Edinburgh Instruments FLS980 User's Guide

Version 2.02

Edited by Dr. Hartmut G. Hedderich

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The following guide describes the use of the Edinburgh FLS980 Fluorometer. The guide is intended to assist instrument users after the initial training session.

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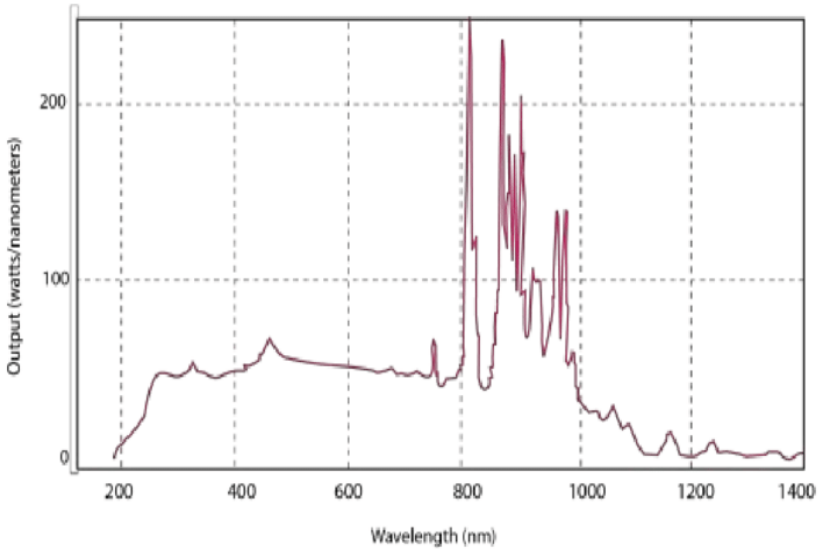
The Edinburgh FLS980 fluorometer is a modular spectrometer that can be utilized to measure fluorescence spectra of liquids and solids. With the insertion of the integration sphere into the sample chamber, quantum yields of liquids can also be obtained.

1. Hardware

The following section describes the FLS980 standard components:

- A 450W continuous xenon arc lamp which produces "white" light composed of a continuum superimposed by Xe excitation lines. The optimal spectral range extends from below 250nm to more than 1000nm.



The Xe arc lamp in the fluorometer is an ozone-free bulb!

- The **excitation monochromator** is a double-grating monochromator which contains 2 gratings. The gratings are:

A.	400nm blaze	1200 grooves/mm	200-1200nm range
B.	1000nm blaze	600 grooves/mm	200-1700nm range

- The **emission monochromator** contains 3 gratings:

1.	500nm blaze	1800 g/mm	200-900nm range	noise above 850nm
2.	1000nm blaze	600 g/mm	500-2100nm range	
3	2000nm blaze	300 g/mm	500-2100nm range	noise below 1100nm

- Useful combinations of the gratings for different spectral regions:

○	(A)(1)	noise below 850nm	best for UV-Vis, PMT detector
○	(A)(2)	500nm – 1700nm	best for Vis to NIR, InGaAs detector
○	(B)(2)	noise <900nm, >1900nm	good for NIR, InGaAs detector
○	(B)(3)	noise below 1100nm	best for NIR, InGaAs detector

The highlighted combination is the standard combination for the fluorometer!

- The sample chamber has accessories for liquid samples (cuvettes), solid samples (slides, thin film, crystals, powders) and for quantum yield measurements. The following sample holders are available:
  - basic cuvette holder, no controls, temperature is only measured
  - cuvette holder with stirring motor and temperature control (default)
  - solid sample setup with slide holder, crystal holder, powder holder
  - integrating sphere for quantum yield measurements

- The fluorometer uses two detectors: a PMT for the UV-Vis and an InGaAs detector for the NIR. The software switches automatically to the correct detector depending on the chosen gratings. There is also a photodiode in the sample chamber. It measures the output of the xenon lamp to calibrate the intensity and wavelength of the emission monochromator.

The users must provide their own cuvettes or powder holders. The cuvettes need to be made from far-UV quartz (also known as Spectrosil® quartz) with either a stopper or a screw cap. Screw cap cuvettes do NOT fit into the integration sphere for quantum yield measurements!

Cuvettes with flat tops are not allowed with this instrument due to serious safety problems in case of spills. Cuvettes can be purchased from Starna Cells (www.starnacells.com). I recommend the following models:

23-Q-10 Spectrosil® Far UV Quartz Windows, 170-2700nm, 3.5ml volume

53-Q-x Spectrosil® Far UV Quartz Windows, micro cells with x indicating the channel thickness in mm (x=1 to 5)

3-Q-10-GL14-C Spectrosil® Far UV Quartz Windows, with screw cap

any fluorometer sub-micro cuvettes (16.xxF-Q-10/Zxx series) where xx describes the size and volume of the cell. These cells have a flat top. Please talk to the trainer before ordering them!

For powder samples a demountable quartz cuvette is needed. ICUVETS Optical Supplies (www.icuvets.com) sells those with a wide range of volumes.

2 Startup

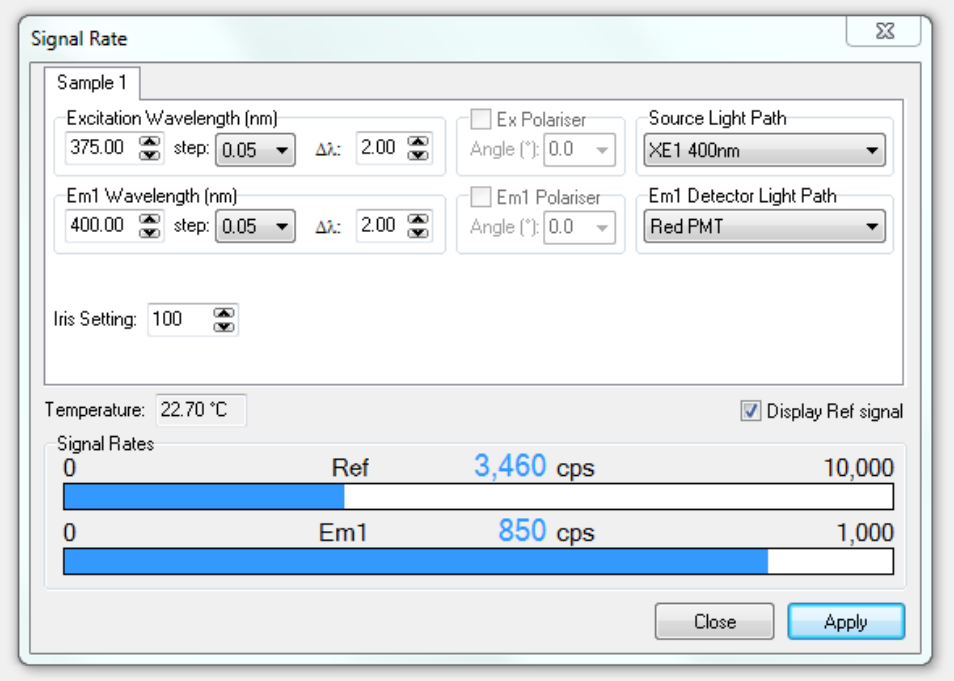
All electronics are turned on with the main power switch on the top unit. The power switch for the PMT cooling is always on! The final temperature for the PMT is -21°C. The user should wait for the detector to reach that temperature for very sensitive measurements (i.e.: low quantum yield percentage, any quantitative data).

The light source has its own power supply. The silver button at the lamp module needs to be pushed once to start the lamp.

Choose the sample holder of your choice. The cuvette holder is the default setting. The holder for solid samples is stored within the sample chamber and can be changed by the user. Carefully remove the cuvette holder from its location and unplug the connector. Place the cuvette holder in the corner of the sample chamber. The solid sample holder goes into the open holder spot. There is only one correct position for the holder. It will click into place at the correct position! Connect the tube to the alignment rod for fine alignment of the sample. Please remember to use a longpass filter for solid sample measurements to protect the PMT from source radiation.

Start the software by clicking on the F980 program icon on the screen. The software will go through a startup calibration procedure. The PIC will make some general setting changes to the software initiation file usually at the users training session. These include changes for calibration setup and filter wheel selection. This needs to be done once.

The program will start with the ***Signal Rate Monitor***.



The ***Signal Rate Monitor*** is the user's best friend. It shows all the information necessary to set up a successful experiment while also protecting the spectrometer from damage.

The window shows the excitation and emission wavelengths with their respective spectral bandwidths as well as their monochromator gratings. It also shows the signal rates for the reference and emission detectors. The iris setting optically attenuates the light incident to the sample. It should be at 100% but can be reduced to decrease signal from very strong sample emissions.

Set the excitation and emission wavelengths to the correct values for your sample. Please start with a small bandwidth (i.e.: 1nm or even 0.5nm) if you don't know how strong the fluorescence signal will be. Choose the correct monochromator settings depending on the spectral range needed for the experiment (see table above). The signal rate for the emission signal should be in the range from a few 1000 to about 800000 counts. If the counts are much higher than 800000, there is a good chance that the signal during the actual scan will be strong enough to damage the PMT! If the signal is too small, increase the bandwidth stepwise. The biggest bandwidth that can be used without losing resolution is 5nm!

Once the signal is optimized the spectral data collection can start. There are 3 choices for the instrument user:

(1) ***Emission Scan***: spectral scans, measured with fixed excitation wavelength and scanning emission wavelength.
(2) ***Excitation Scan***: spectral scans, measured with fixed emission wavelength and scanning excitation wavelength.

(3) ***Synchronous Scan***: Spectral scans, measured with excitation and emission monochromators scanning simultaneously, using a fixed offset (10nm) between excitation and emission wavelengths.

The ***Correction Scan*** option is only for administrator use! Please do not start any scans of this type since this will overwrite the correction files of the spectrometer!

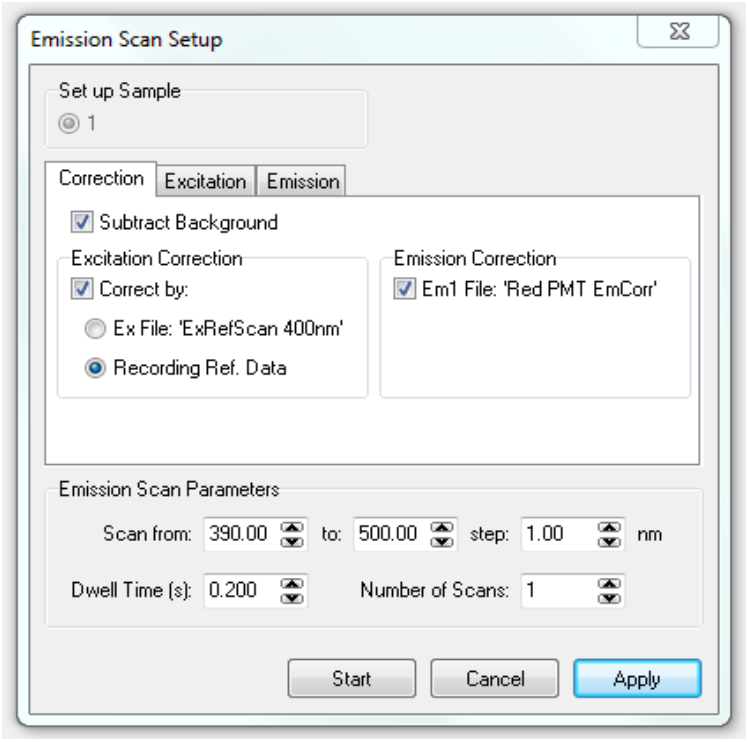
3. Measurements

The toolbar has three scan icons: 

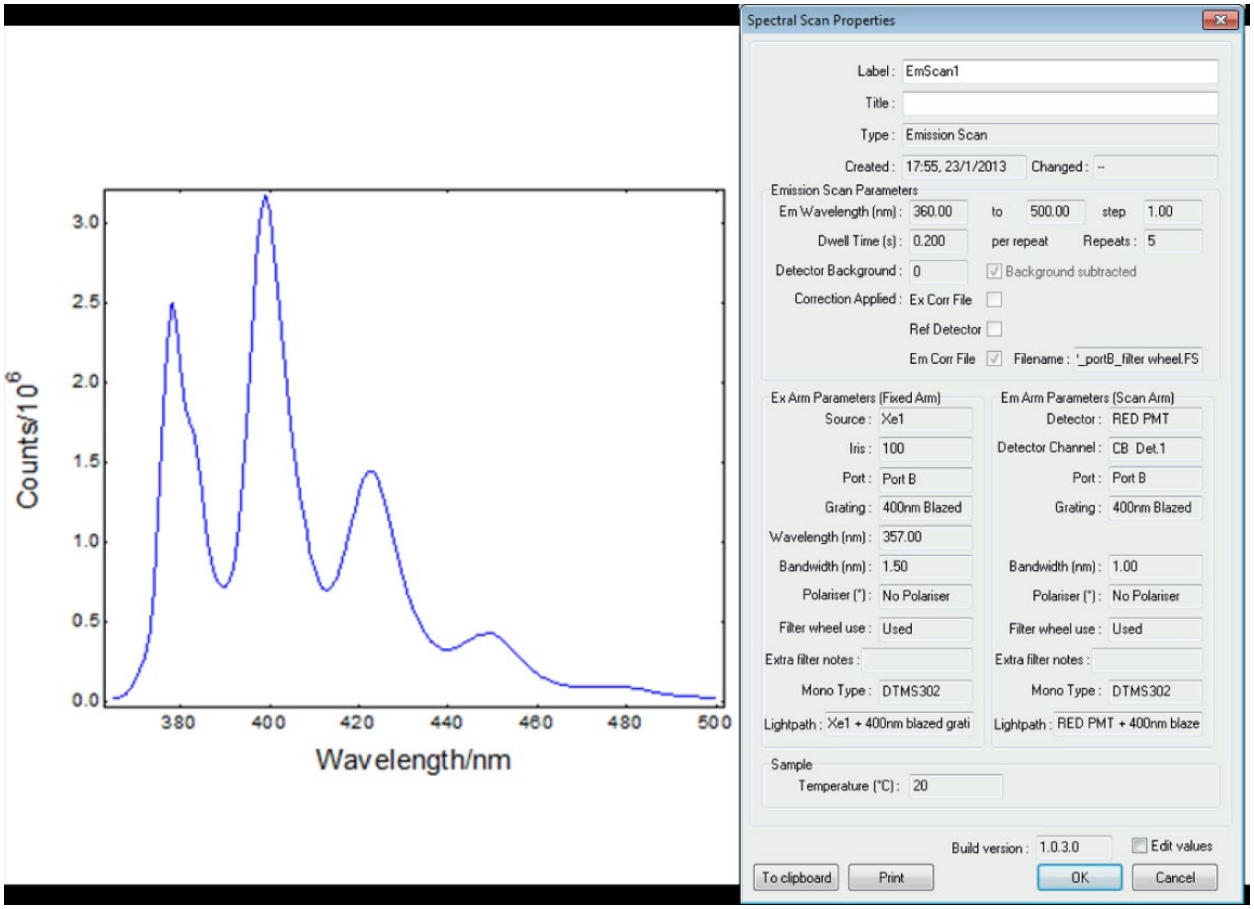
They open from left to right the Signal Rate Monitor, the spectral measurement options and the lifetime measurement options. Due to the CW light source only lifetimes in the milli second range can be measured! A planned future addition of a fast laser source will change this option to measure short lifetimes, too.

3.1. Emission Scan

Emission scans are the typical data collection scans for users. They are measured with a fixed excitation wavelength and a scanning emission wavelength. The offset between the excitation wavelength and the start of the emission spectrum should be at least 10nm to avoid excitation light hitting the detector!



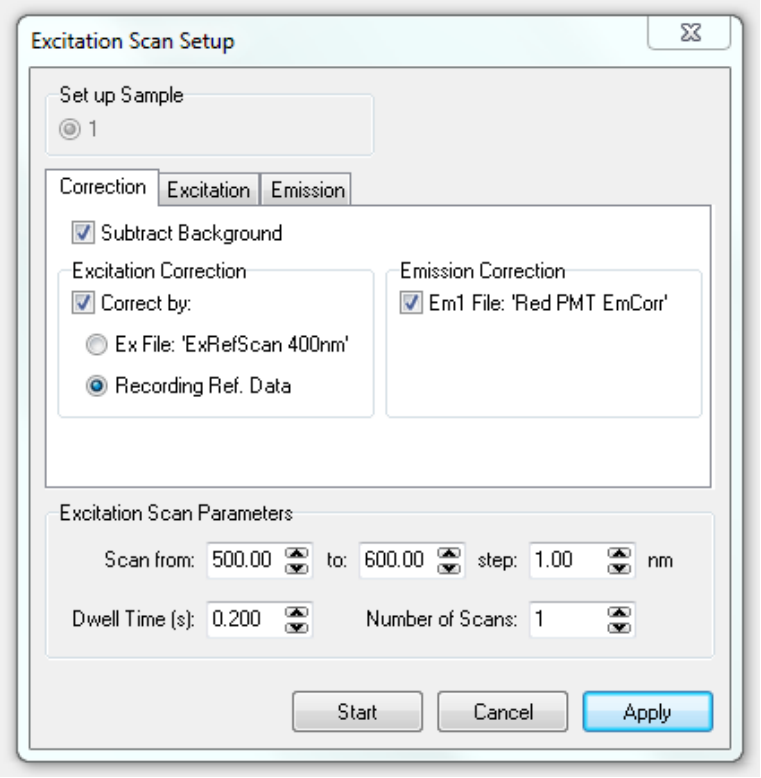
Clicking on this option will open a new window in which the user adds the necessary information for their experiment: emission range, dwell time, resolution, and repeats (co-adding spectra). The calibration of the data is done via live data from the photodiode for the excitation arm and using a calibration file for the emission arm.



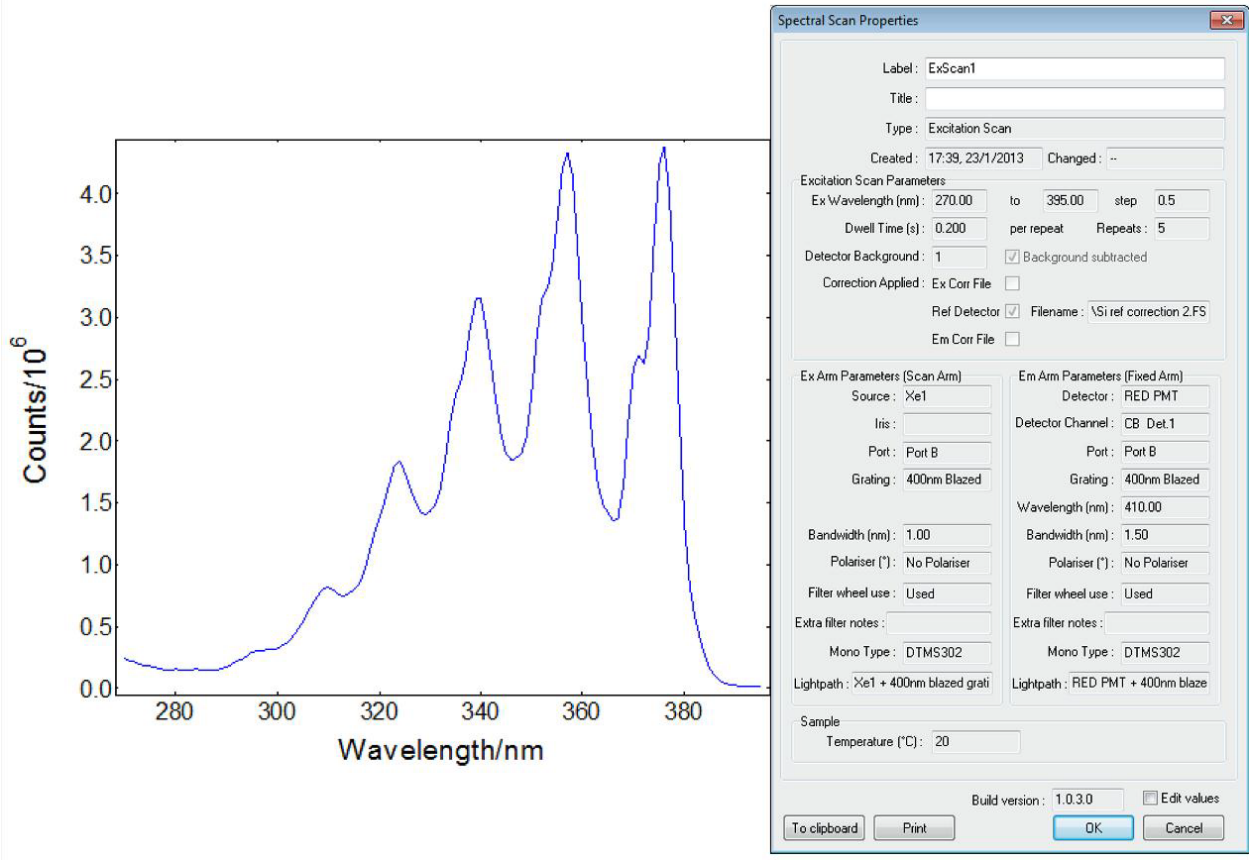
The graph depicts a typical emission scan display and the scan properties.

3.2. Excitation Scan

Excitation scans are often used to optimize the excitation wavelengths to get a better emission spectrum. It is measured with fixed emission wavelength and scanning excitation wavelength. There should always be an offset of at least 10nm between the end of the excitation scan and the emission wavelength!



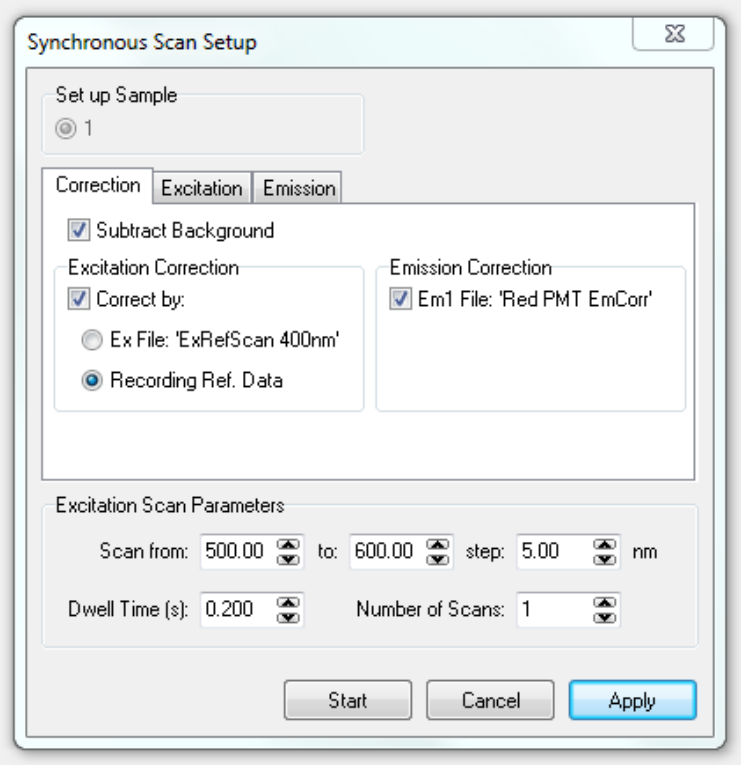
Clicking on this option will open a new window in which the user adds the necessary information for their experiment: excitation range, dwell time, resolution, and repeats (co-adding spectra). The calibration of the data is done via live data from the photodiode for the excitation arm and using a calibration file for the emission arm.



The graph depicts a typical excitation scan display and its scan properties.

3.3. Synchronous Scan

Synchronous scans can be useful if the user has no information about the spectroscopic properties of their sample. It measures a spectrum with excitation and emission monochromator scanning simultaneously, using a fixed offset between the respective wavelengths of at least 10nm.



Clicking on this option will open a new window in which the user adds the necessary information for their experiment: excitation range, dwell time, resolution, and repeats (co-adding spectra). The monochromator offset defines the emission range. The calibration of the data is done via live data from the photodiode for the excitation arm and using a calibration file for the emission arm.

3.4. More Helpful Tool Bar Icons

The tool bar contains several helpful icons for the user:

- File Icons:  to open, save and print data
- Measurement Plot Icons:  to zoom in and out of the spectrum, show cursor, add grid, plot peak position
- Measurement Container Icons:  to join scans into a single measurement window (multiple scans), split multiple scans and extract individual scans from measurement container.

3.5. Saving Data

The data can be saved with "save as" in the program specific format. To receive a standard ASCII/CSV text file the data needs to be exported in CSV format. It contains the instrument settings as well as the actual data. There are two ways to save data – either save every window individually or combine the spectra in a data container (window) and save the spectra together in one file. Important: data can only be saved together in one file if all spectra have the same resolution!

4. Shutdown of spectrometer

Save all your data files, close the program. Turn off the electronics with the on/off switch on the top module. Turn the xenon lamp off by pushing the silver push button. The lamp will go into shutdown mode, which means that the fans will run until a certain safe temperature is reached. Afterwards the lamp module will go into a count down mode (900 to 0 seconds) during which the lamp cannot be restarted. This feature protects the source since starting of a hot xenon lamp can result of an explosion of the lamp.

Please remove your cuvettes or solid samples from the sample chamber.

5. Integration Sphere and Quantum Yield Measurements

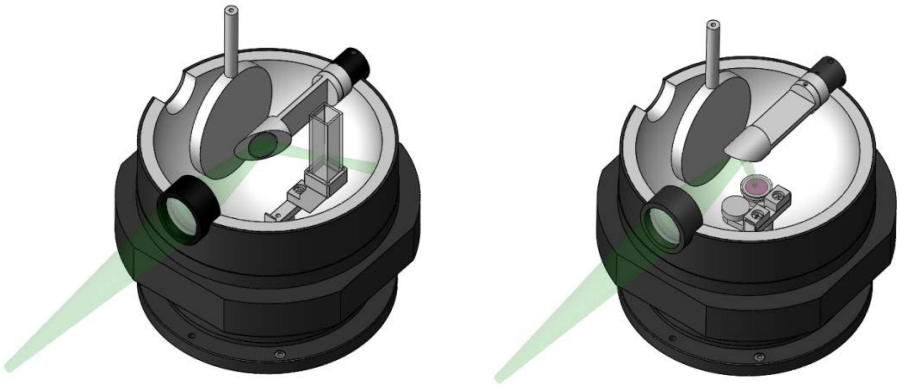
5.1. Hardware

The integrating sphere assembly, F-M01, is an accessory to the FLS980 spectrometer. It is designed for the measurement of the absolute quantum yield of liquid samples, powders, films and bulk samples.

The integration sphere consists of a 120mm inside diameter spherical cavity, which is manufactured from a PTFE like material with a reflectance of >99% over the spectral range of 400-1500nm and with >95% reflectance within 250-2500nm. The material is very delicate. The user should never touch the inner surface of the integration sphere. Extreme caution is to be applied to avoid spilling any material within the sphere. Any contamination will ruin the sphere for future measurements. Refurbishing the surface is time consuming and very expensive! If contamination happens, please inform the person-in-charge right away. Do NOT try to clean the sphere!

The integrating sphere has two ports 90° apart, one with a lens to focus the excitation beam into the sample, the other being an open aperture through which the emission or scatter can be monitored. There is a baffle near the exit port which guarantees that only diffuse scattered radiation can exit the sphere.

The sphere has an internal mirror directing the light either to the side for measuring liquid samples in a cuvette or toward the bottom for the measurement of solid samples.



Beam path, mirror and sample position for measurement of liquid samples (left) and bulk, powder, or film samples (right)

5.2 Principle of Absolute Quantum Yield Measurements

The absolute fluorescence quantum yield η, is, by definition, the ratio of the number of photons emitted to the number of photons absorbed:

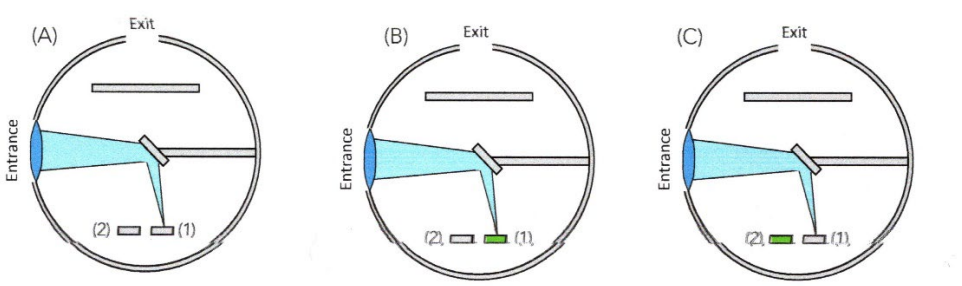
$$\eta = N^{em}/N^{ex}$$

There are two different methods to measure the absolute quantum yield: "**direct excitation**" measurements and "**direct & indirect excitation**".

"Direct Excitation" Method

*Two different measurement configurations required for **Direct Excitation** measurements:*
(A) Reference sample (solvent only) in position 1
(B) Test sample in position 1
(position 2 remains empty for both measurements)

"Direct Excitation & Indirect" Method



*Three different measurement configurations required for **Direct and Indirect Excitation** measurements:*

(A) Reference samples (blanking plugs) in both positions 1 and 2
(B) Direct: test sample in position 1 and blanking plug in position 2
(C) Indirect: blanking plug in position 1 and test sample in position 2

Each measurement gives a spectrum containing a scatter **S-region** (excitation wavelength peak) and an emission **E-region**. The ratios of the integrated areas of those scans define the absolute quantum yield.

For ***Direct Excitation*** the ratio is:

$$\Phi_{D\_Ex} = (E_B - E_A) / (S_A - S_B)$$

with A denoting the reference scan and B the sample scan.

For ***Direct & Indirect Excitation*** the ratio is:

$$\Phi_{DI\_Ex} = [S_B (E_C - E_A) - S_C (E_B - E_A)] \ / \ [(S_B - S_C) S_A]$$

with A describing the reference, B the direct measurement and C the indirect measurement scans.

The actual calculations are done by the ***Quantum Yield Wizard***. After starting the wizard, the file names of the different scans need to be selected. The next step defines the scatter range followed by the emission range. In both cases a frame is put with the mouse around the desired regions. The last step automatically calculates the quantum yield on the chosen parameters.

Using the FLS980 and the integrating sphere for fluorescence quantum yields offers great flexibility, in particular for measurements of absolute quantum yield in the near infrared spectral range or for samples with very low quantum yield or low absorbance. However, this also has the consequence that a large variety of measurement parameters of the FLS980 must be optimized for precise absolute quantum yield measurements. This requires practice, experience, careful planning and often patience, as measurements can be time-consuming!

6. Notes