FL 6500/8500 Integrating Sphere Installation Instructions

This instruction sheet describes the installation of this accessory which is used with the FL 6500/8500 Fluorescence Spectrometer.

NOTE: *Read these instructions before you install this accessory.*

Contacting PerkinElmer

Supplies, replacement parts, and accessories can be ordered directly from PerkinElmer, using the part numbers.

See our website:

http://perkinelmer.com

PerkinElmer's catalog service offers a full selection of high-quality supplies.

To place an order for supplies and many replacement parts, request a free catalog, or ask for information:

If you are located within the U.S., call toll free 1-800-762-4000, 8 a.m. to 8 p.m. EST. Your order will be shipped promptly, usually within 24 hours.

If you are located outside of the U.S., call your local PerkinElmer sales or service office.

Features

- Used for getting Quantum Yield
- Available sample type: Powder/Liquid
- Provide the Simplification and the de Mello's method

Figure 1 FL 6500/8500 Integrating Sphere [P/N:N4201017]

PerkinElmer, 710 Bridgeport Avenue,
Shelton, CT 06484-4794, U.S.A

Produced in the USA.

Dimensions and Specifications

Physical characteristic		Specification
Outline	Dimensions (mm)	130 x 267 x 170 (WDH)
Weight	Kg	2.86
Integrating Sphere	Diameter (mm)	101.6
Sample Holder size	mm	12.5 x 12.5

Connectable Cells

Description
Standard cell, 10 x 10 mm
Micro cell, 10 x 10 mm

Configuration of the Integrating Sphere

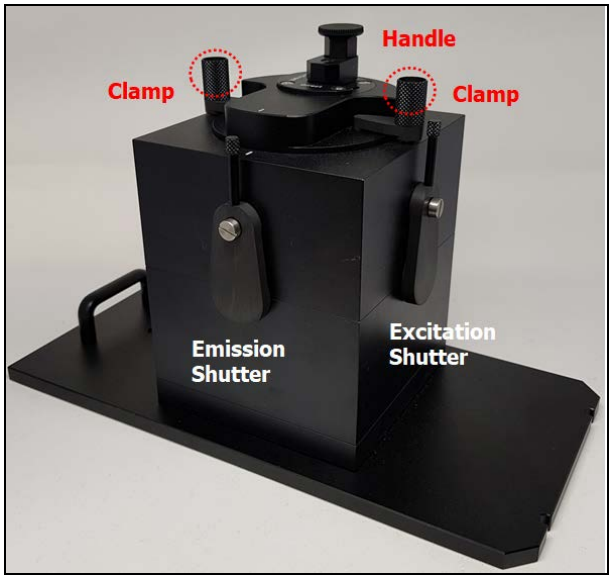


Figure 2 Integrating Sphere Configuration

Figure 3 Quartz Cell with Low Profile Stopper [P/N: N4202030]

NOTE: *When using the integrating Sphere, it is recommended to use the enclosed Quartz Cell to prevent scratching of the lid.*

Figure 4 Lid and Cell Holder Cap

Installation

1. Prepare the FL 6500/8500 Fluorescence Spectrometer to install this accessory.
2. Connect the power cord and the communication cable.
3. Loosen the accessory fixing bolt and remove the current sample accessory.

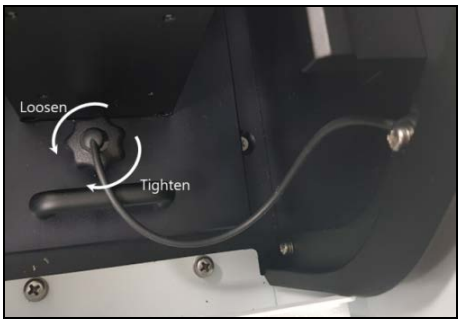


Figure 5 Loosening the Accessory Fixing Bolt

4. Pull out the cell holder by hand.

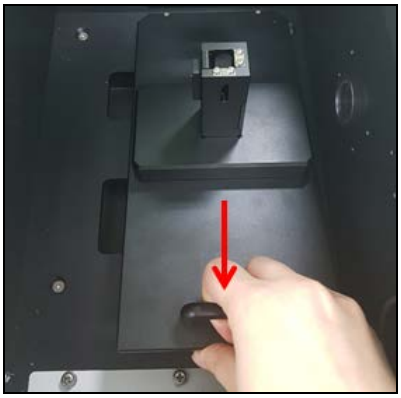


Figure 6 Pulling Out the Cell Holder

5. Prepare an Integrating Sphere. Make sure that there is no sample inside before mounting the Integrating Sphere.

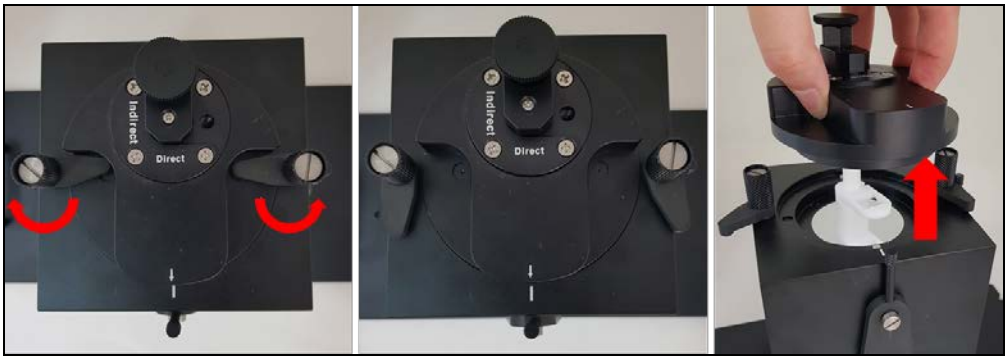


Figure 7 Checking the Sample Holder

6. After checking the pogo pin position of the sample compartment, place the Integrating Sphere into the pogo pin well.

Figure 8 Install the Accessory

7. Tighten the accessory fixing bolt.

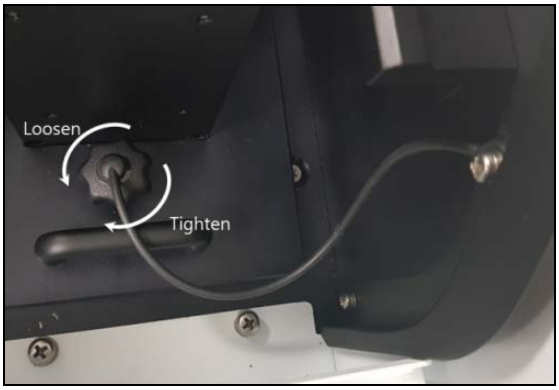


Figure 9 Tightening the Accessory Fixing Bolt

8. Remove the lid part of the Integrating Sphere and separate the cell holder cap.

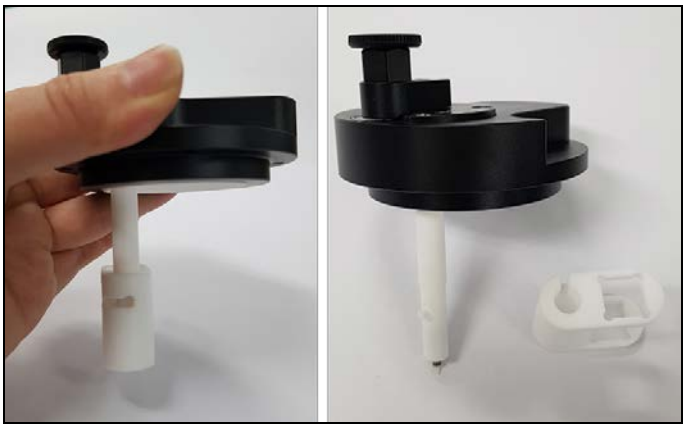


Figure 10 Remove the Lid Part

9. After loading the sample cell into the separated cell holder cap, mount it in the accessory and measure it.

Correction Spectra for Integrating Sphere

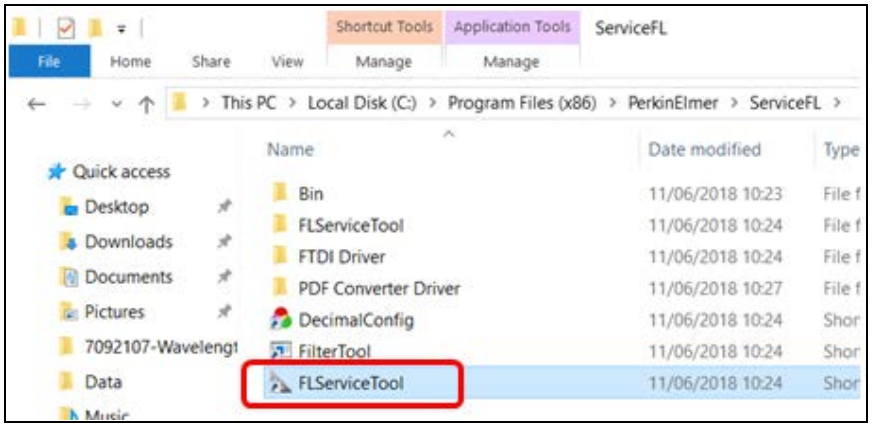
NOTE: *When using the integrating Sphere for the first time, a correction factor for the integrating sphere must be created and stored.*

The spectrum could be altered because of light scattering, or instrumental condition like lamp fluctuation. It could be corrected by Rhodamine 101 and MgO diffuser as reference material. The correction factor is then produced and saved. This factor is various among instruments (because of influence of the devices such as lamp, detector, and so on), so it can't be used with other instrument even in the same model or maker. Spectra function is used to compensate the spectra's distortion which may be caused by the differences in reflectance, absorptivity, sensitivity of each component of the system such as PMT, grating, mirror, lens, etc.

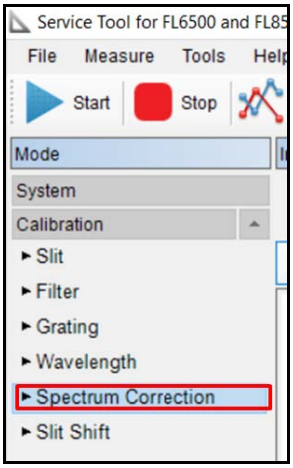
NOTE: *Correction tools (Rhodamine 101 cell, MgO diffuser) are optional accessory.*
To correct spectra, Rhodamine 101 is required as a separate purchased. The MgO diffuser is prepared by user. Prepare a solution of about 2 mg MgO in 10 mL distilled water.

1. How to measure the Excitation and Emission correction factors

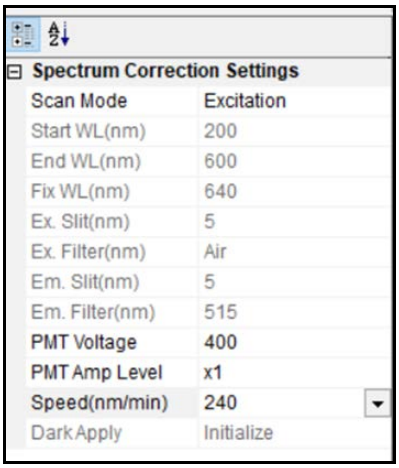
1. Execute the **FLServiceTool** software.



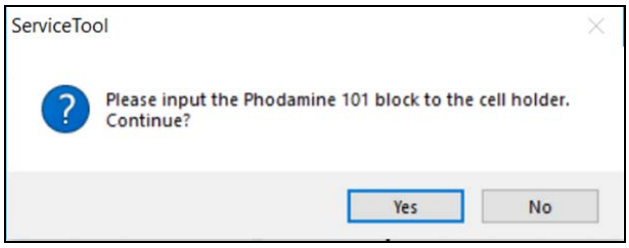
2. Select the **Calibration** tab in the Mode, click **Spectrum Correction**.



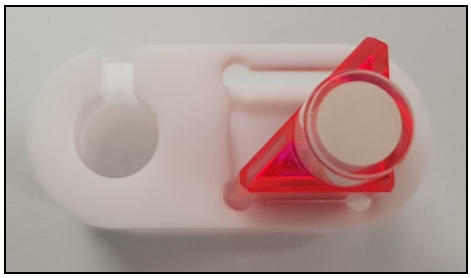
3. After setting parameters as below picture, click the **Max RFU** icon. When the Max RFU measurement is finished, click the **Start** button.

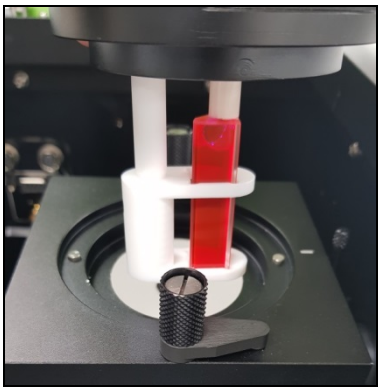


4. When the pop-up window appears, place the Rhodamine 101 triangular cell in the integrating sphere, Click **Yes** to start measurement.

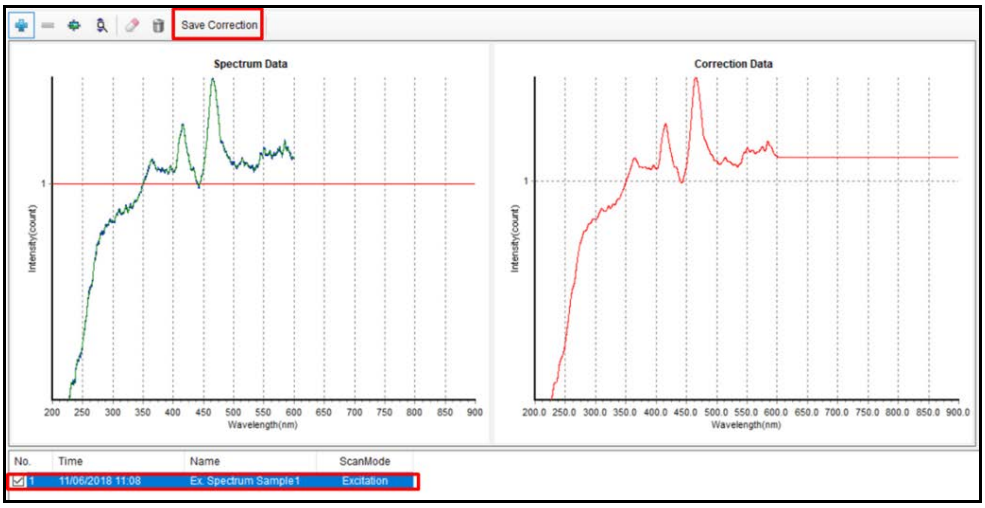


NOTE: *The mounting direction of Rhodamine 101 is as follows.*



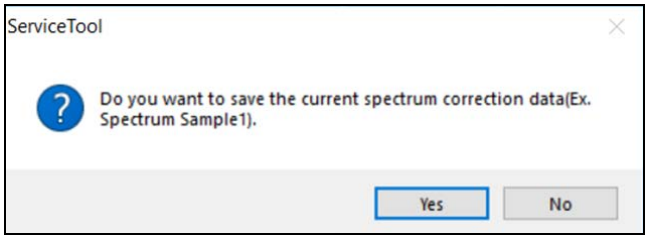


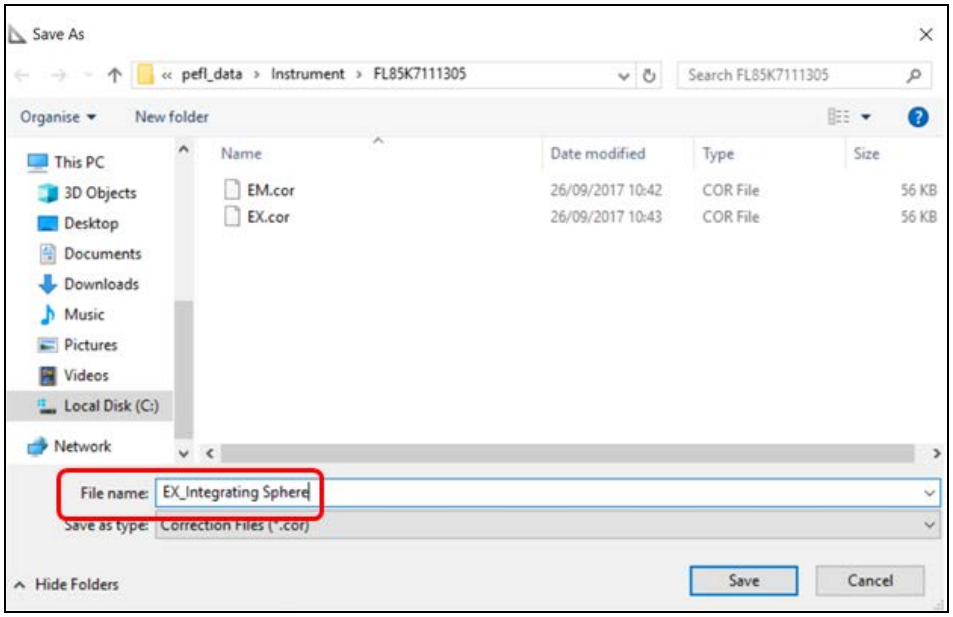
5. After the measurement is finished, select the measured graph and click the **Save Correction** button.



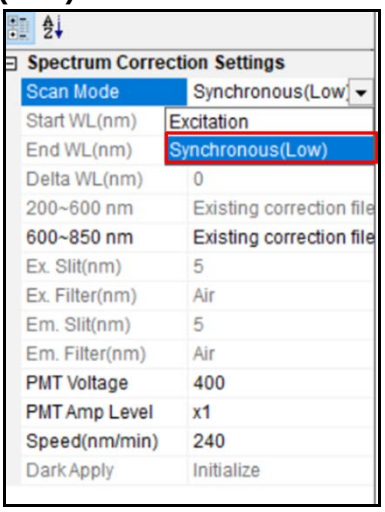
6. Click **Yes** to the Ex Correction factor for Integrating Sphere.

NOTE: *Be careful not to save the file name as an existing EX.cor.*

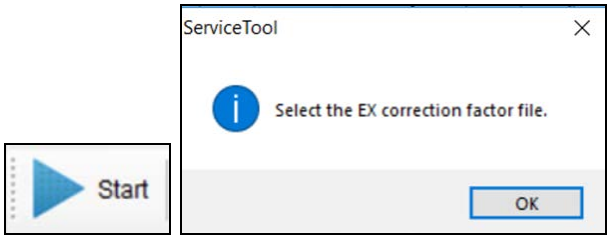




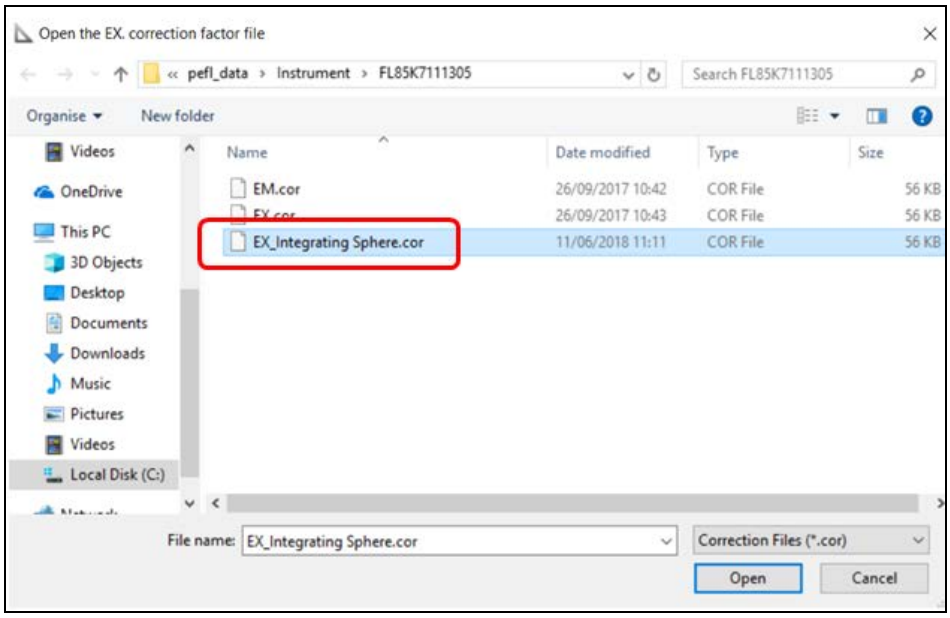
7. After saving the excitation correction factor for Integrating Sphere, select **Synchronous (Low)** in the **Scan Mode** of the **Spectrum Correction Settings** tab.



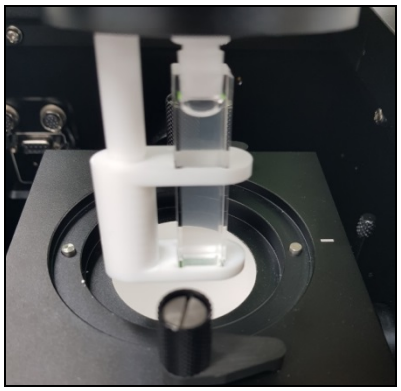
8. Click **Start**. When pop up window appears, click **OK**.

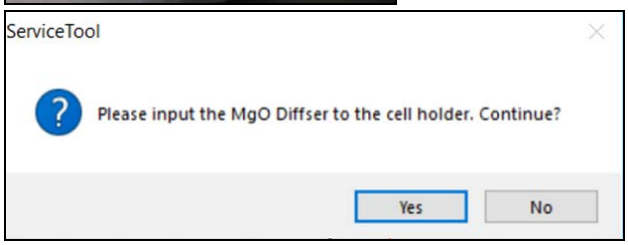


9. Select the saved excitation factor for the integrating sphere and click **Open**.

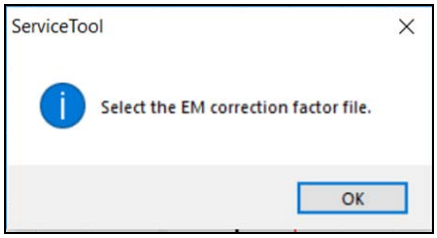


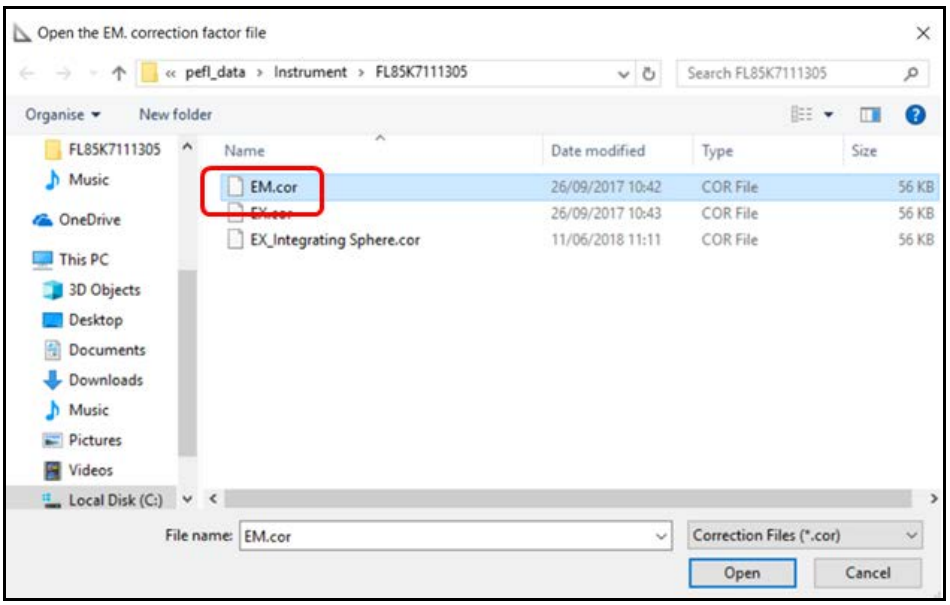
10. When the pop up window appears, after shaking the prepared MgO diffuser well and mount it. Click **Yes**.



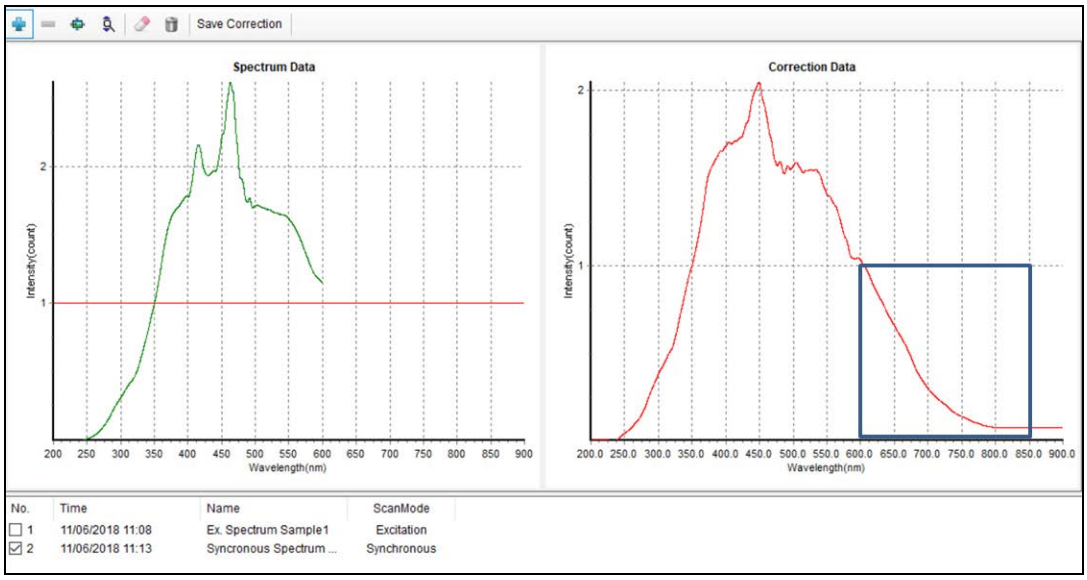


11. When the measurement is completed from 200 to 600 nm, the following pop-up window will appear. Click **OK** and select the saved **EM. cor** File in the existing device.

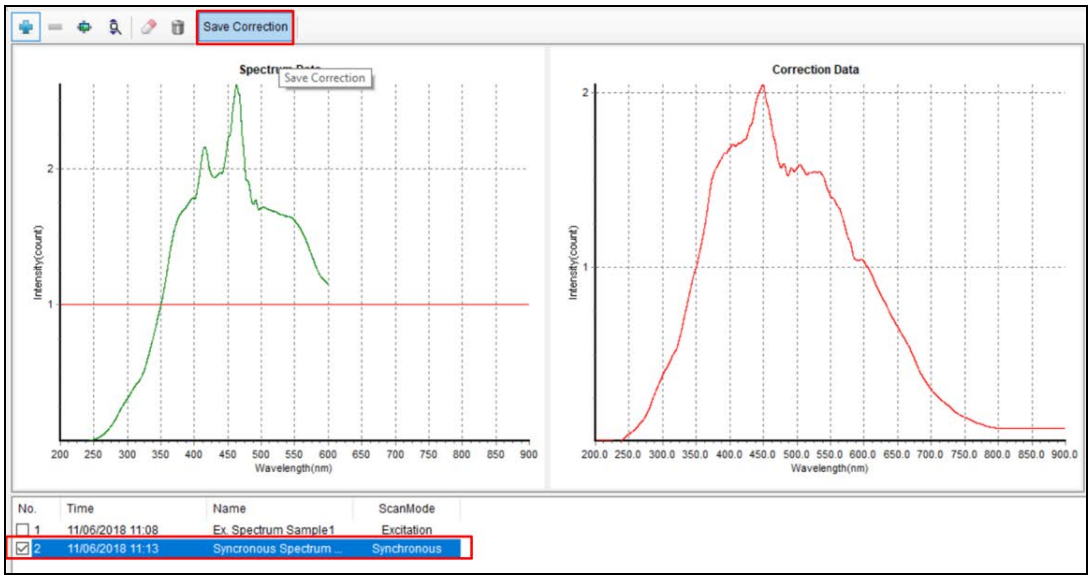




12. Confirm that data of 600 ~ 850 nm is added.

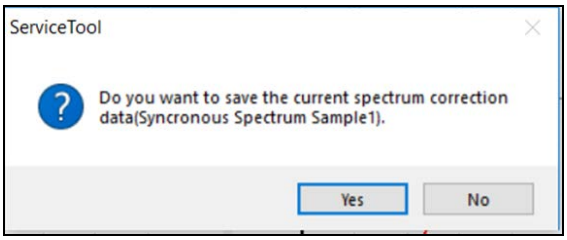


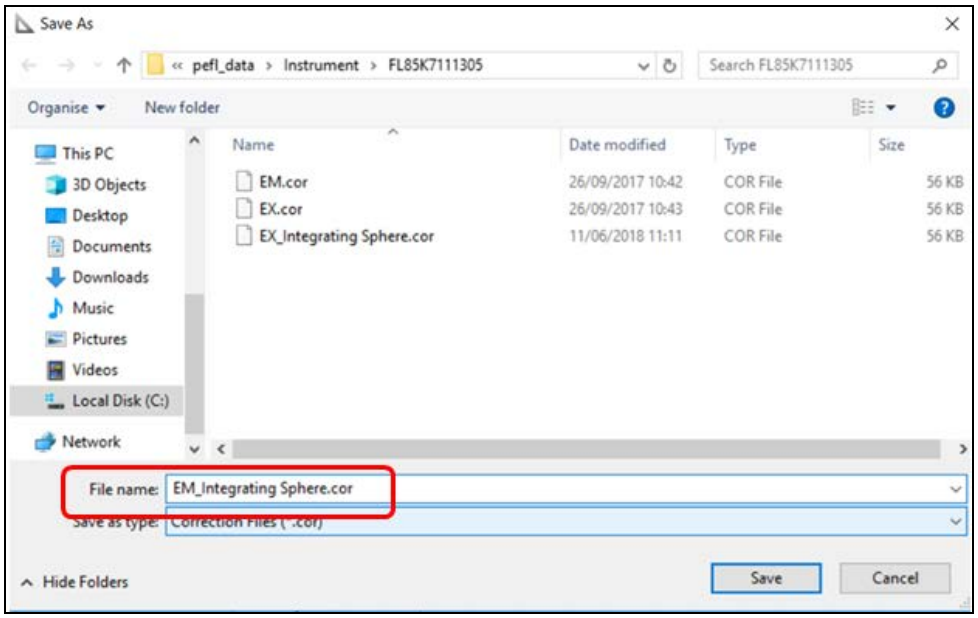
13. Select the measured Synchronous spectrum and click **Save Correction** button.



14. Click **Yes** to save the Emission Correction factor for Integrating Sphere.

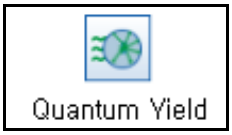
NOTE: *Be careful not to save the file name as an existing EM.cor.*



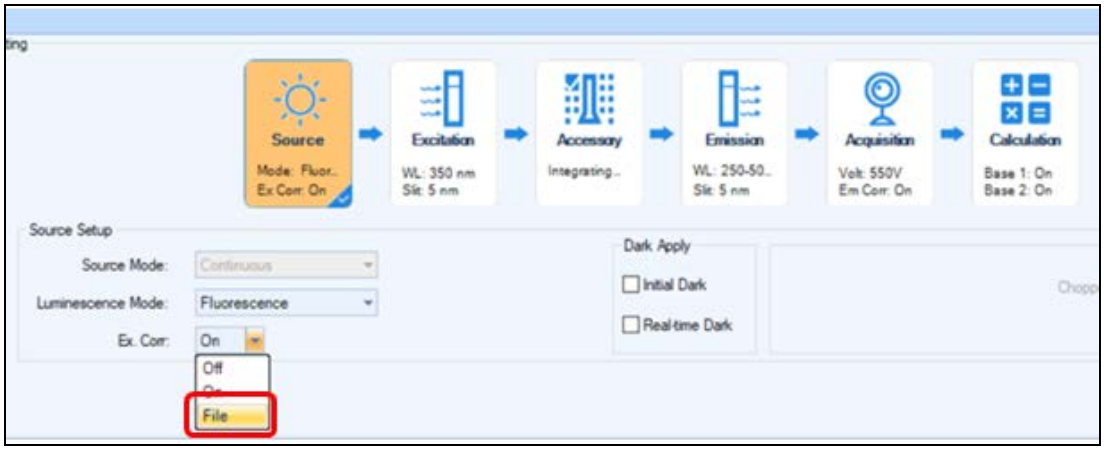


2. How to apply the correction factor in measurement

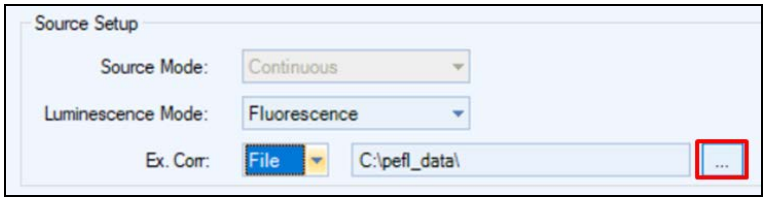
1. Execute the **Spectrum FL** software.
2. Select the **Quantum Yield** mode.

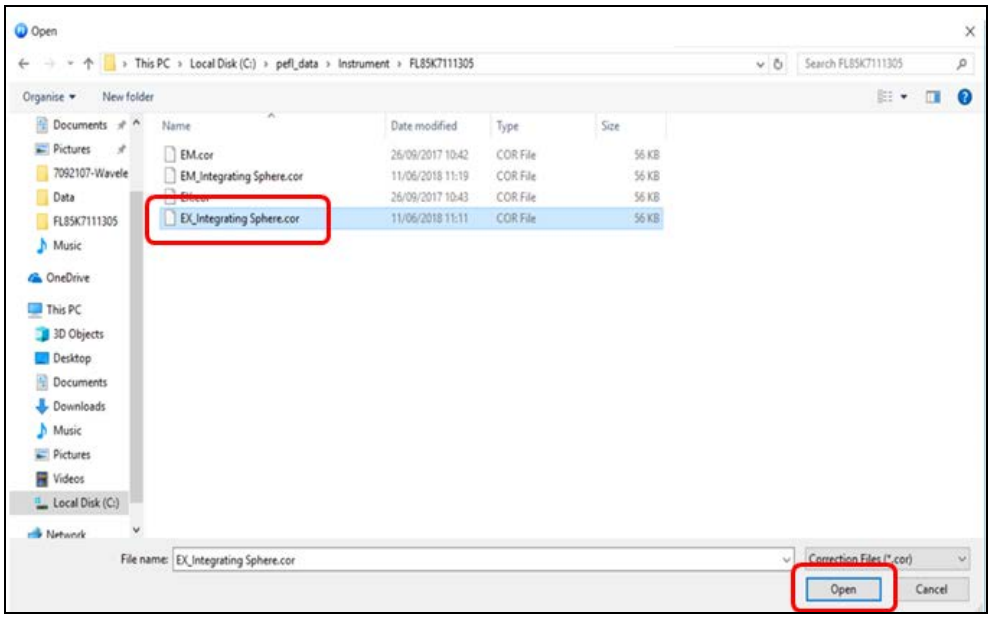


3. When setting parameters, select **File** in the **Ex.corr** tab.



4. Click the **File select** button and select the saved correction factor file. Click **OK**.





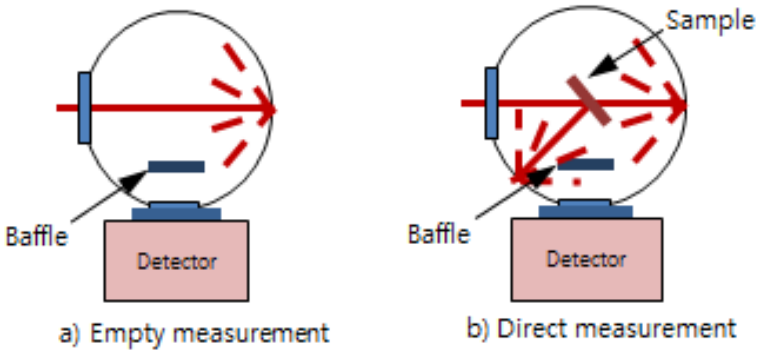
5. Apply the correction factor in the same way as in steps 3-4 for the Emission.

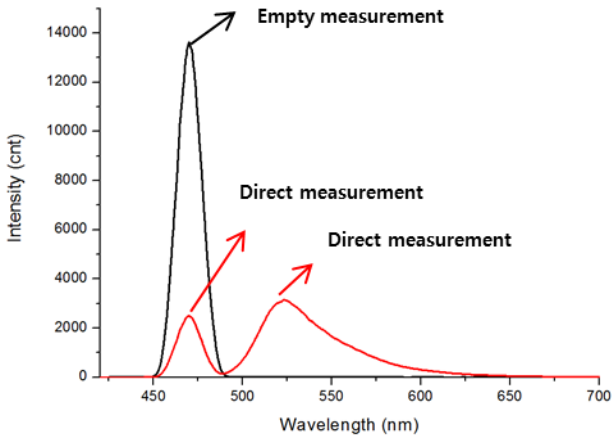
Measurement

Perform the measurements according to the purpose of the experiment.

- **Absolute mode: Simplification method**

Quantum yield = Ec/(La-Lc)



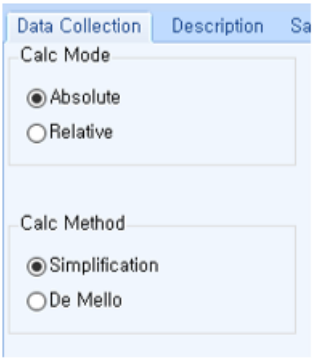


1. Double click on the **Spectrum FL** software.

2. Check the recognition of Accessory.

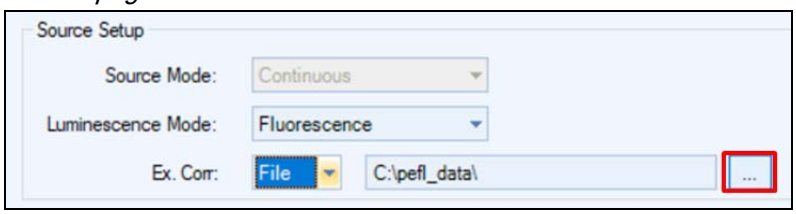
3. Click **Quantum Yield** mode.

4. Select **Absolute** Calc Mode and **Simplification** Calc Method in the **Data Collection** tab.



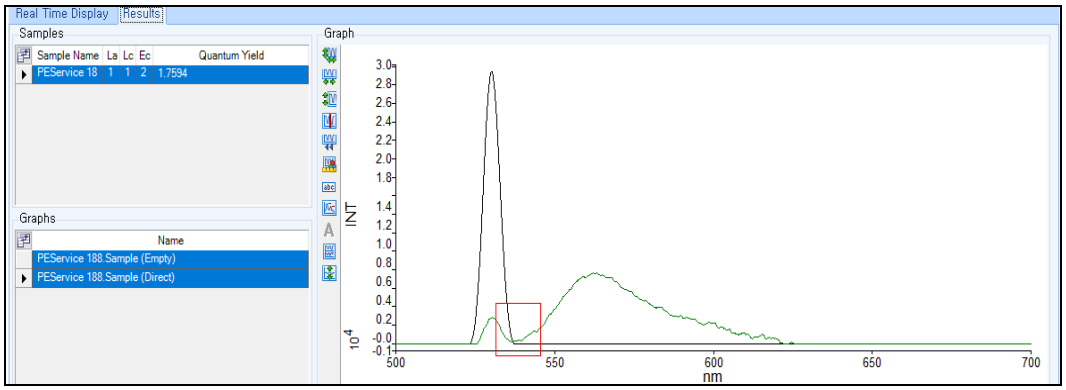
5. Set up the measurement parameters in turn.

NOTE: *In Absolute mode, select **File** from Ex. Corr and Em. Corr tab, then select the correction factor for integrating sphere. Refer to the **2. How to apply the correction factor in measurement** on the page 10.*

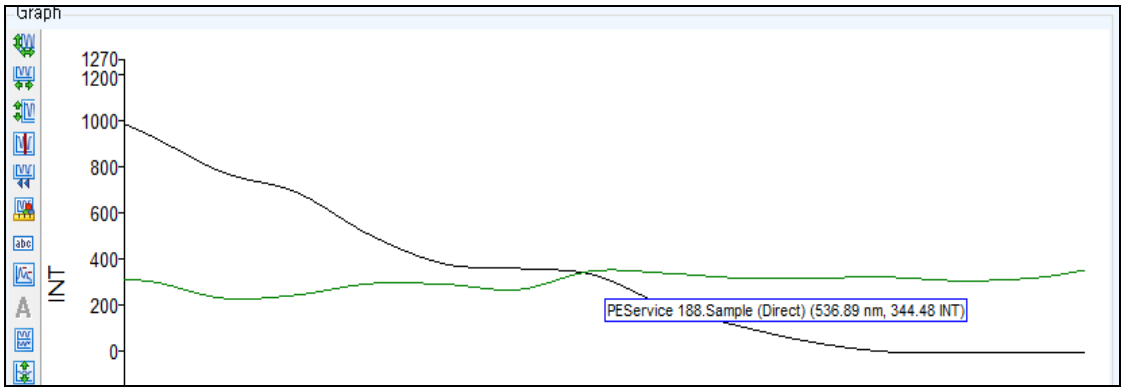


6. Click **Save** to save the method after setting up the parameters.
7. Select the **Run** icon.
8. Input the sample name and click **OK**.
9. Input Solvent or Empty cell. And click **OK**.
10. Remove Solvent or empty cell and input the Sample (Direct position) click **OK**.
11. Confirm the spectrum and results.
12. Calculate the quantum yield in the result window.

13. Enlarge the graph intersection.



14. Check the intersection value and separates the excitation and emission areas of each graph based on the intersection value.



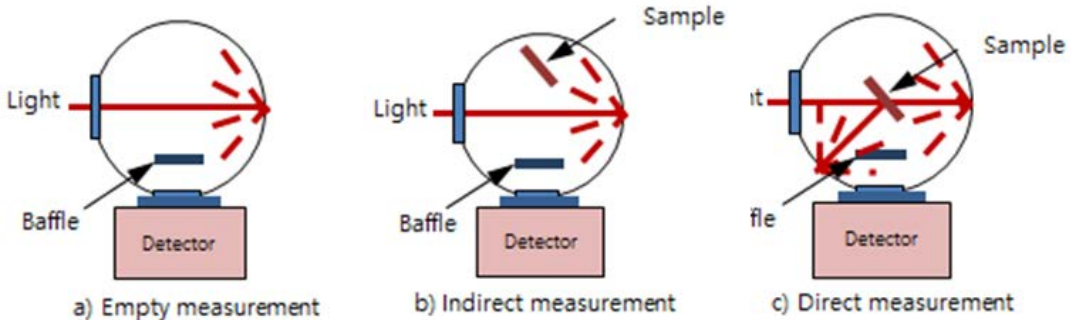
15. When the area is set, the Quantum yield value is automatically calculated and displayed.

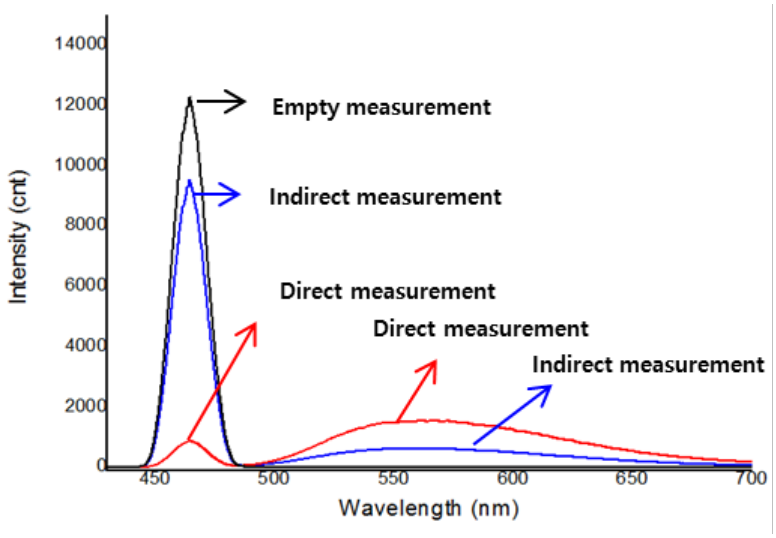
16. Save or print the data.

- **Absolute mode : De Mello method**

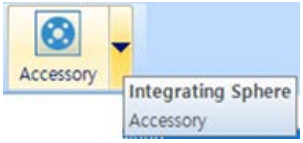
Quantum yield = Ec-[(1-A)*Eb]/La*A

A = 1-(Lc/Lb)





1. Double click on the **Spectrum FL** software and select the measurement mode.
2. Check the recognition of Accessory.



3. Click the **Quantum Yield** mode.

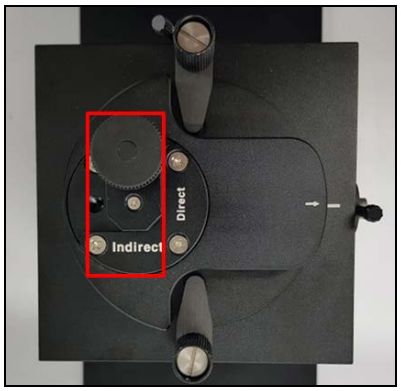
4. Select **Absolute** Calc Mode and **De Mello** Calc Method in the **Data Collection** tab.

5. Set up the measurement parameters.

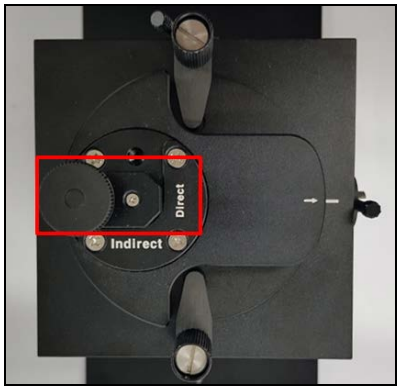
NOTE: *For more detail of method, refer to Spectrum FL Software Users Guide.*

6. Click **Save** to save the method after setting up the parameters.
7. Select the **Run** icon.
8. Input the sample name and select **OK**.
9. Input Solvent or Empty cell. And select **OK.**
10. Remove Solvent or empty cell and input the Sample.

11. Turn the knob to **Indirect position**, select **OK.**



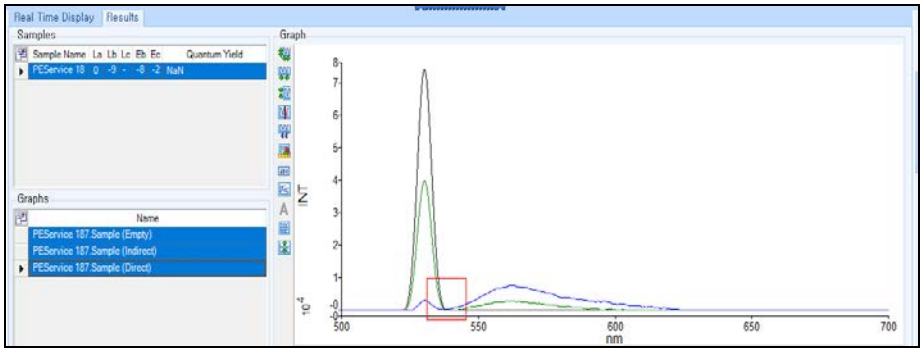
12. Turn the Knob to **Direct position**, select **OK**.



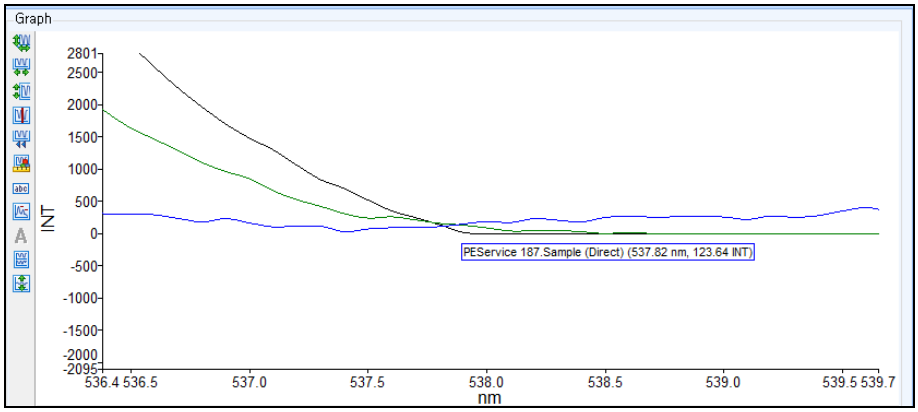
13. Confirm the spectrum and results.

14. Calculate the quantum yield in the result window.

15. Enlarge the graph intersection.



16. Check the intersection value and separates the excitation and emission areas of each graph based on the intersection value.

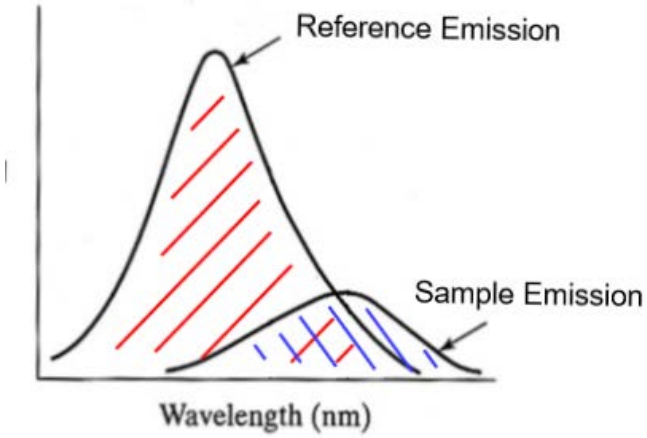


17.When the area is set, the Quantum yield value is automatically calculated and displayed.

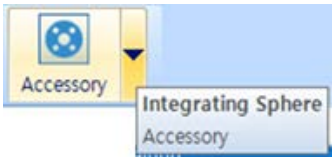
- **Relative Quantum Yield**

Relative Quantum Yield method is not required an Integrating Sphere accessory. This method is determined by comparing the reference with known quantum yield with the sample in question. And it is needed Absorbance and Refractive Index values.

$$\Phi = \Phi_R \times \frac{Int}{Int_R} \frac{1 - 10^{-A_R}}{1 - 10^{-A}} \frac{n^2}{{n_R}^2}$$

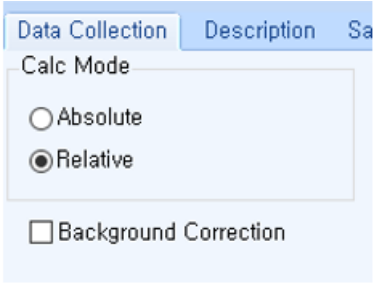


1. Double click on the **Spectrum FL** software and select the measurement mode.
2. Check the recognition of Accessory.



3. Click the **Quantum Yield** mode.

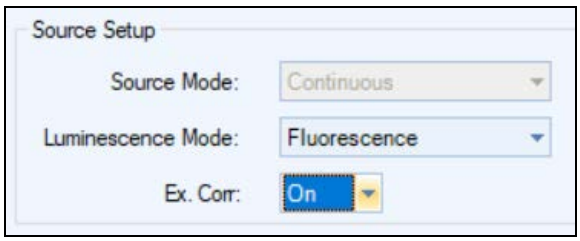
4. Select the **Relative** Calc Mode in the **Data Collection** tab.



NOTE: *Standard and sample are measured and compared under the same conditions. Therefore, errors can be minimized by using substances with similar emission ranges.*

5. Set up the measurement parameters.

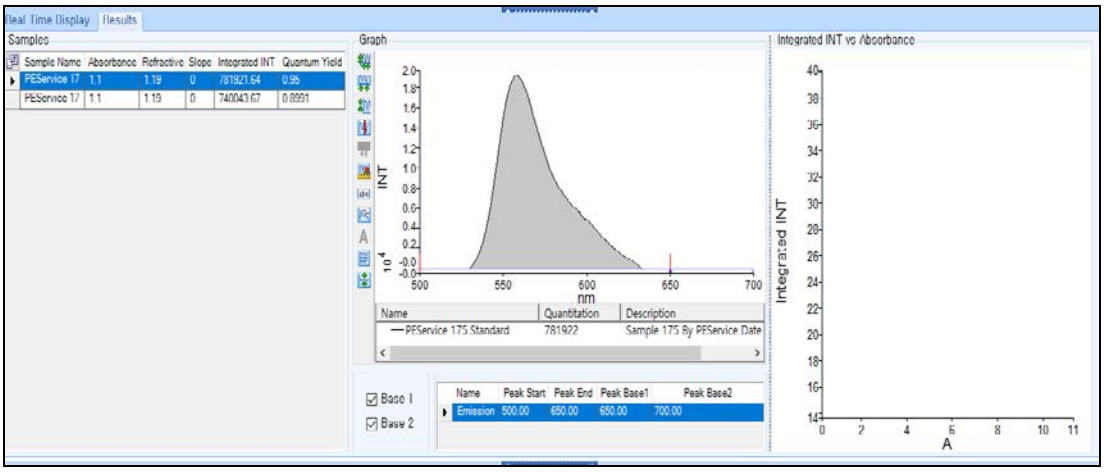
NOTE: *In Relative Quantum Yield mode, select **On** from Ex. Corr and Em. Corr tab.*



6. Select **sample table** Tab and add the sample.

NOTE: *One of the samples must be set to standard, and the Absorbance, Refractive and Quantum Yield values must be entered.*

7. Click **Save** to save the method after setting up the parameters.
8. Select the **Run** icon.
9. Input the sample name and click **OK**.
10. Input Standard sample cell. And click **OK**.
11. Remove Standard sample cell and input the Sample, click **OK**.
12. Confirm the spectrum and results.
13. Calculate the quantum yield in the result window.



14. Save or print the data.