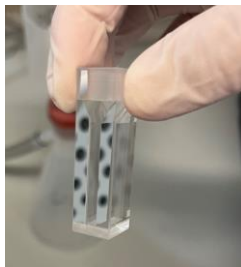


Fluorospectrometer FS5

Standard Operating Protocol

Precaution

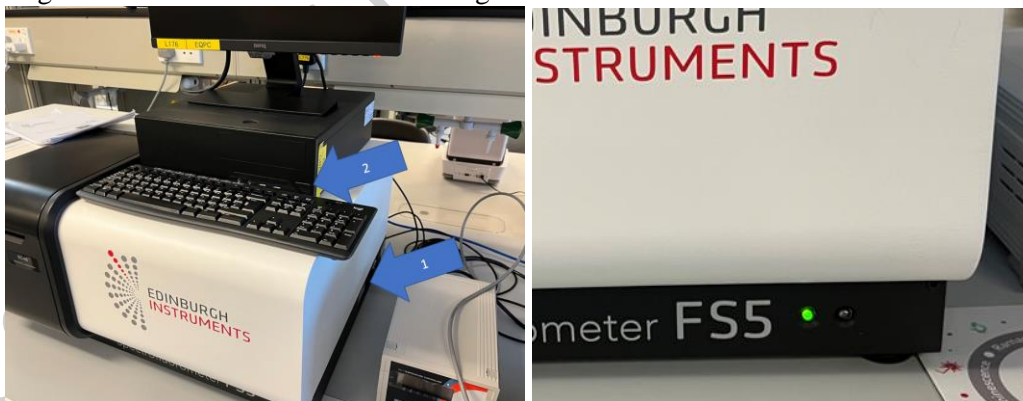
- Please be careful when handling the CPOS quartz cuvettes, any damage beyond repair will be charged to the user's PI account.
- Always hold the cuvette with gloves and only hold on to the top, do not hold the cuvette on the sample liquid level.



- Please bring your own acetone / absolute ethanol/ water/ buffers for cuvette washing.
- Make sure the **Signal Intensity do not exceed tolerable value** to protect the detector.
(Emission signal intensity < 1,000,000 cps / Transmission signal intensity < 2,000,000 a.u.)

I. Initialization of instrument

1. Sign in the Log Book.
2. Power ON FS5 with the switch at the lower right side and the instrument computer. Login to Windows with the account information at the bottom of the monitor of the instrument computer. Log in PPMS tracker. Green indicator light will be on for the instrument.



(Optional: Setting up of temperature controller and chiller)

If you would like to have temperature control for your experiments, please contact our staff to arrange 2 days in advance.

*The chiller and the temperature controller operate independently, make sure to switch on the chiller pump for fluid circulation and temperature controller for cuvette temperature control.

<u>Temperature required</u>	<u>Chiller</u>	<u>Temperature Controller</u>
Below 5°C	Please contact out staff at least 2 weeks in advance	
5 – 20 °C	Set target temperature for both device	
Above 20 °C	Set 20 °C	Set target temperature

1. Switch on the temperature controller (next to the FS5) and the chiller.

Temperature Controller



Chiller

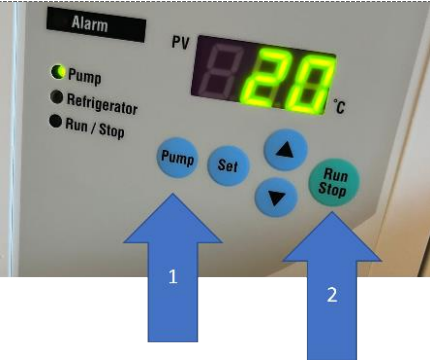


Chiller

2. Press the [Set] key, the displayed value on the temperature indicator blinks and temperature can be set. Press the up and down key to increase/ decrease the value.
3. Press [Set] key again to confirm the set temperature of the chiller.

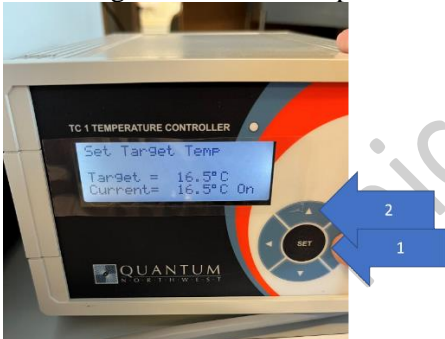


4. Press [Pump] to start the circulation pump, the LED next to Pump will be on. (Check for leakage)
5. Press [Run/ Stop] to start the chilling process, this button only controls the chilling, not the circulation pump.



Temperature Controller

6. Press the left or right arrows on the Menu Button, until [Set Target Temp], press up or down arrows to set the Target Temperature.
7. Press [SET] to retain the new Target and initiate temperature control.



8. Wait until [Current] reaches your target temperature.

Software - Fluoracle

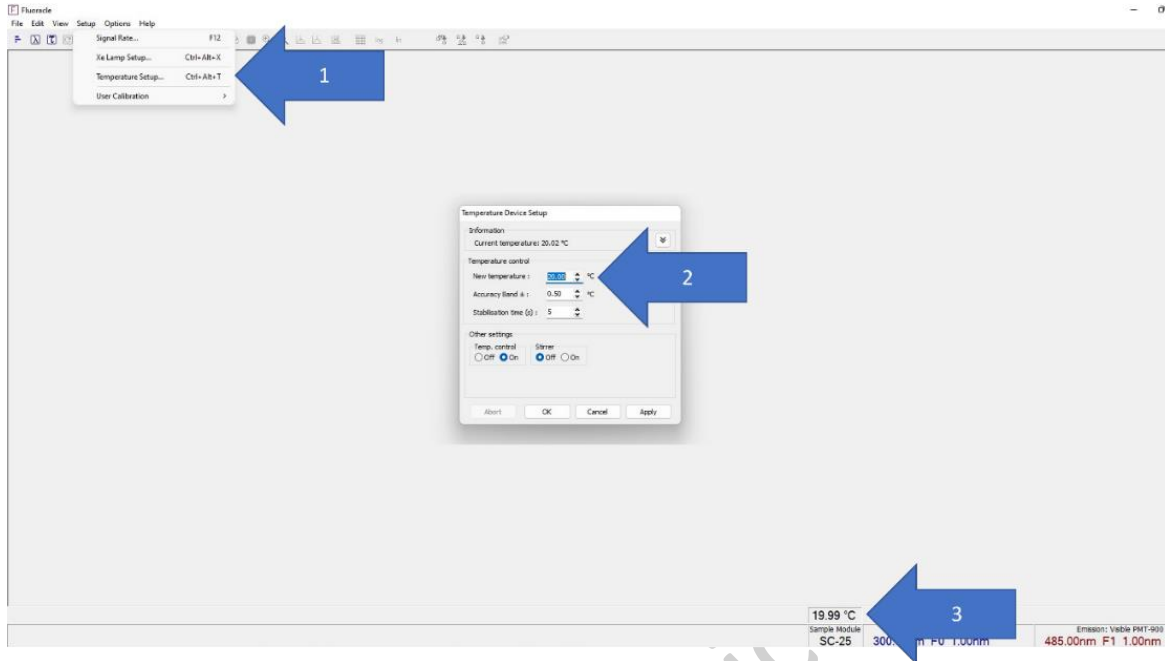
9. Double click on the software **Fluoracle** to launch the controlling software. The white indicate light on the instrument will turn on upon successful connection. This will turn on the Xenon lamp.



10. Wait 10 minutes for the Xenon lamp and detector to stabilize.

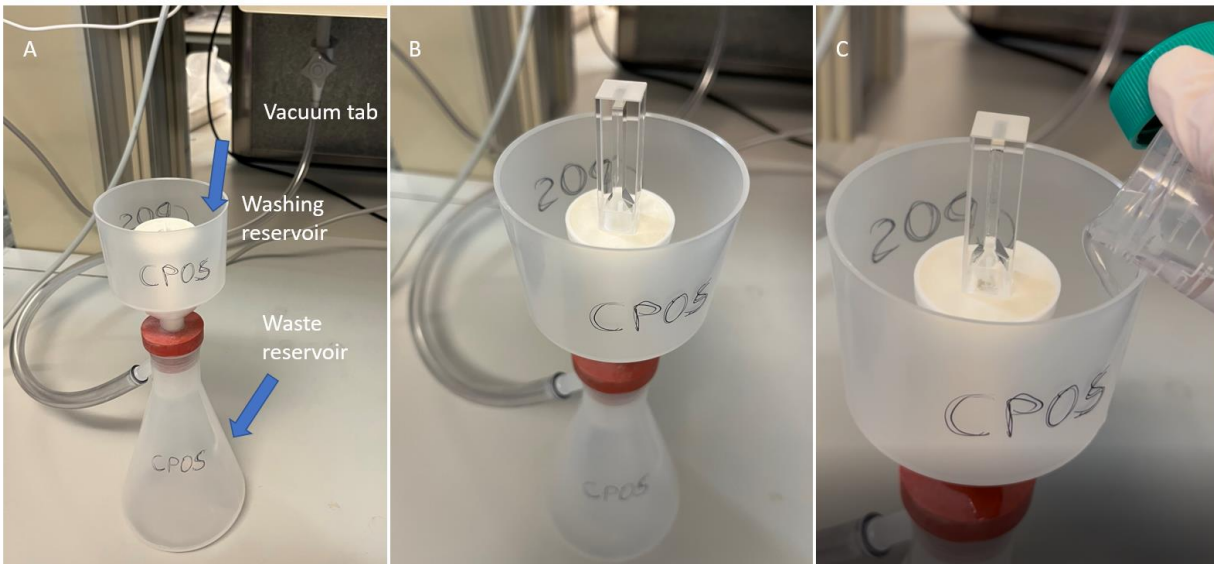
(If you are using temperature controller)

11. In Fluoracle, click **Setup**, select **Temperature Setup**, in the pop up box under temperature control, type in the New Temperature. The temperature of the cuvette module will be shown in above Sample Module SC-25 (only if Temperature controller is switch on), wait for it to reach the target temperature.



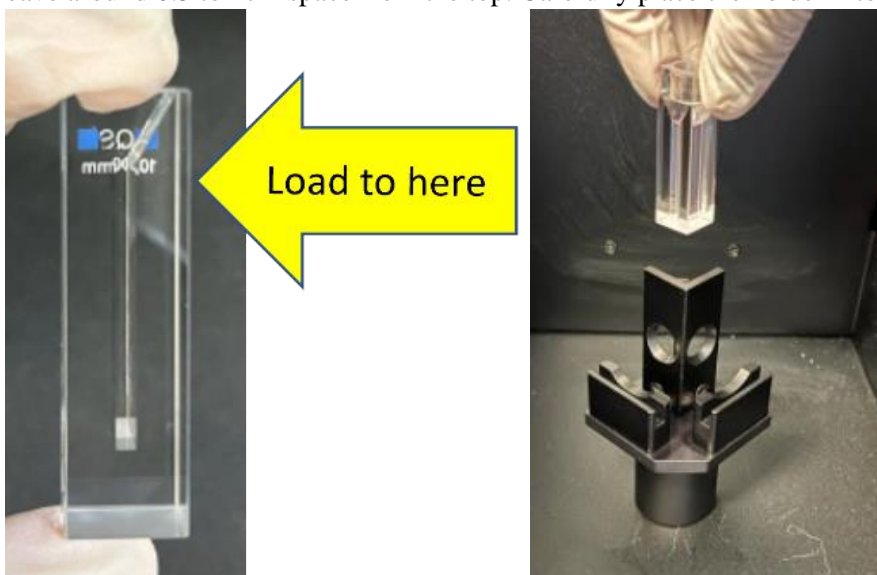
II. Setting up a measurement

12. Connect the cuvette cleaner to the grey vacuum tab, turn on the vacuum tab [A].
13. Pour out any contents from the cuvette into a container.
14. Carefully invert the quartz cuvette on to the washing port with the small spout, make sure the cuvette opening covers the inner opening circle of the white stage [B].
15. Gently pour 10ml of Milli-Q water into the washing reservoir (not on the white stage) [C].




16. Gently pour 10ml of Acetone or absolute ethanol into the reservoir (not on the white stage)
17. Repeat step 17-18 for two times.
18. Let the cuvette stay on the white stay for the air to blow dry.

19. Load your testing sample into the cuvette up to the designated region (yellow arrow), do not load to the top, leave around 0.5 to 1cm space from the top. Carefully place the holder into the cuvette.

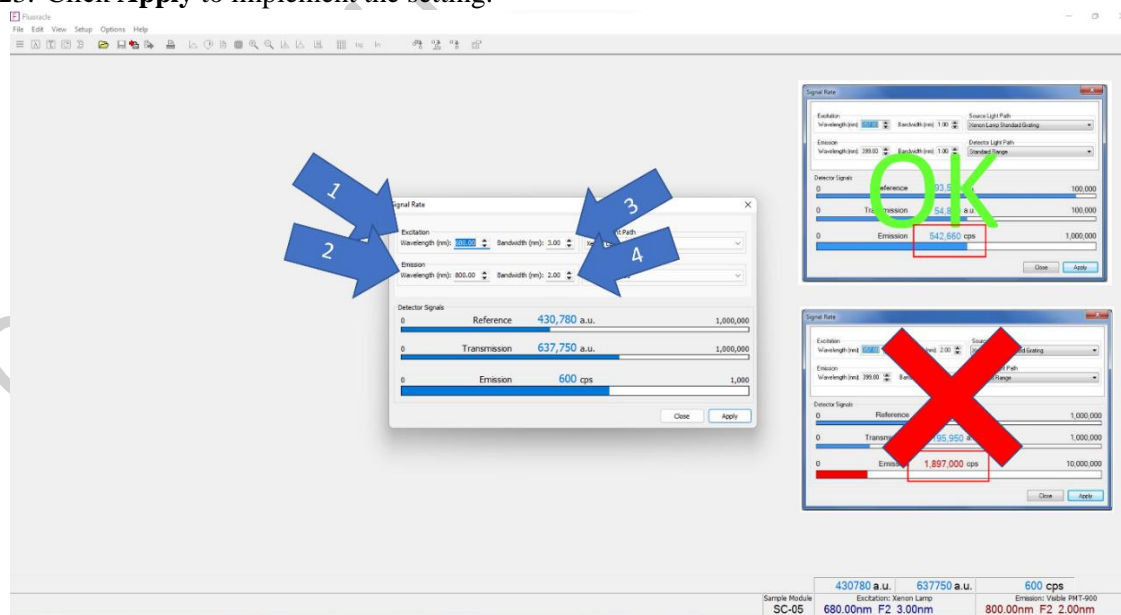


III. Setting up of Signal Rate

20. To prepare for a spectral scan, use the **Signal Rate** dialogue box, set in your targeted Excitation and Emission wavelength, the **Emission Signal Intensity should not exceed 1,000,000 cps**.

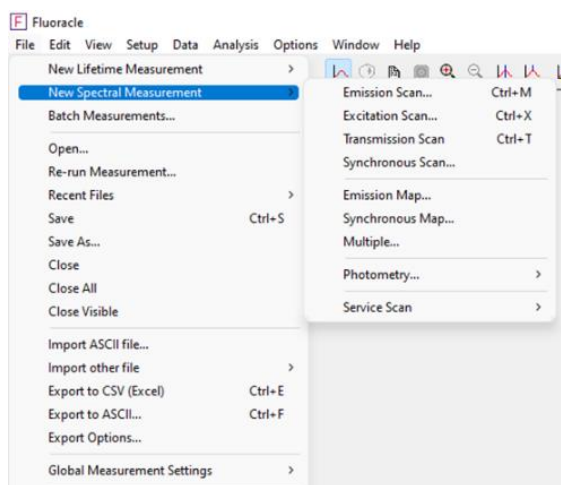
(The signal rate dialogue box can be opened by clicking this  button).

21. Start by increasing the Bandwidth value by 0.1nm, gradually adjusting the value.
22. Make sure the **Emission Signal Intensity does not exceed 1,000,000 cps** before you start a scan.
Signal intensity higher than allowed will damage the detector.
23. Click **Apply** to implement the setting.

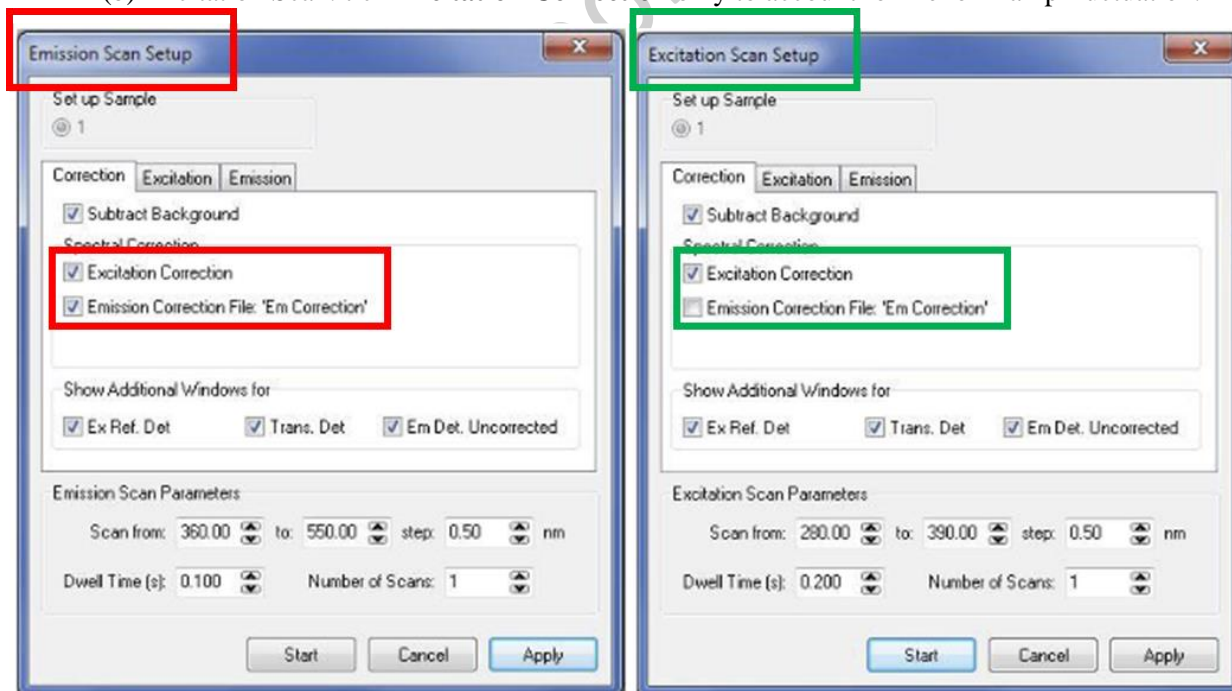


IV. Setting up an Emission or Excitation Scan

24. Check the Signal Rate dialogue box, make sure the expected maximum excitation and emission wavelength signal is set with the **Emission signal intensity does not exceed 1,000,000 cps**. The photon count can be controlled by increasing or decreasing the bandwidth.
25. At **File, New Spectral Measurement**, select **Emission /Excitation Scan**. A dialogue screen for Emission /Excitation Scan Setup will appear.



26. (a) Emission Scan: tick both **Excitation Correction** and **Emission Correction**.
- (b) Excitation Scan: tick **Excitation Correction** only to account for Xenon Lamp fluctuation.



27. Select the scan parameter, press **Apply** button to save the excitation /Excitation scan parameter.
28. Press **Start** for the spectral scan.
29. Click the **Stop** button on the top right corner of Fluoracle software or Esc to stop the scan.
30. After the scan, select **File, Save** to save the scan.

V. Setting up a Transmission Scan

31. Open the Signal Rate dialogue box and enter the excitation wavelength which you expect the maximum signal at the transmission detector. The **signal does not exceed 2,000,000 a.u.** If your scan range crosses 468nm, then also check 468nm does not exceed 2,000,000 a.u.
32. At **File, New Spectral Measurement**, select **Transmission Scan**. A dialogue screen for Transmission Scan Setup will appear.
33. In the **Correction** Tab, tick the **Excitation Correction** to account for Xenon Lamp fluctuation.



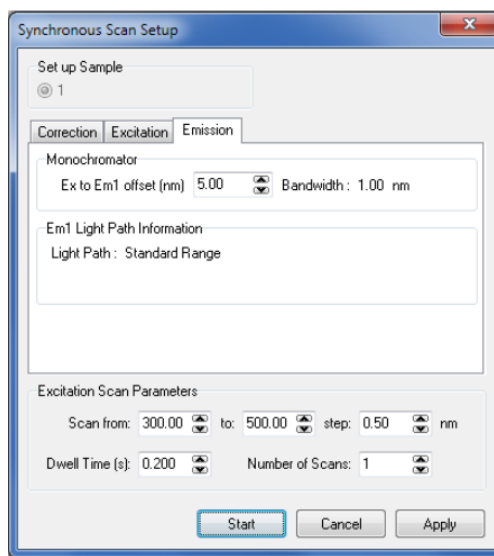
34. Tick **Subtract Background** for the recording of background signal prior to each scan.
35. In the Excitation tab confirms the spectral bandpass of your excitation
36. Select the scan parameter, press **Apply** button to save the scan parameter.
37. Press **Start** for the spectral scan.
38. Click the **Stop** button on the top right corner of Fluoracle software or Esc to stop the scan.
39. After the scan, select **File, Save** to save the scan.

VI. Setting up a Synchronous Scan

Synchronous scans are where both excitation and emission wavelengths are scanned simultaneously, with a zero or fixed offset between the emission and excitation wavelength. For zero offset, samples need to have negligible scattering properties. For fixed offset, the wavelength of emission must be greater than excitation.

40. Check the Signal Rate dialogue box, make sure the expected maximum excitation and emission wavelength signal is set with the **Emission signal intensity does not exceed 1,000,000 cps.**
41. At **File, New Spectral Measurement**, select **Synchronous Scan**. A dialogue screen for Synchronous Scan Setup will appear.

42. At **Emission** tab, type in the wavelength offset between excitation and emission.



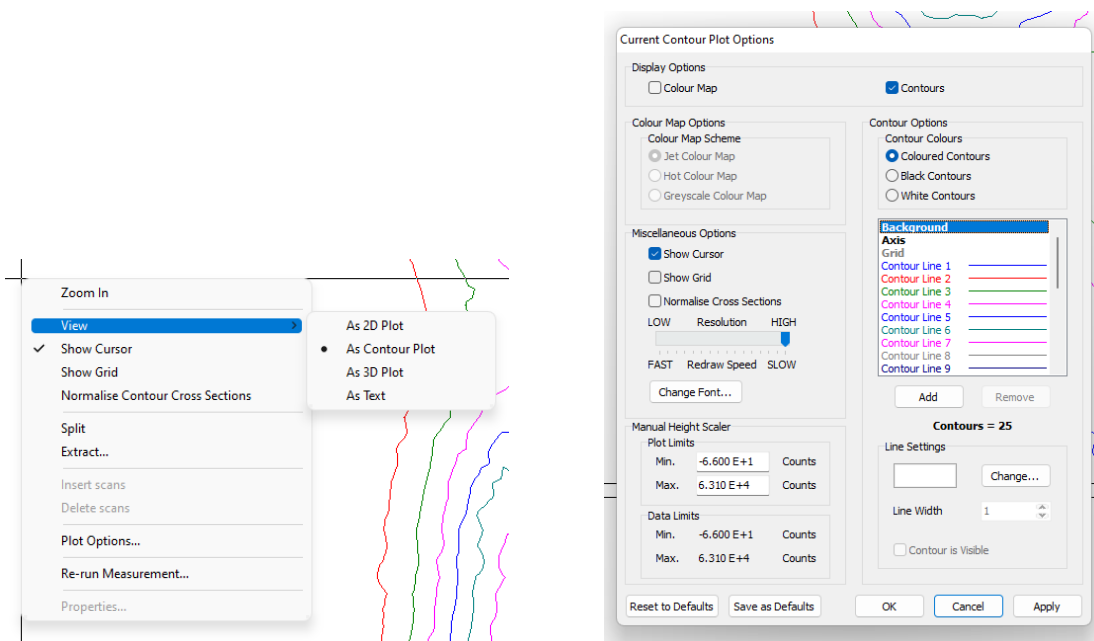
43. Select the scan parameter, press **Apply** button to save the scan parameter.
44. Press **Start** for the spectral scan.
45. Click the **Stop** button on the top right corner of Fluoracle software or Esc to stop the scan.
46. After the scan, select **File, Save** to save the scan.

VII. Setting up an Excitation Emission Map

Excitation Emission Map is a series of emission scans with systematic changes in the excitation wavelength aiming to find the set of excitation and emission wavelength with maximum signal.

47. Check the Signal Rate dialogue box, make sure the expected maximum excitation and emission wavelength signal is set with the **Emission signal intensity does not exceed 1,000,000 cps**. It is appropriate to set identical bandwidth for both excitation and emission.
48. At **File, New Spectral Measurement**, select **Emission Map**. A dialogue screen for Synchronous Scan Setup will appear.
49. In Emission Tab, keep the “Avoid 1st order excitation (Em1 start > Ex)” tick and no need select the “Avoid 2nd order excitation (Em1 stop < Ex^{x2})” as the automatic filter wheel are in place.
50. Select the scan parameter, press **Apply** button to save the scan parameter.
51. Press **Start** for the spectral scan.
52. Click the **Stop** button on the top right corner of Fluoracle software or Esc to stop the scan
Only the last active scan will be lost, all previous scans of the map will remain and can be further processed.
53. After the scan, select **File, Save** to save the scan.

54. Different display format for the image can be selected, and further configured with right click **Plot Options**.



VIII. Setting Up a Synchronous Map

Synchronous Maps is a series of synchronous scans with offsets between excitation and emission wavelength being systematically increased.

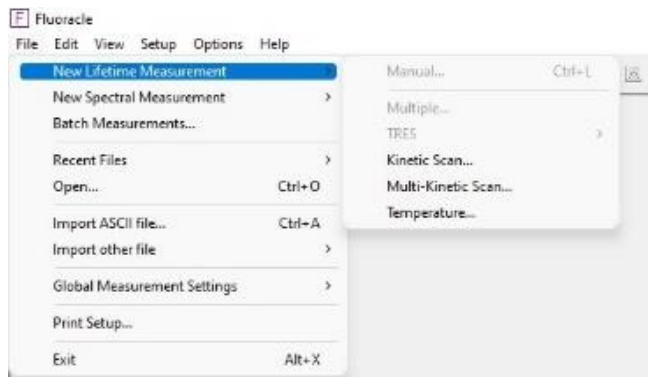
Refer to VI and VII, for similar set up.

IX. Setting up Multiple Spectral Measurements

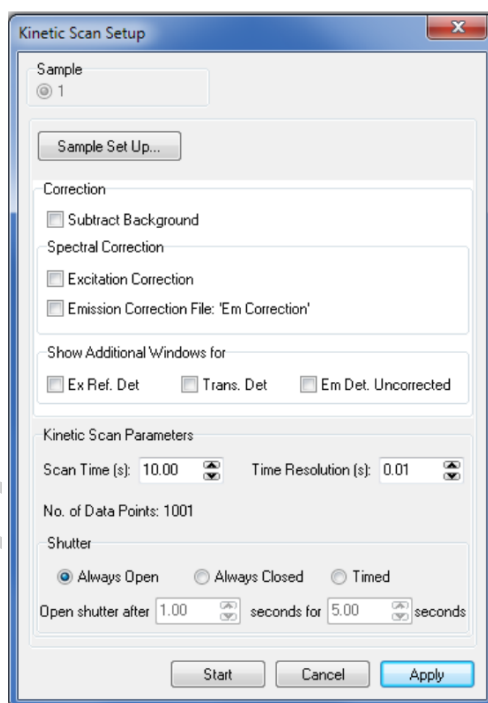
Multiple Spectral measurements are used for repeated measurements of either Emission Scans, Excitation scans, Transmission scans or Synchronous scans. Ideal use for slow kinetic studies/ photobleaching/ long term stability effects.

X. Setting up Kinetic Measurements

Kinetic Measurements is a time course of signal followed over a period, the excitation and emission wavelength remain fixed for the time of measurement.



55. Check the Signal Rate dialogue box, make sure the expected maximum excitation and emission wavelength signal is set with the **Emission signal intensity does not exceed 1,000,000 cps**. It is appropriate to set identical bandwidth for both excitation and emission.
56. At **File, New Lifetime Measurement**, select **Kinetic Scan**. A dialogue screen for Kinetic Scan Setup will appear.

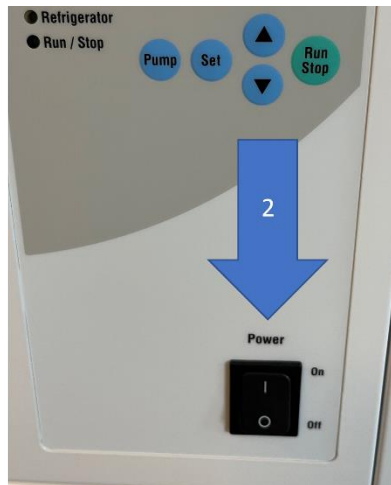
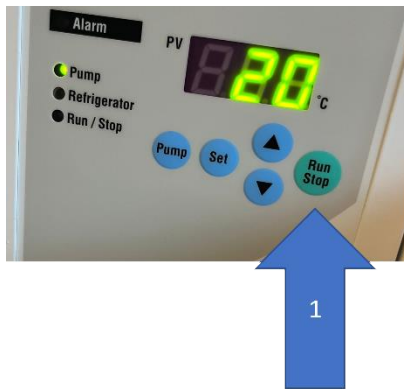


57. Set up the (overall) **Scan Time** and the **Time Resolution**. The combination of two will affect the total No. of Data Points. **The number cannot exceed 10,000**.
58. Press **Start** for the Kinetic scan.
59. Click the **Stop** button on the top right corner of Fluoracle software or Esc to stop the scan
Only the last active scan will be lost, all previous scans of the map will remain and can be further processed.
60. After the scan, select **File, Save** to save the scan.

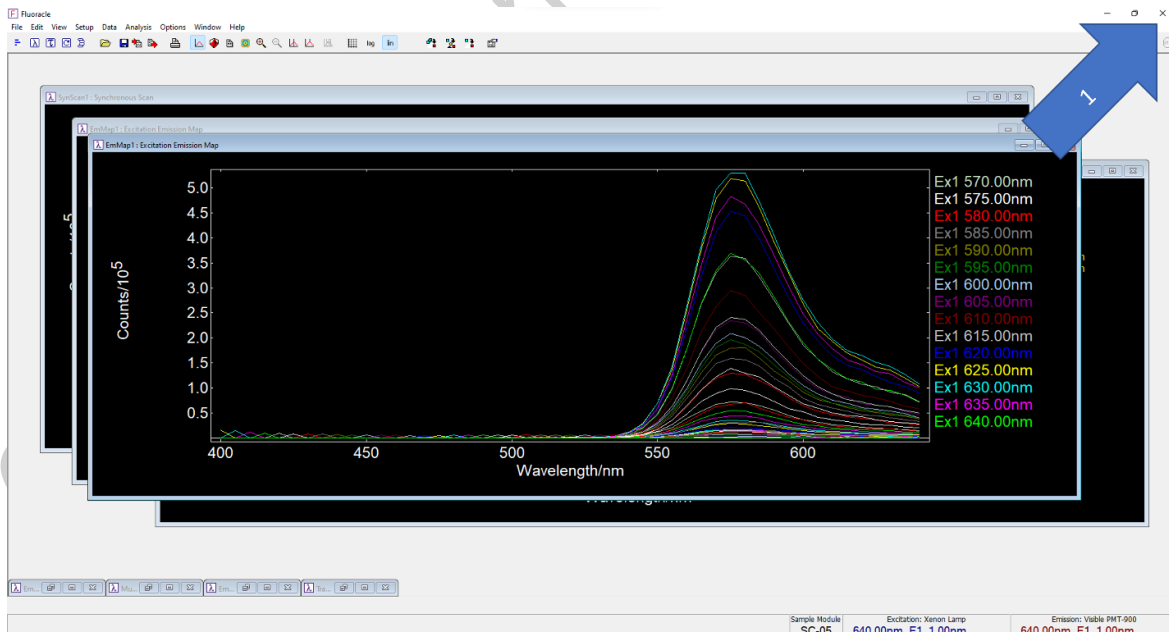
XI. Switch off instruments

61. Switch off the temperature control and chiller pump if it was used:

1. Stop the chiller pump by pressing the [Run/ Stop] button.
2. Power off the chiller pump by the power switch.
3. Power off the temperature control unit with the power button on the back of the temperature control.



62. Quit software session first, this will stop the lamp. If you need to restart the lamp, please wait at least 5 minutes.





63. Power OFF FS5 with the switch at the lower right side and the computer.



64. Wash the CPOS cuvette with acetone/ absolute ethanol, and Milli-Q water for 2 times, dry the cuvette and return the cuvette and cuvette washer to CPOS in L601.

65. Discard the chemical waste in designated container.

Tips for making a good measurement

- Liquid is easier to measure than solid or film samples, with less scatter and alignment effect.
- Do not use high sample concentration and choose an excitation wavelength that is in range with sample absorbance reduced.