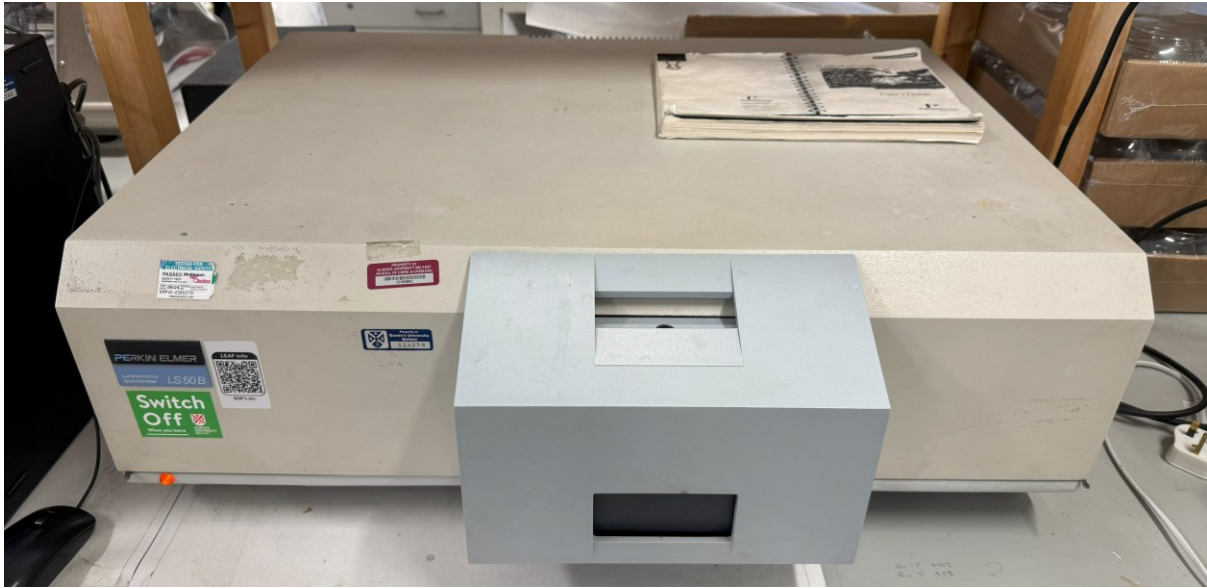


PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure

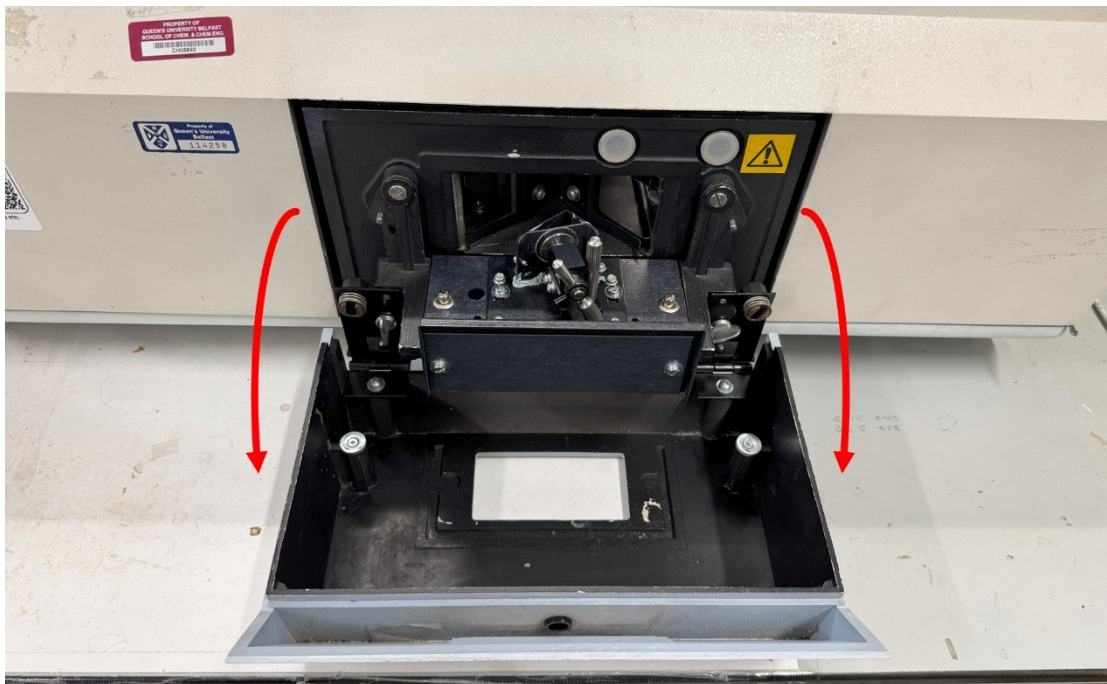
NOTE: Before reading this you MUST read the 'SOP - Energy and environmental impacts under normal, abnormal and emergency conditions' which is Mills group web site, <https://www.profandrewmills.com/leaf-documents/>. This addresses general energy and environmental impacts under normal, abnormal and emergency conditions considerations which you NEED to be cognisant of before conducting any experiment. If you identify anything in an SOP which can be improved, please contact the LO and PI to discuss the proposed change(s) before putting them into effect.

PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure

PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure (01.003)

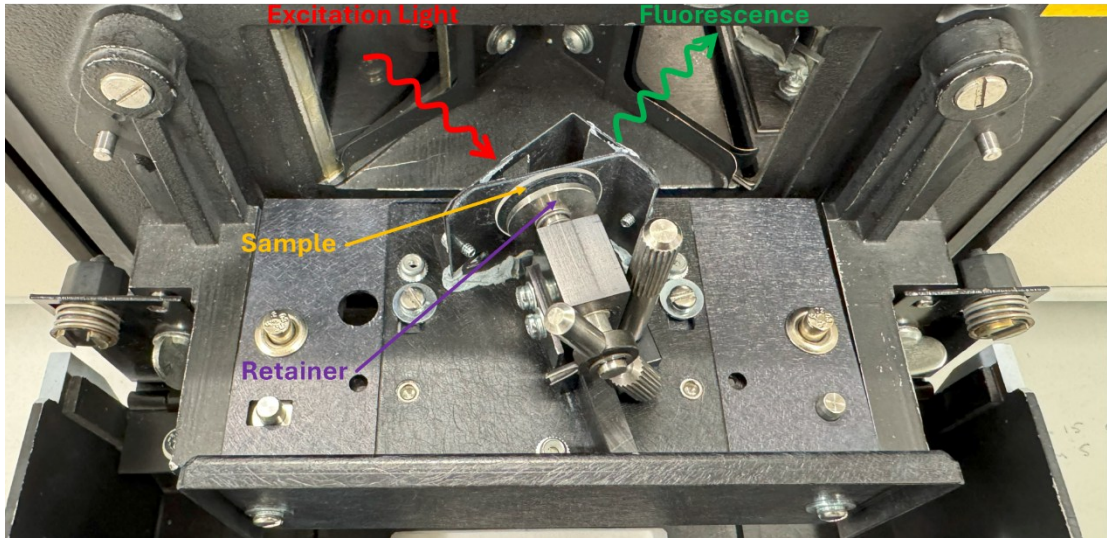


1. Switch the fluorimeter on using the switch located at the back left-hand side of the instrument. The instrument will start making loud noises – this is normal when setting up.
2. To insert your sample, open the sample area door by pulling towards you. The door will swing downwards.

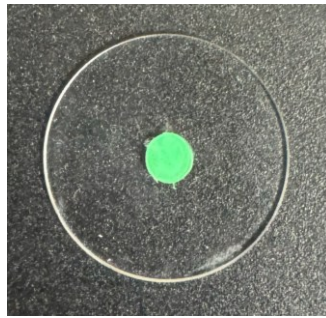


3. The installed sample holder is for solid samples, i.e. sensor films. The sample is held in place by a spring loaded retainer plate.

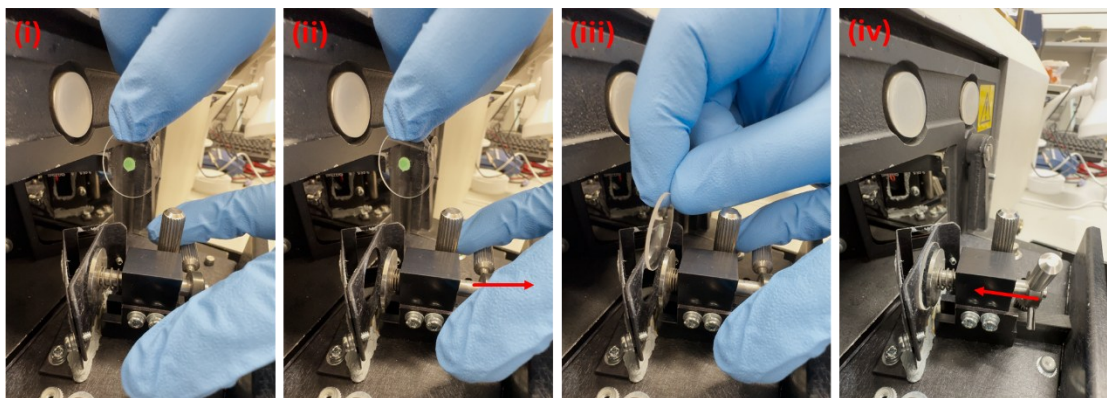
PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure



4. If the sample is smaller than 2.5 cm, it can be supported on a microscope slide or a glass disc. The image shows a 5 mm oxygen sensor (Pyroscience) supported on a 2.5 cm diameter glass disc using a little piece of Blu Tack.



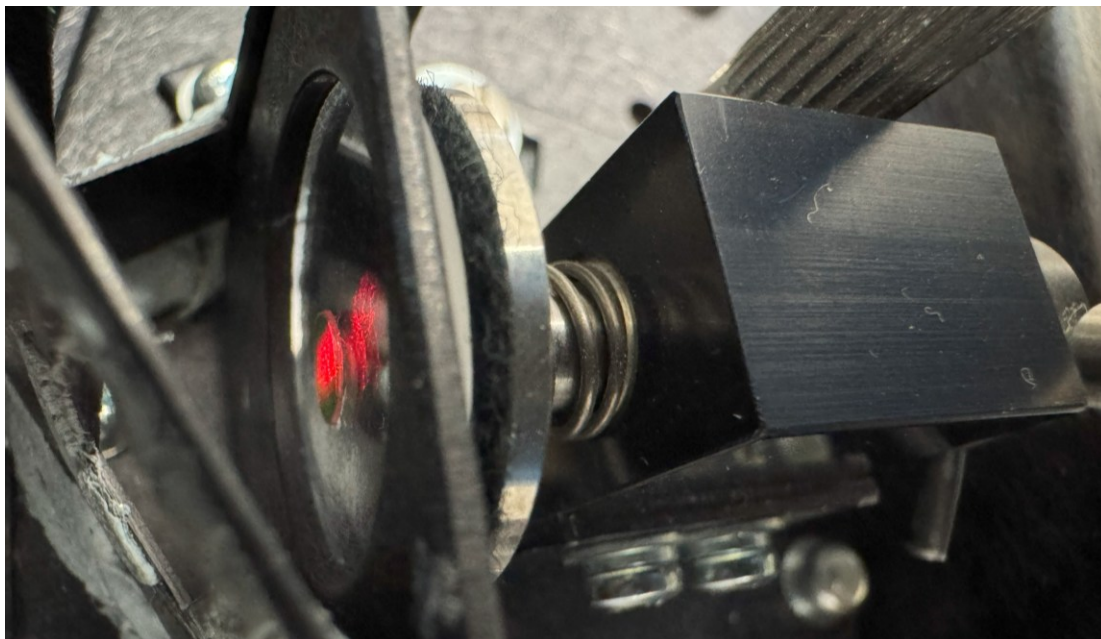
5. In order to load the sample **(i)** hold the back of the retainer and **(ii)** pull it back gently. **(iii)** Slide the sample down in front of the retainer plate (with the sample facing towards the spectrometer) and **(iv)** gently release the retainer, which will now hold the sample in place.



6. If you look at the front of your sample from above the retainer, you should be able to see the excitation light hitting the centre (620 nm, red light in this demonstration). If it isn't

PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure

centred, you should reposition your sample in the holder before closing the sample area door.



7. Switch on the PC. On the desktop, double click the 'virtual box' icon. The software cannot be run on the latest operating systems, therefore, it has to be run on a virtual machine running Windows XP.

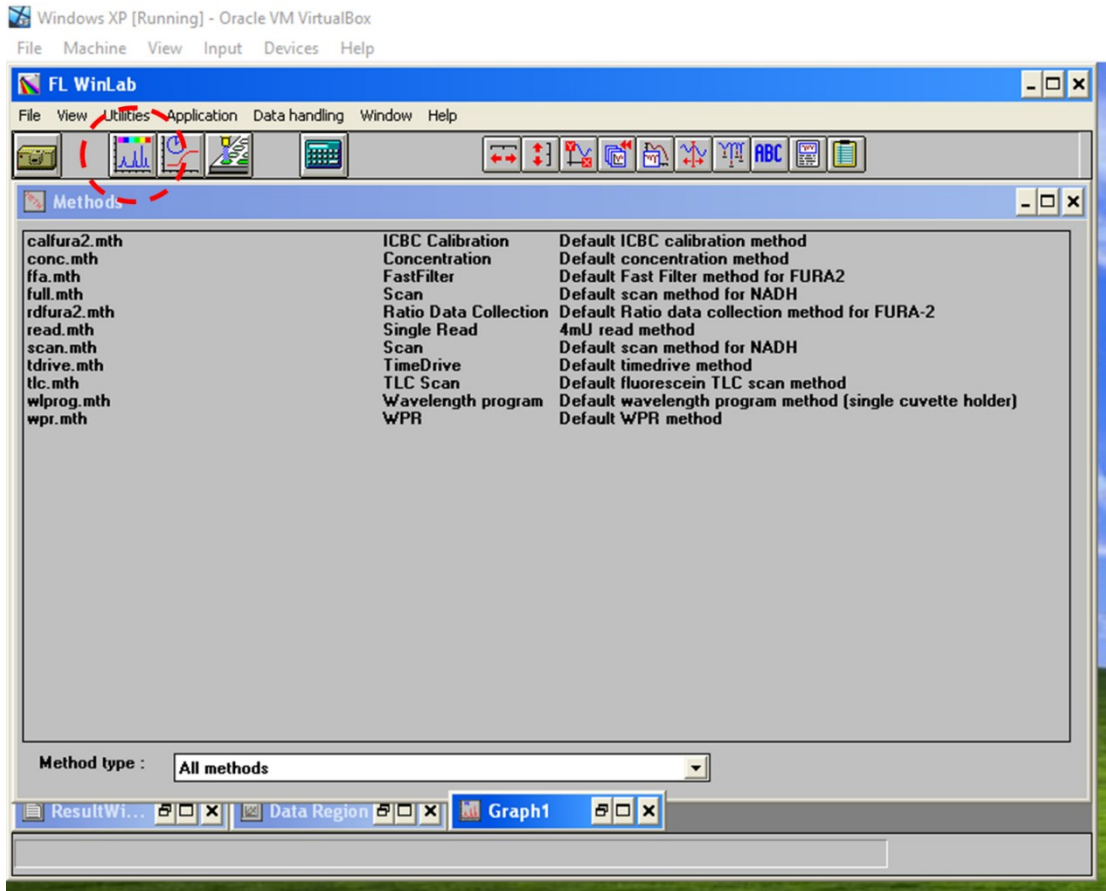


8. When the Windows XP desktop is displayed, double click on the 'FL WinLab' icon.

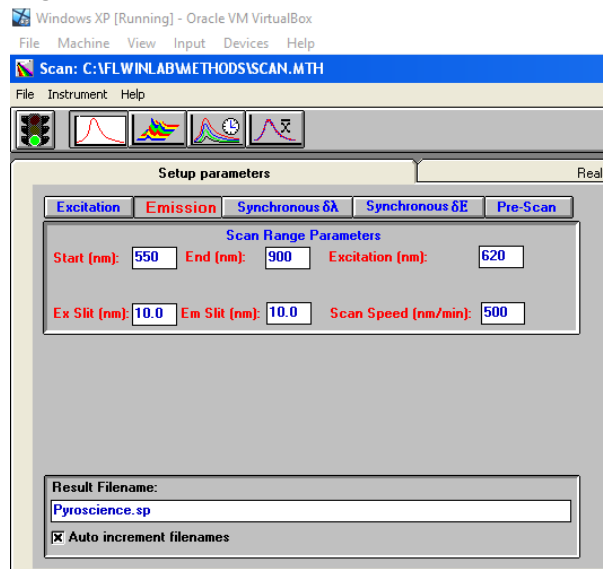


PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure

9. The following window will open displaying a list of default methods. Click on the highlighted button at the top to open the window to open up the ‘Setup parameters’ tab.

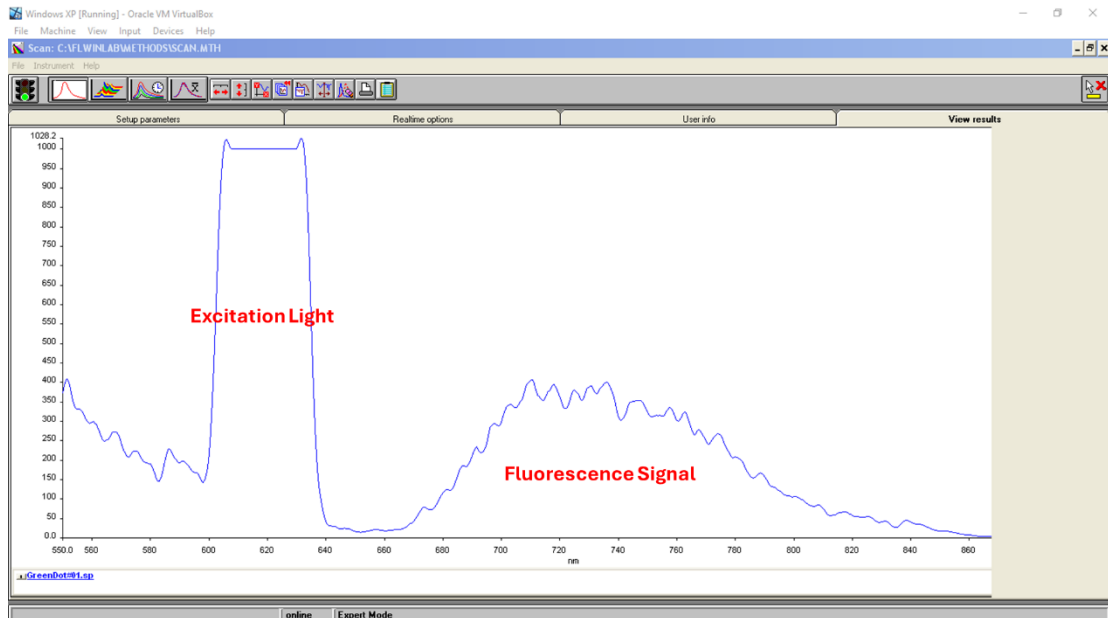


10. In this tab, ensure that the ‘Emission’ section is selected (highlighted in red text). Enter in the desired wavelength range (max. range = 200 nm – 900 nm), and the excitation wavelength. In this demonstration a green oxygen sensor (Pyroscience) is being measured. This sensor is usually excited by 620 nm (red) light. The slits can be adjusted, however, for the best quality signals, these should be left at 10.0. The lower the scan speed the better the signal, therefore, ca. 500 nm/min is recommended.



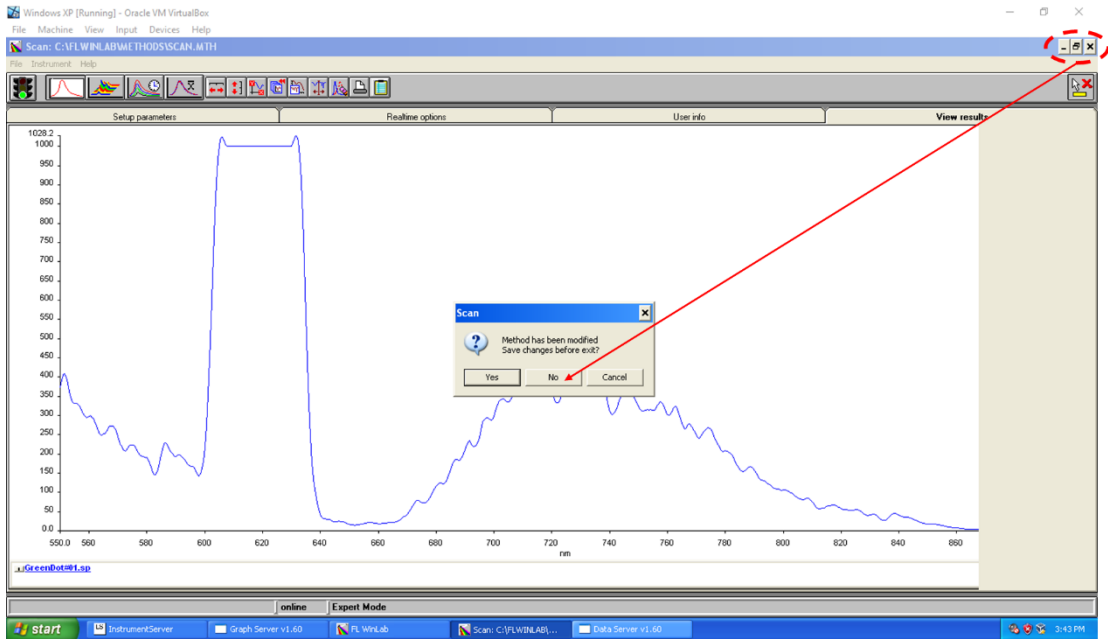
PerkinElmer LS 50 B Fluorimeter – Standard Operating Procedure

11. Enter your sample name in the 'Result Filename' box at the bottom and then click the traffic light button at the top left of the window to start the scan. The traffic light will turn red.
12. Once the scan is complete the traffic light button will turn green again.

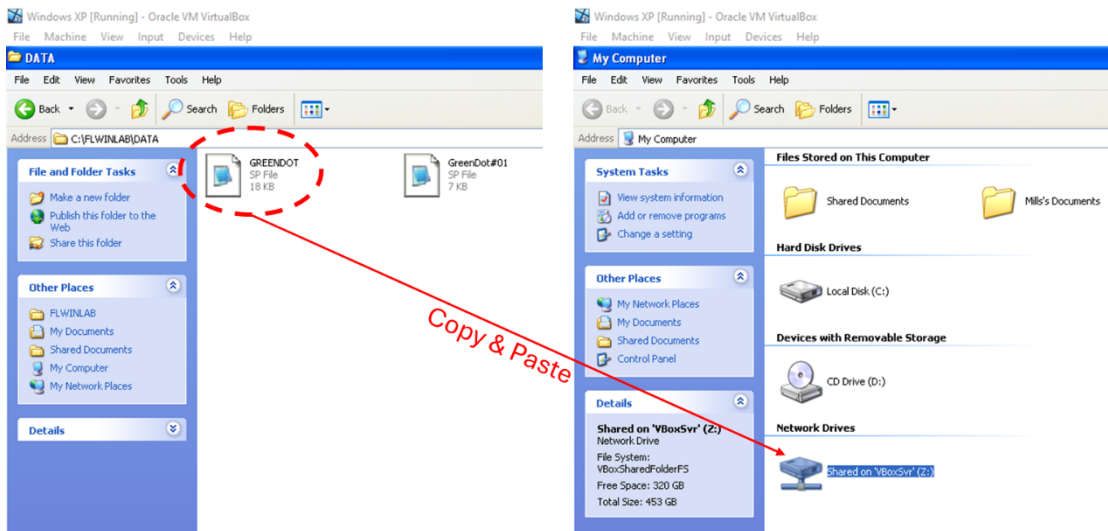


13. If you have more scans to record, you can simply click on the traffic light button again and the new spectra will overlay on the last.
14. If you have to change the scan range or the excitation wavelength, you can do so by clicking on the 'Setup parameters' tab above the spectra.
15. When finished, the data is saved automatically, therefore, the software can be closed using the cross at the top right of the screen. Note: ensure that it is the cross for the software and not for the virtual machine which is located just above. A box will pop up asking to save changes – just click 'No' to exit the software back to the Windows XP desktop.

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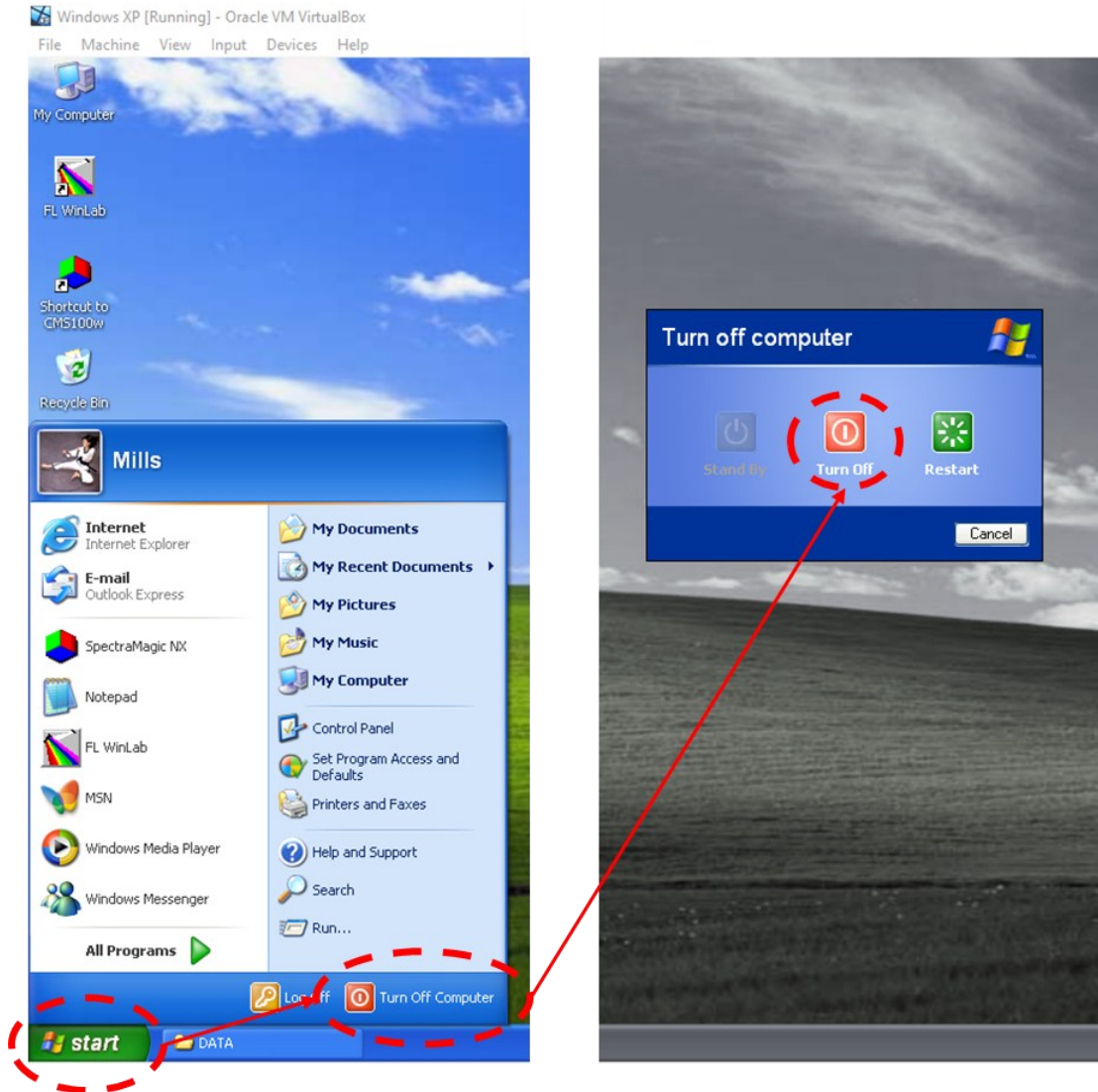


16. Double click on 'My Computer' and navigate to 'C:\FLWINLAB\DATA' where you will find all the saved data. The larger of the .sp files will be the one that contains the spectra data. Copy and paste this over to the shared network drive located in 'My Computer'. This allows you to export the files out of the virtual machine.

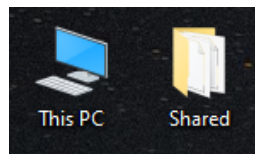


17. The virtual machine can now be shut down by clicking the Windows 'Start' button and then clicking on 'Turn Off Computer'. Lastly, click on 'Turn Off' on the popup window. This will take you back to the main PC (Windows 11).

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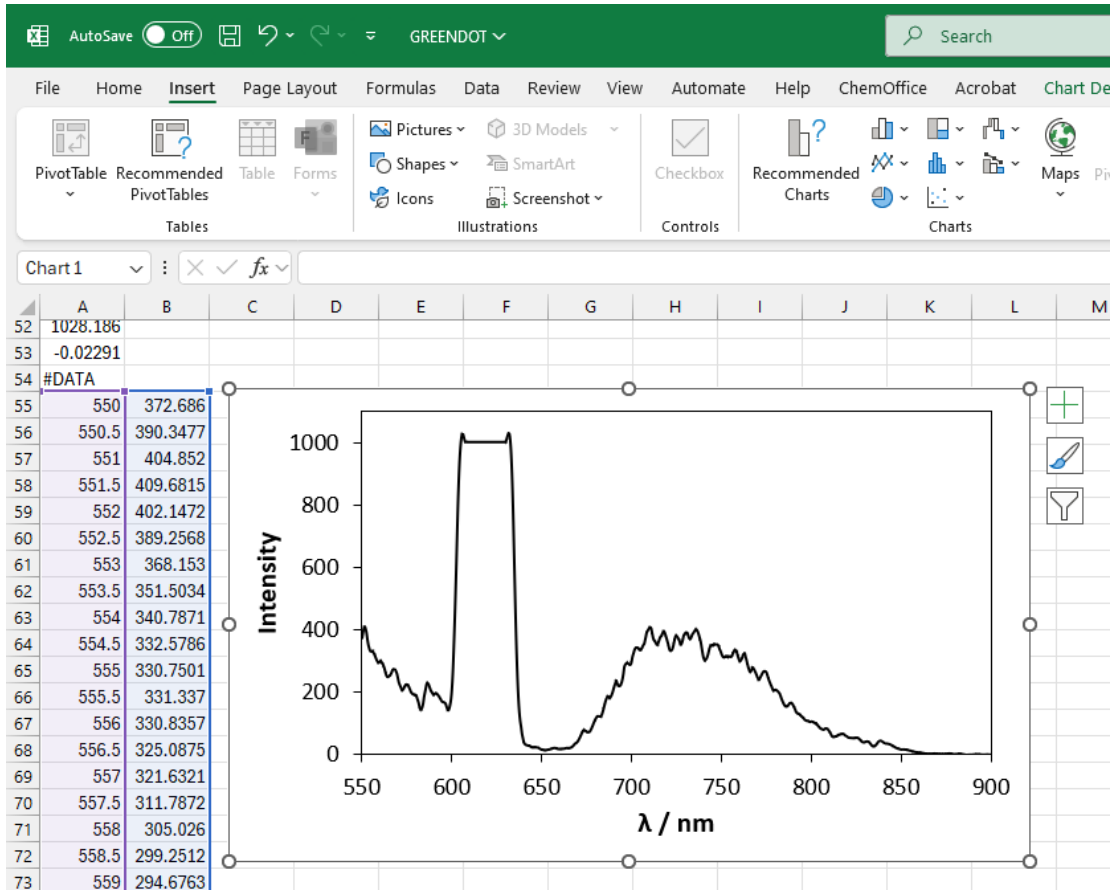


18. The spectra files can be obtained from the 'Shared' folder located beside the 'This PC' icon at the top left of the desktop.



19. The .SP files can be opened in Excel without any conversion etc. When opened in Excel, scroll down to row 55, where you will find the data to plot your fluorescence spectra.

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20. When finished, the fluorimeter should be switched off using the switch located at the back left-hand side of the instrument, and the computer should be shut down.
21. If any further information on the operation of the fluorimeter is needed, i.e. if samples are too intense, weak, or noisy, there is a copy of the manual on top of the instrument, or you can ask a PDRA who can advise on how to modulate the light signal and run signal averaging.