should be specified in the "***to***" text box.

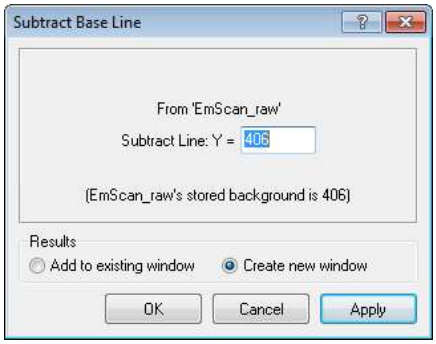
The "to"-value can be manually edited. Alternatively a curve is selected in the list of files below. As soon as a single measurement is highlighted, the intensity value at specified wavelength will be found and will be displayed in the "to" box.



Normalisation to negative numbers will invert the data. Normalisation to zero will set all curves to zero.

Subtract Baseline

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



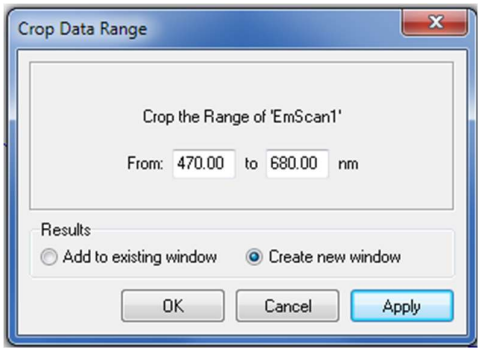
The number that is displayed in the editable field will be subtracted from the data of the active data window. Upon opening the dialogue box the editable field is normally blank. An exception is a raw single scan that was measured without automatic background subtraction. As the background had been recorded,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

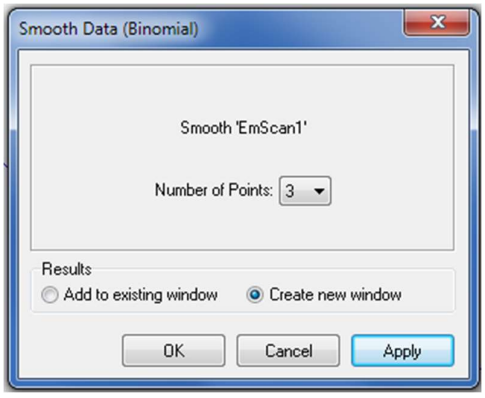
Spectral scans may be cropped, i.e. the spectral range may be cut back. This function is useful for removing unwanted data from the left or the right side of the graph, in particular if the ***Append*** function (see section 9.2) is to be used afterwards.

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.



9.4. 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).

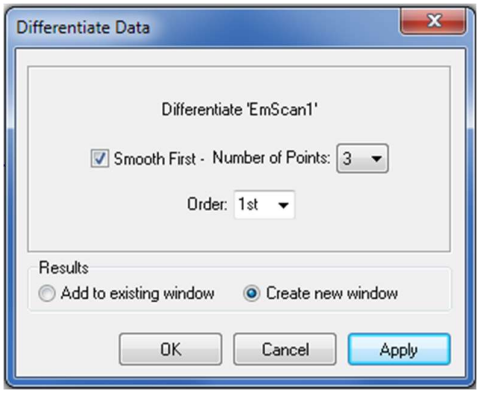


Note: excessive smoothing may alter real spectral features. Smoothing is best performed on oversampled data, i.e. curves with high number of data points for the spectral features involved.

9.5. 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.

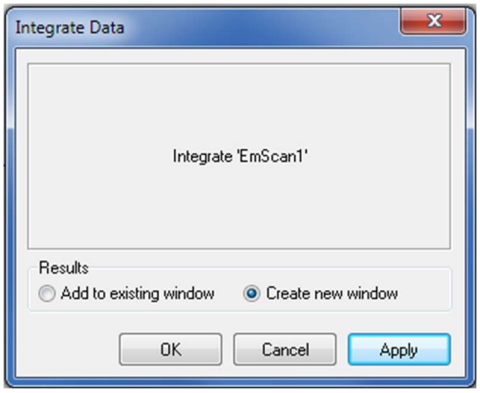


Integrate

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.

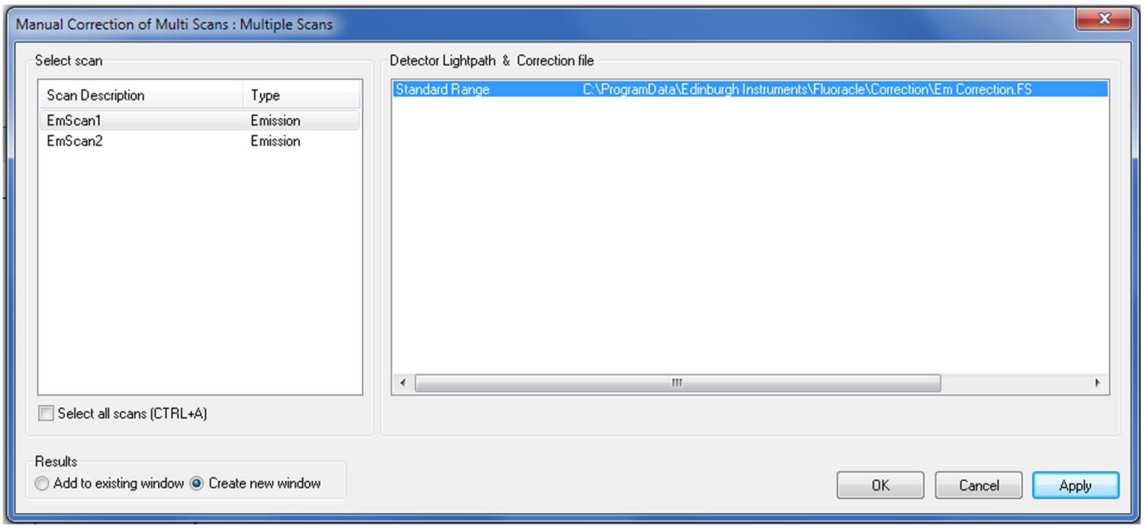


10. Data Analysis

10.1. Manual Spectral Correction

This dialogue enables you to perform spectral correction post acquisition, using the stored correction file specific to your spectrometer.

When the active window contains one or more spectral scans, the option for manual post acquisition correction will be available (*Analysis > Correction...*).



The left panel of the dialogue will show a list of all scans of the active data window. You can select one scan or a group of scans of the same type to correct.

10.2. Enabling Special Analysis Options

Use this dialogue to enable or disable the analysis options that apply to your system. Some analysis features only apply if the correct sample **module** is available. For instance, absolute quantum yield calculations will only be needed if an SC-30 integrating sphere module is available (refer to *FS5 Series Reference Guide – SC-30 Integrating Sphere*). The option for polarisation measurements analysis should be selected for analysis anisotropy measurements performed using non-standard polarisers such as dichroic sheet polarisers (Refer to *FS5 Series Reference Guide – Anisotropy*).

10.3. Chromaticity Plots

The chromaticity of a colour is the hue (the property that distinguishes red from purple) and saturation or purity (the property that distinguishes red from pink). It is used to quantify the perception of colour.

The FLUORACLE software can produce chromaticity plots according to CIE 1931 and CIE 1976. CIE: Commission Internationale de l'Eclairage – International Commission on Illumination.

The human eye has photoreceptors called cone cells for medium to high brightness colour vision. The Cone cells have sensitivity peaks at blue (420 nm – 440 nm), green (530 nm – 540 nm) and red (560 nm – 580 nm). All perceived colours can be translated into differing amounts of three primary colours corresponding to the cone sensitivities. The different levels of stimulus to the three types of

cone cell, or the amount of each primary colour, give three parameters which can describe any perceived colour, these are the tristimulus values, X, Y and Z.

The formulas for the calculation of the tristimulus values from the measured spectrum and the three standard observer functions are given in Appendix *Formulas and Definitions*, equations *1 - 3*. For the calculation of the CIE 1931 coordinates, x and y, refer to equations *4* and *5*. For the calculation of the CIE 1976 coordinates, u' and v', refer to equations *6* and *7*.

Procedure 16: To generate a chromaticity plot

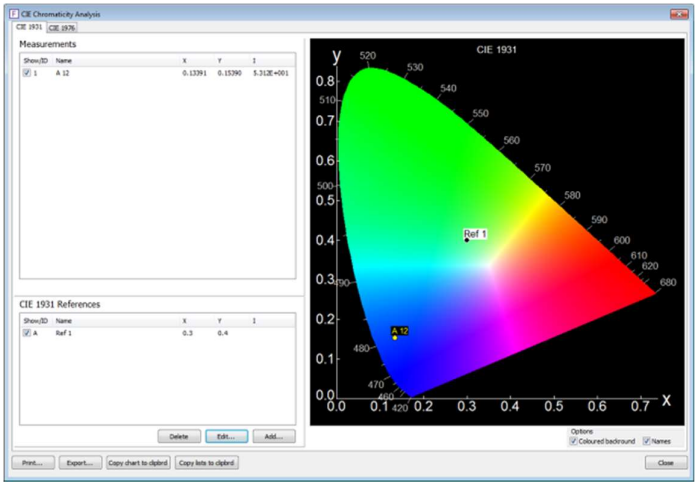
16-1 Enable chromaticity calculations in analysis options. *Options > Analysis Options > Enable CIE Chromaticity Calculation.*

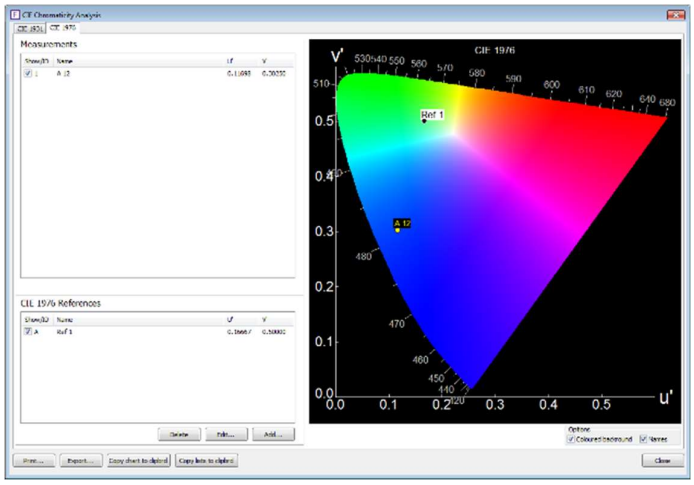
16-2 Open the emission scans on which to perform the analysis. Perform the calculations in the analysis menu; *Analysis > Chromaticity*. A warning message will be displayed if the scan(s) are not emission scans.

Scans can be measured with any step size. The colour-matching functions are used to interpolate any further data required.

16-3 Using the two tabs on the top of the Chromaticity window, choose between the CIE 1931 and CIE 1976 standard.

16-4 You can set reference points. Use the *Delete, Edit, Add* buttons on the bottom of the screen for this purpose.





10.4. Absorbance

The absorption spectrum can be calculated using data recorded with the transmission detector. Typically this is made with solvents using sample modules SC-05, SC-20, SC-25, SC-80.

Two measurements are required:

(1) a transmission scan of your sample

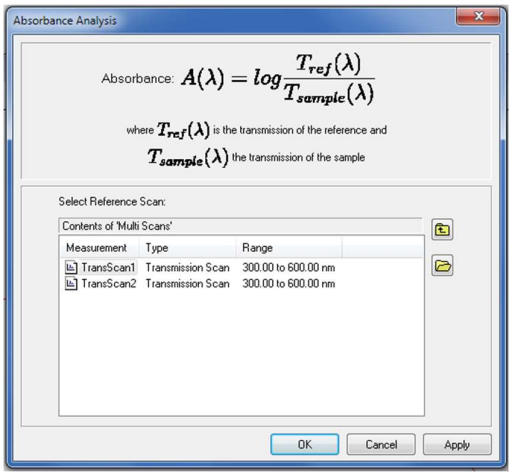
(2) a transmission scan of the cuvette filled with the solvent only (blank)

Section 6.4 on page 41 describes how to make these measurements.

Procedure 17: To calculate the absorption spectrum

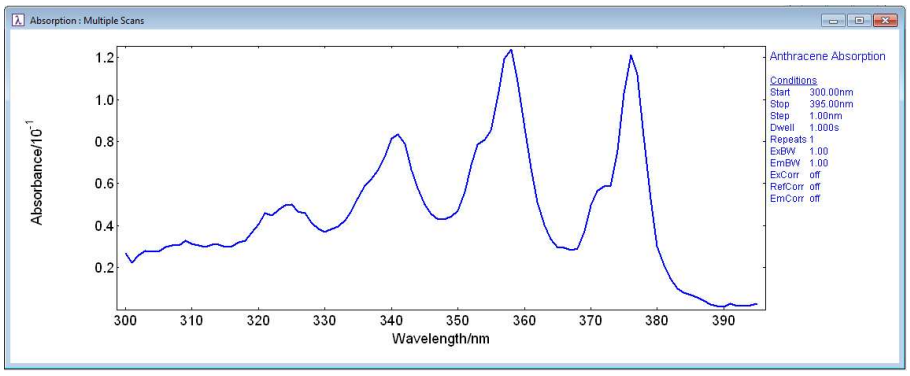
17-1 Enable your active data window contains two or more transmission scans, one of which being the blank measurement.

17-2 Open the dialogue *Analysis* > *Absorbance*…



17-3 In the list of scans presented, select the reference scan (or blank measurement)

17-4 Use *Apply* or *OK* to calculate the absorption measurement(s), they will be shown in a new data window. *Apply* will keep the dialogue box open, *OK* will immediately close the dialogue.



11. Maintenance

11.1. General Maintenance

The FS5 requires a minimum of regular maintenance:

- Ensure that ventilation holes are not blocked by any obstructions.
- The covers and the sample module may be wiped with a damp cloth. A small amount of soap may be used. The paint of the covers is epoxy based and should be resistant to alcohol based solvents. The sample module plastic must be protected from acetone based solvents. This will cause markings.
- The quartz windows inside the sample compartment may be cleaned with a soft, dry and clean cloth. Fingerprints may be removed with a small amount of ethanol or isopropanol.

11.2. Xenon Lamp Replacement

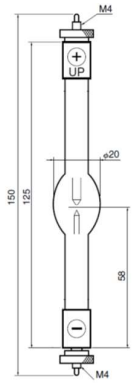
When is it time to replace the xenon lamp?

The xenon lamp has an average life of 3000 hours. With regular use of the instrument the xenon lamp may have to be changed approximately once every 2 years.

It is recommended to measure the water Raman spectrum regularly (for example once every month). The peak signal will drop over time. With a new lamp the peak of the water Raman signal at 397 nm should be ≥400,000 cps. If the signal drops below 100,000 cps then it is time to change the xenon arc lamp.

Which Xenon Lamp?

The FS5 uses 150 W ozone free xenon bulbs. The stabilised operating current is 8 A. The principle physical dimensions of the xenon arc lamp is shown in the adjacent figure. Note that the lower screw will be replaced by a different fitting.



Warning !

New xenon lamps are supplied with a protective cover. Do not remove the protective cover from the replacement lamp until you are instructed to do so.

Which tools are required?

Use a flat-bladed screw driver suitable to fix M3 and M4 slotted head screws.

Procedure 18: To replace the xenon lamp . . .

 **Caution** **Risk of Explosion**	Xenon lamps are pressurised and therefore are an explosion hazard. Wear explosion proof face shield and protective clothing when handling xenon lamps.
 **Caution** High Voltage	Xenon lamps are ignited with voltage spikes of thousands of Volts. An accidental ignition of the xenon lamp during the procedure is a hazard to life. Ensure the mains is disconnected from the instrument.
 **Caution** **Hot Surface**	The xenon lamp remains hot for approximately half an hour after operation. Do not touch the lamp or its metal parts until the lamp is fully cooled down.
 **Caution** **High Brightness, UV Radiation**	The xenon lamp is a very bright light source with a high content of UV Radiation. Never look directly at the xenon arc or reflections of the arc. Protect your eyes with UV eliminating goggles when working with the unshielded xenon lamp.

18-1 Switch the FS5 off.

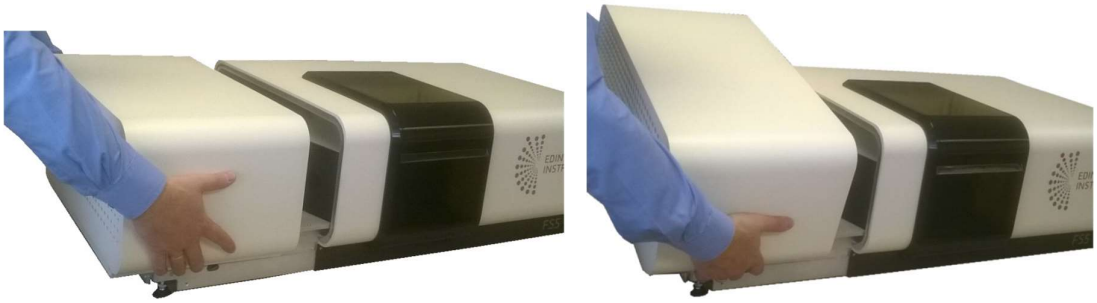
A) Be sure the FS5 and its host computer are turned off.
B) Unplug the mains connector from the FS5.
C) Unplug the USB cable from the FS5.

18-2 Remove the left hand side cover.

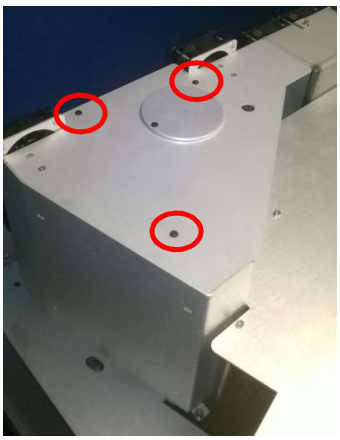
A) Remove the black trim strips on the front left and on the left hand side of the instrument (5 slotted screws).

B) Remove three black screws on the back side of the FS5, fixing the left-hand-side cover.

C) Hold the left service cover as shown in the figure, pull it about 10 cm to the left of the instrument, then tilt it (see picture below) to remove it from the instrument.



18-3 Remove the lid from the lamp housing

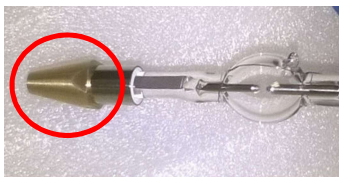


A) Remove the three slotted screws highlighted in the adjacent picture.
B) Carefully lift the lid and remove it from the lamp housing.

Be aware that the lamp housing contains delicate optics, this is the lamp AND THE REFLECTOR MIRROR. Use gloves for your own protection (explosion hazard), but also to avoid accidental fingerprints on BOTH of the two optical elements.

18-4 Removal of the xenon lamp

A) Undo the two countersunk screws highlighted in the picture.
B) Hold the top metal electrode of the lamp (use gloves!), then remove the upper plastic clamp from the lamp housing.
C) Lift the lamp out of the bottom holder and undo the cable on the upper electrode holder by unscrewing the knurled nut. (The removal of the lower electrode is required if the screw on the upper electrode is hard to remove. The removal from the lower electrode avoids mechanical stress to the lamp quartz envelope while unscrewing the top electrode cable.)
D) Unscrew the cone-shaped lower electrode holder. You may need a spanner for this.



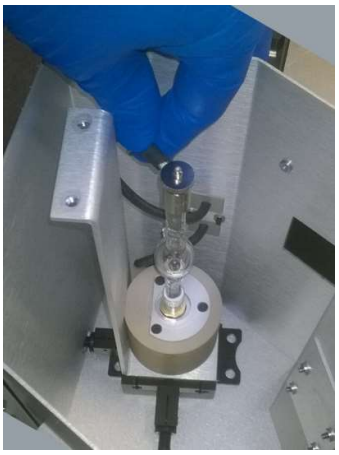
18-5 Safely dispose of the old lamp. Follow local regulations

18-6 Preparing the new lamp

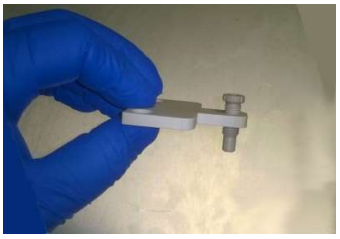
A) Remove the new lamp from the original packaging.
B) Locate the UPPER electrode, typically marked with '⊕' and/or 'UP'.
C) Fix the cone-shaped electrode holder to the LOWER electrode, see adjacent figure.
D) Remove the screw ring from the upper electrode and keep it close for the next step in the procedure.

Note the thin wire across the quartz envelope of the lamp. When the lamp will be installed in the next step this thin wire must not obstruct the beam (that goes to the mirror first before it exits the lamp housing).

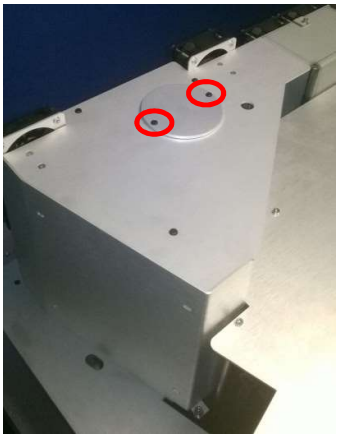
18-7 Mounting the new lamp to the lamp housing



A) Insert the cone into the cathode socket (lower electrode socket).
B) Connect the cable to the top electrode and use the screw-ring to tighten the cable. Ensure the cable does not obstruct the beam path. Also ensure the thin wire across the arc is not in the beam path.
The screw-ring must be tightened thoroughly to ensure good electrical connection; still ensure not to put physical stress onto the bulb.
C) The upper plastic clamp contains an adjuster screw for the height of the xenon arc. Ensure the height adjuster screw is in the middle position, as shown in the adjacent picture.
D) Using the two countersunk M4 screws, fix the plastic clamp to the lamp assembly. Ensure that the upper electrode holder of the xenon lamp sits securely in the recess of the adjuster screw.
E) Close the lid of the xenon lamp housing using the three M3 screws – refer to the picture in step 16-3.

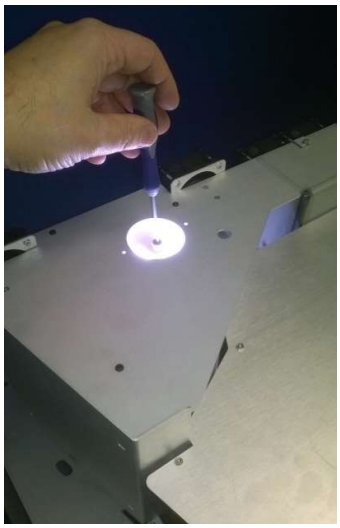


18-8 Alignment of the new xenon lamp



A) Remove the two screws from the lamp housing. With the round lid removed you will have access to the Z-adjustment of the lamp. Remember that the lamp will produce bright light with a high UV content. Use UV-eliminating goggles to protect your eyes.
B) With the left-hand-side cover of the FS5 still removed, reconnect mains and USB cables, then switch the instrument on and restart the software.
C) View the Raman signal of the standard water sample using the Signal Rate dialogue box (λ_{exc}=350 nm, λ_{em}=397 nm, $\Delta\lambda_{exc}=\Delta\lambda$em = 5 nm). Adjust X, Y and Z of the xenon lamp to obtain maximum signal.
The picture on the side shows the Z adjustment. You will find the X adjustment through a hole on the bottom front of the

FS5 and the Y adjustment on the bottom left of the instrument.
Note that each of the adjustments should be turned a maximum of two rotations clockwise and two rotations anti-clockwise.



18-9 Final refitting work

A) Close the xenon lamp hatch lid, secure the two screws.
B) Refit the left-hand-side cover cover of the FS5.
C) Refit the trim strips on the front and the left of the instrument.

12. Validating System Performance

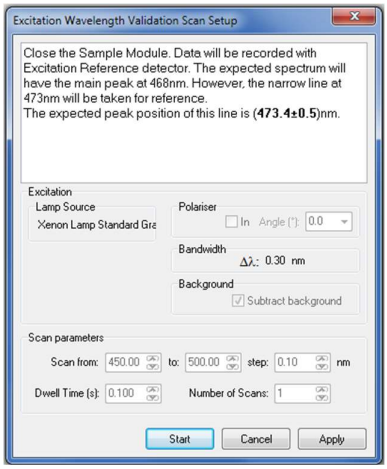
12.1. Wavelength Calibration of Excitation Path

Use the *Excitation Wavelength Validation Scan* from the *Service Scan* menu to verify that the calibration of the excitation monochromator is correct.

You can access and perform the *Excitation Wavelength Calibration Scan* at any time during your session without affecting your normal settings, such as wavelength, bandwidth, dwell time an step size.

Procedure 19: Validating the spectral calibration of the excitation beam path.

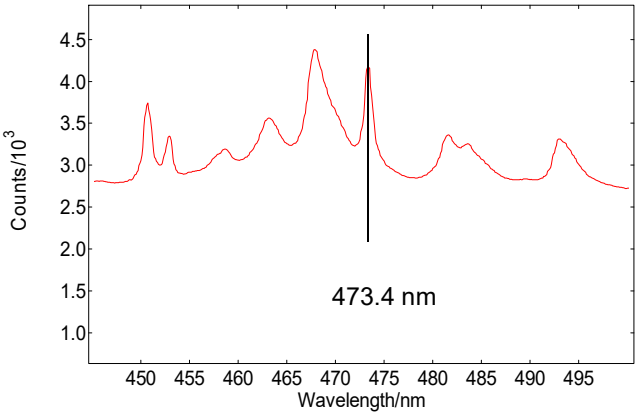
19-1 Access the *Excitation Wavelength Calibration Scan* through *File* > *Spectral Measurement* > *Service Scan* . Note that this special scan, like all service scans, is not accessible through the  button.



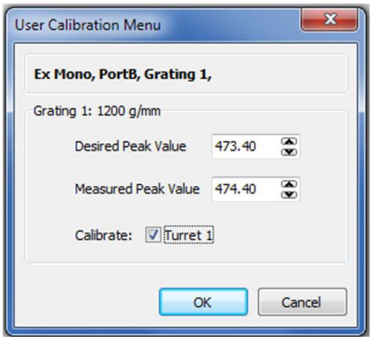
19-2 Some instructions and information is given in the upper section of the dialogue box. You will NOT be required to select settings. Instead all standard settings will be displayed only.

19-3 Start the scan: *Start*

The wavelength of the excitation beam path is correctly calibrated if the narrow line on the right hand side of the bigger but broader line is 473.4 nm ± 0.5 nm.



19-4 If the wavelength setting requires re-adjustment proceed with *Setup* > *User Calibration Setup* > *Calibrate Ex Grating*.



19-5 Enter actually measured peak value and upon pressing Ok the system will automatically re-adjust monochromator parameters.

12.2. Wavelength Calibration of Emission Path

Use the *Emission Wavelength Validation Scan* from the *Service Scan* menu to verify that the calibration of the emission monochromator is correct.

You can access and perform the *Emission Wavelength Calibration Scan* at any time during your session without affecting your normal settings, such as wavelength, bandwidth, dwell time an step size.

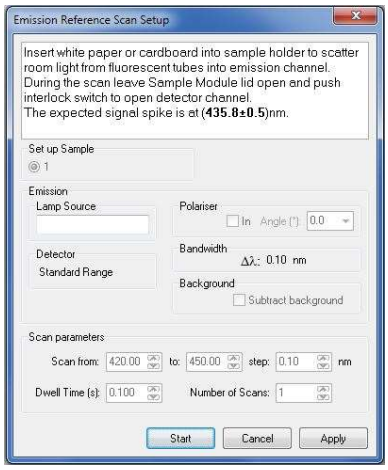
The validation of the emission wavelength calibration is based on the 435.8 nm mercury line. This narrow lines of the mercury emission spectrum are readily available in practically all laboratories, as fluorescent strip lights contain a small amount of mercury. The brought white background of the phosphor emission of these light sources is typically at longer (visible) wavelengths, so the 435.8 nm line shows very low background. You will need a piece of paper or cardboard to scatter the light into the emission channel.

Procedure 20: Validating the spectral calibration of the emission beam path.

20-1 Access the *Emission Reference Scan* through *File* > *Spectral Measurement* > *Service Scan* .

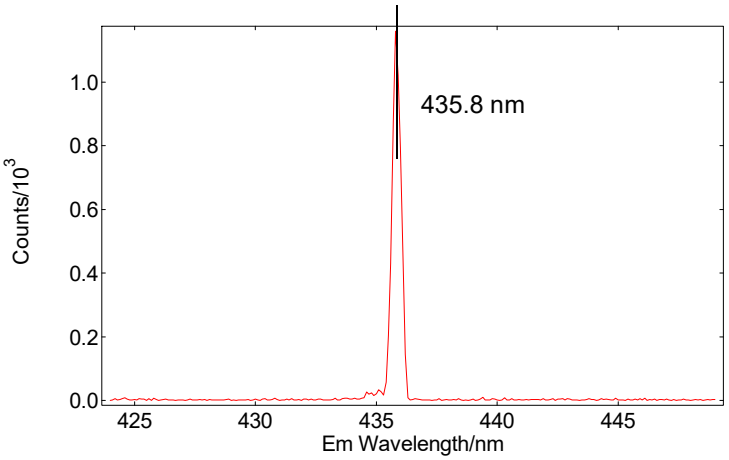
Note that this special scan, like all service scans, is not available through the  button.

20-2 Follow the instructions given in the upper section of the dialogue box. You will NOT be required to select settings. This is automatically done for you and the default standard settings will be displayed.

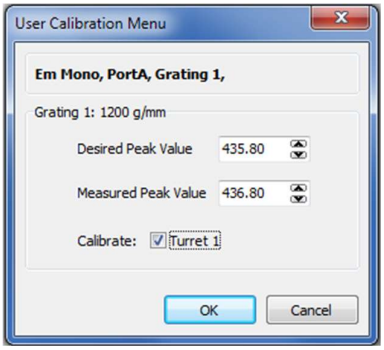


20-3 Start the scan: *Start*

20-4 The wavelength of the emission beam path is correctly calibrated if the peak is at 435.8 nm ± 0.5 nm.



20-5 If the wavelength setting requires re-adjustment proceed with *Setup > User Calibration Setup > Calibrate Em Grating*.



20-6 Enter actually measured peak value and upon pressing *Ok* the system will automatically re-adjust emission monochromator parameters.

12.3. Validation of Sensitivity

Use the *Sensitivity Validation* facility from the *Service Scan* menu verify instrument sensitivity.

You can access and perform the *Sensitivity Validation Scan* at any time during your session without affecting your normal settings, such as wavelength, bandwidth, dwell time an step size.

The standard Water Raman scan is used for validating the instrument sensitivity.

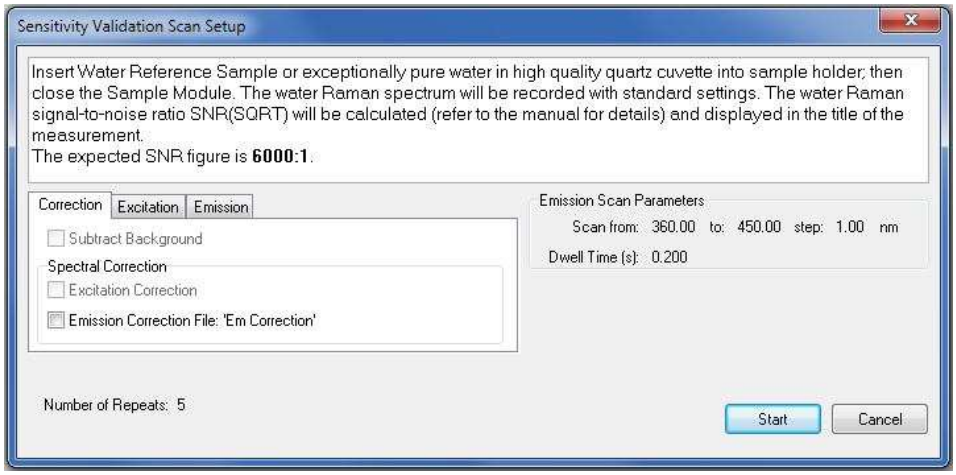
You will need a Water Reference sample or an exceptionally pure water sample in a high quality fluorescence quartz cuvette for this validation scan.

This validation procedure will produce the water Raman Spectrum, will measure the noise by means of a kinetic scan, and will finally present two different Water Raman Signal-to-Noise figures.

Procedure 21: Validating the sensitivity

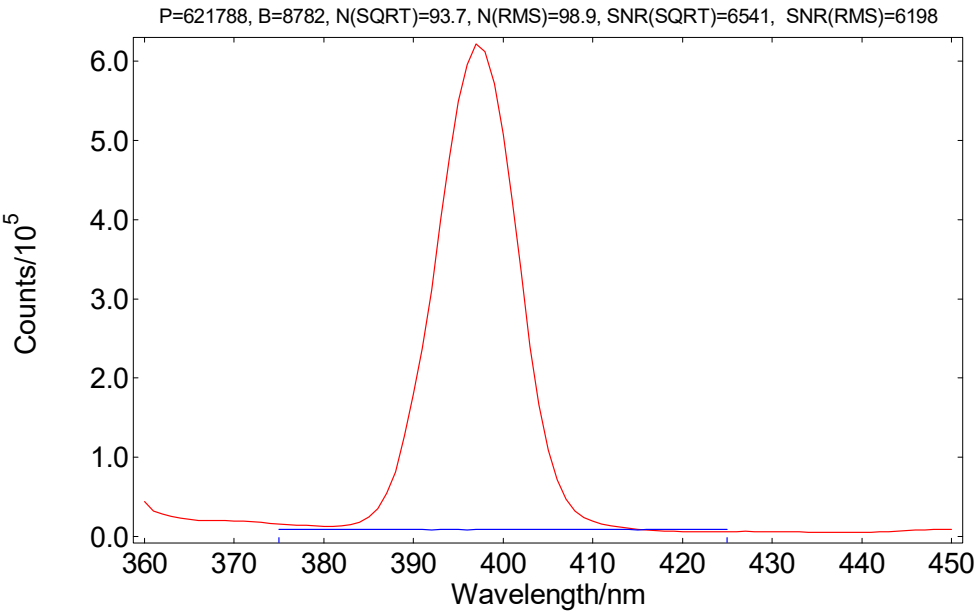
21-1 Access the *Sensitivity Validation Scan* through *File* > *Spectral Measurement* > *Service Scan* .

Note that this special scan, like all service scans, is not available through the button.



21-2 Follow the instructions in the upper section of the dialogue box. You will NOT be required to select settings. This is automatically done for you and the default standard settings will be displayed.

21-3 Start the scan: *Start*



The values presented at the top of the graph are:

P	Peak value of the Raman scan (red scan in above picture)
B	Background value = last data point of Raman scan at 450nm
N(SQRT)	Theoretical noise level, calculated using N(SQRT)= $\sqrt{B}$
N(RMS)	Experimental noise level, using 50 data points recorded at 450nm (blue scan in graph above) and calculated using the RMS formula.
SNR(SQRT)	Water Raman Signal-to-Noise Ratio, calculated using $SNR(SQRT)= \frac{P - B}{\sqrt{B}}$
SNR(RMS)	Water Raman Signal-to-Noise Ratio, calculated using $SNR(RMS)= \frac{P - B}{RMS}$

Edinburgh Instruments uses the SNR(SQRT) value for assessment of the instrument's sensitivity.

The sensitivity of the instrument is verified when the peak of the Raman scan exceeds 400,000counts and the signal-to-noise ratio SNR(SQRT) is greater than 6000 (6000:1).

13. Technical Specifications

Optics:	all-reflective for a wavelength independent focus with high brightness (small focus) at the sample
Source:	150 W ozone free xenon lamp in sealed excitation path for best UV performance
Monochromators:	Czerny-Turner design with plane gratings for accurate focus at all wavelength and minimum stray light
Spectral Coverage – Excitation:	230 nm – 1000 nm (short wavelength limited by ozone free xenon lamp)
Spectral Coverage – Emission:	230 nm – 870 nm (long wavelengths limited by detector responsivity)
Bandpass – Excitation/Emission:	0* to 30 nm, continuously adjustable
Wavelength Accuracy – Excitation/Emission:	± 0.5 nm
Scan Speed – Excitation/Emission:	100 nm/s
Integration Time:	1 ms – 200 s
Emission Detector:	photomultiplier R928P, spectral coverage 200 nm – 870 nm, cooled and stabilised
Reference Detector:	UV enhanced silicon photodiode
Transmission Detector:	UV enhanced silicon photodiode
Water Raman Signal:	400,000 cps at 397 nm emission, excitation 350 nm, 5 nm bandpass, 1 s integration time
Signal-Noise Ratio of Water Raman Signal:	6000:1
Dimensions:	104 cm (w) x 61 cm (d) x 35 cm (h)
Weight:	55 kg

* Resolution limit is 0.3 nm

Formulas and Definitions

Definitions of Chromaticity	
Tristimulus functions	$$X = \int_0^\infty I(\lambda)\,\overline{x}(\lambda)\,d\lambda \quad (1)$$ $$Y = \int_0^\infty I(\lambda)\,\overline{y}(\lambda)\,d\lambda \quad (2)$$ $$Z = \int_0^\infty I(\lambda)\,\overline{z}(\lambda)\,d\lambda \quad (3)$$ $I(\lambda)$ – measured spectrum $x(\lambda)$ – function of the blue sensitivity of the human eye $y(\lambda)$ – function of the green sensitivity of the human eye $z(\lambda)$ – function of the red sensitivity of the human eye
CIE1931 Chromaticity functions x, y	$$x = \frac{X}{X+Y+Z} \quad (4)$$ $$y = \frac{Y}{X+Y+Z} \quad (5)$$
CIE 1976 Chromaticity functions u′, v′	$$u' = \frac{4X}{X+15Y+3Z} \quad (6)$$ $$v' = \frac{9Y}{X+15Y+3Z} \quad (7)$$

Calculation of the Absorption Spectrum	
Absorbance	$$A(\lambda) = \log_{10}\left(\frac{T_{Ref}(\lambda)}{T_{Sam}(\lambda)}\right) \quad (8)$$ $T_{Ref}(\lambda)$ – transmission scan of the blank $T_{Ref}(\lambda)$ – transmission scan of the sample

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