SHARED RESEARCH FACILITIES

HORIBA SPECTROFLUOROMETER

CALIBRATION

Purpose of this Instrument: Essential tool for characterizing the relationship between absorbed and emitted photos at specified wavelengths.

Location: 381 Chemistry Research laboratory building

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The Shared Research Facilities are operated for the benefit of all researchers. If you encounter any problems with this piece of equipment, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.

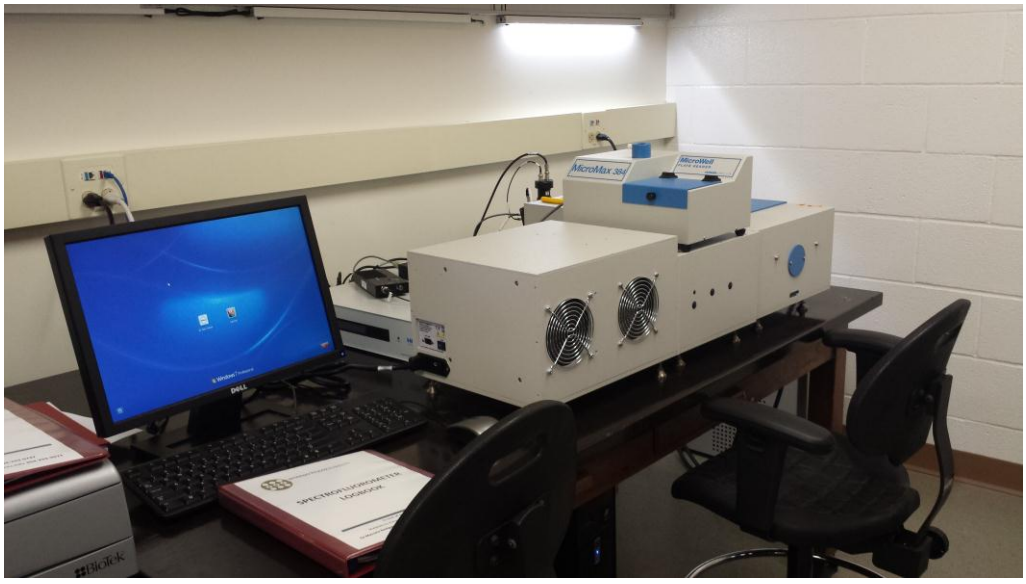

Figure 1 Horiba Fluorolog-3 spectrofluorometer at 381 CRL

Exitation calibration

1. Start instrument as described in SOP.
2. Click Measurement. Then choose SPECTRA.

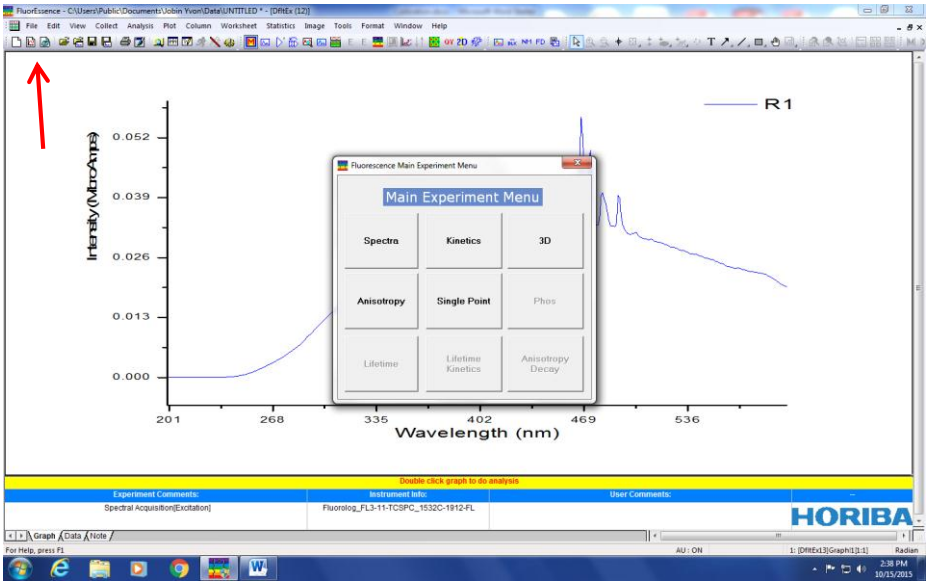

Figure 2 Measure type window.

3. Choose EXCITATION.

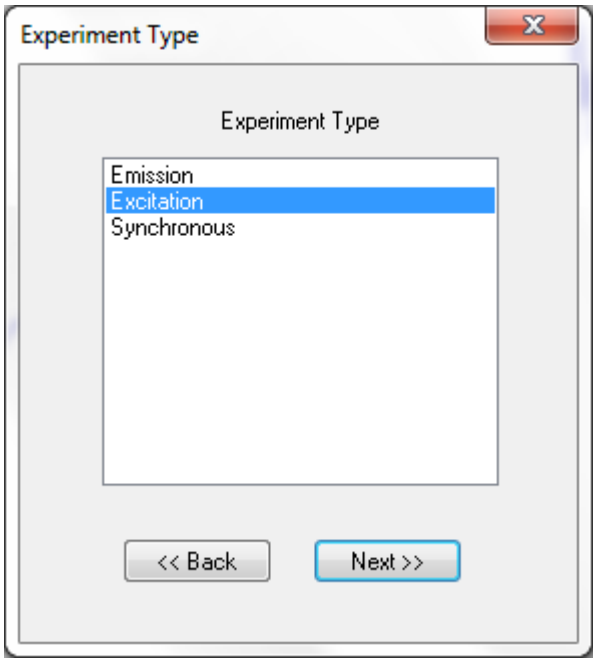

Figure 3 Choose Excitation.

4. Use all default parameter. Excitation 200-600 nm, Emission 350 nm. Then hit RUN.

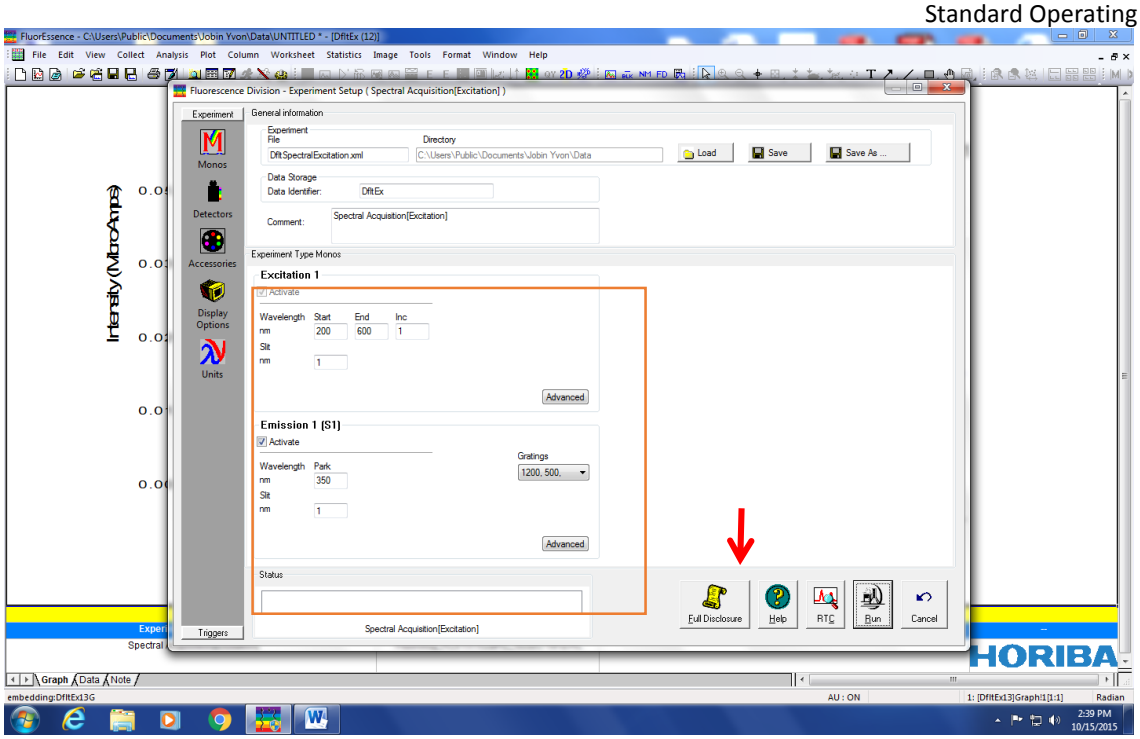

Figure 4 Use default settings for excitation measurement.

You will see real time data acquisition window.

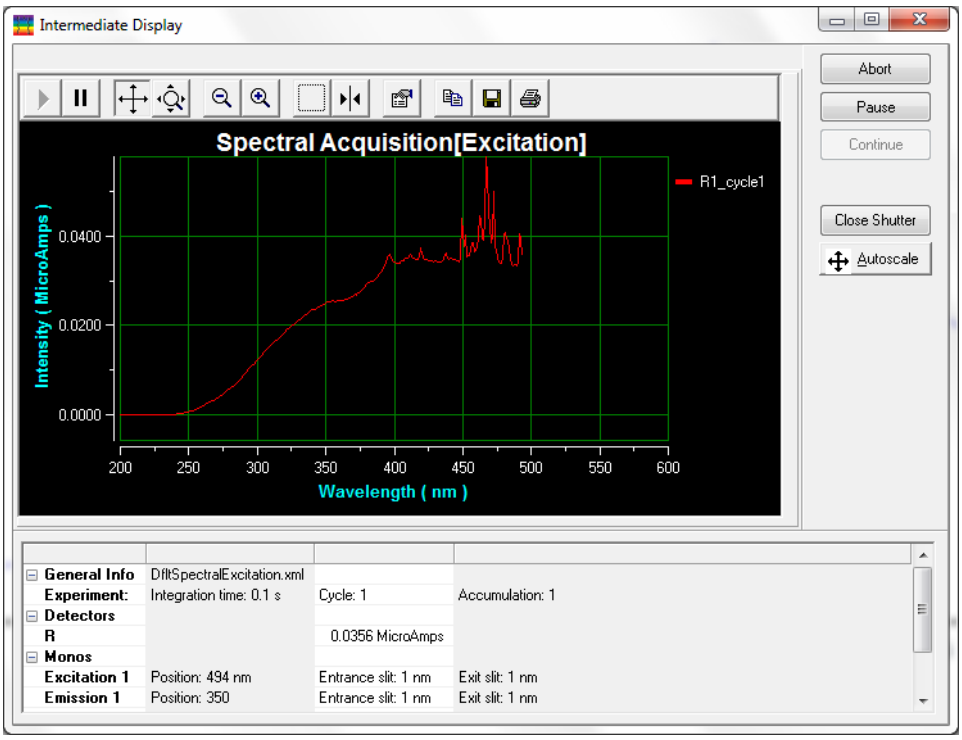

Figure 5 Real time spectra window.

5. Then you will see data window.

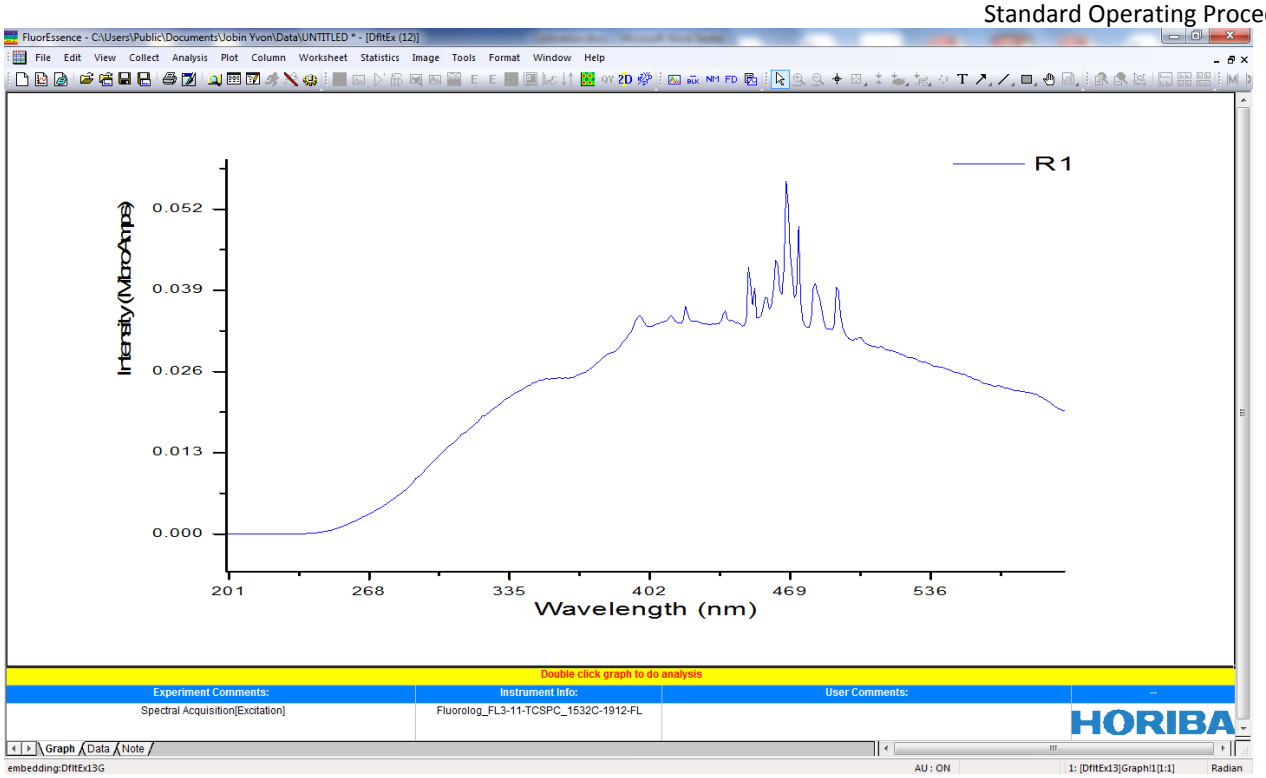

Figure 6 Data file window.

6. Double click on the data window.

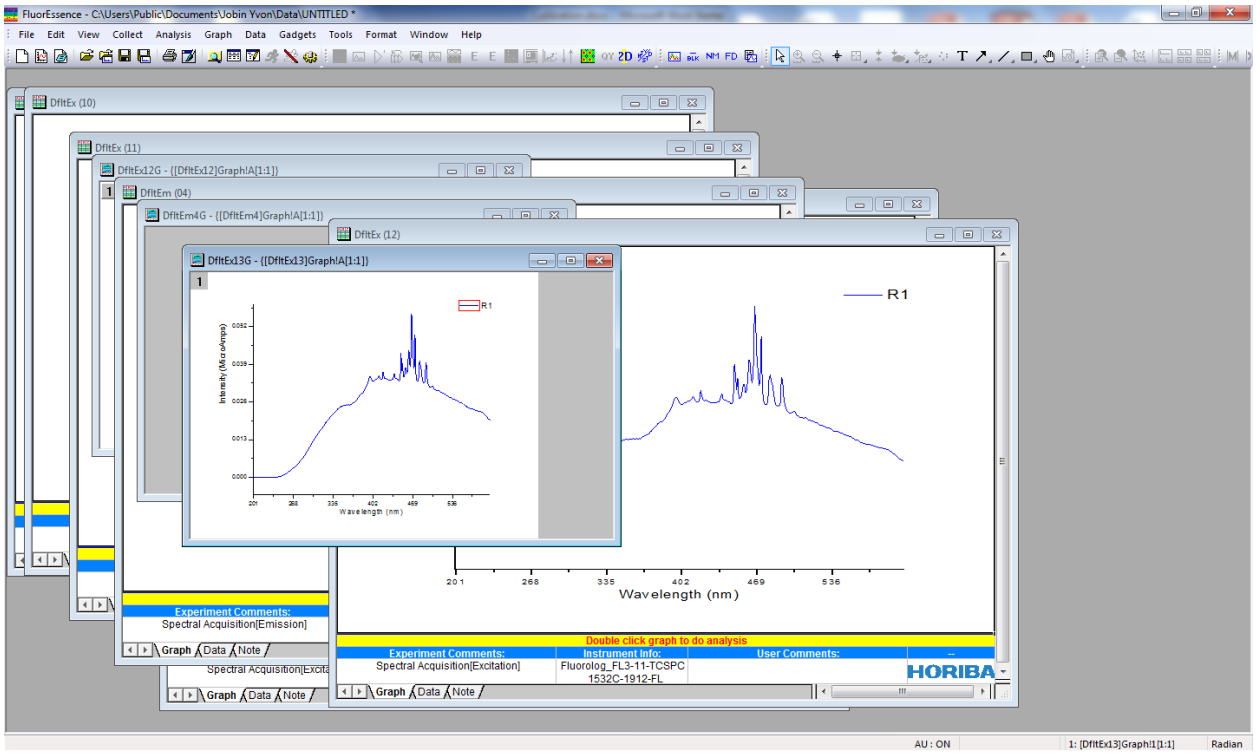

Figure 7 Data file analysis window.

7. You will activate the analytical tool. Place Cursor TOOL on the tip of the peak. You could read the wavelength and intensity of the peak.

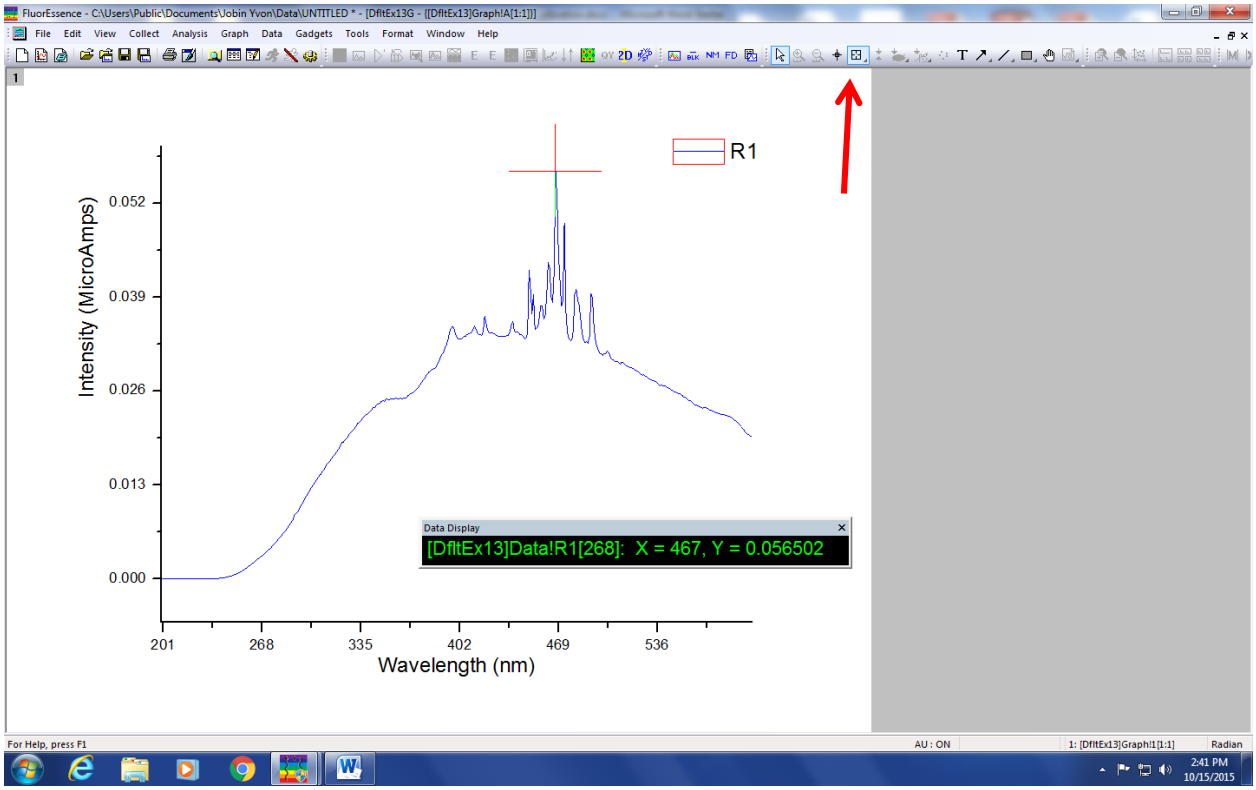

Figure 8 Data analysis window

8. The peak should be at 467. If this peak is not at 467 nm, you should calibrate. Click on the previous experiment button. Then click RTC (real time control).

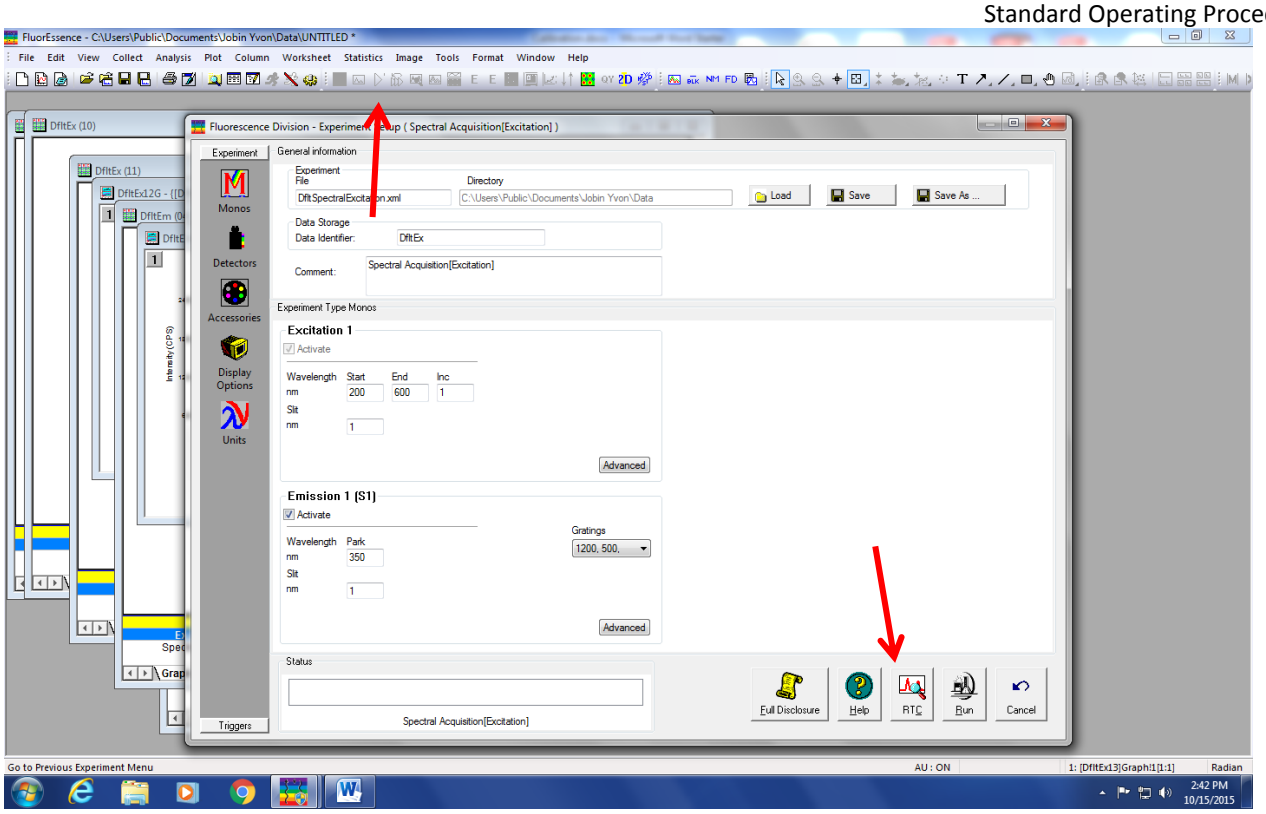

Figure 9 Run real time control (RTC)

9. Click on the Monos. You will see this window (Figure 10). Choose Excitation tab. Tap the number you read from your last excitation reading.

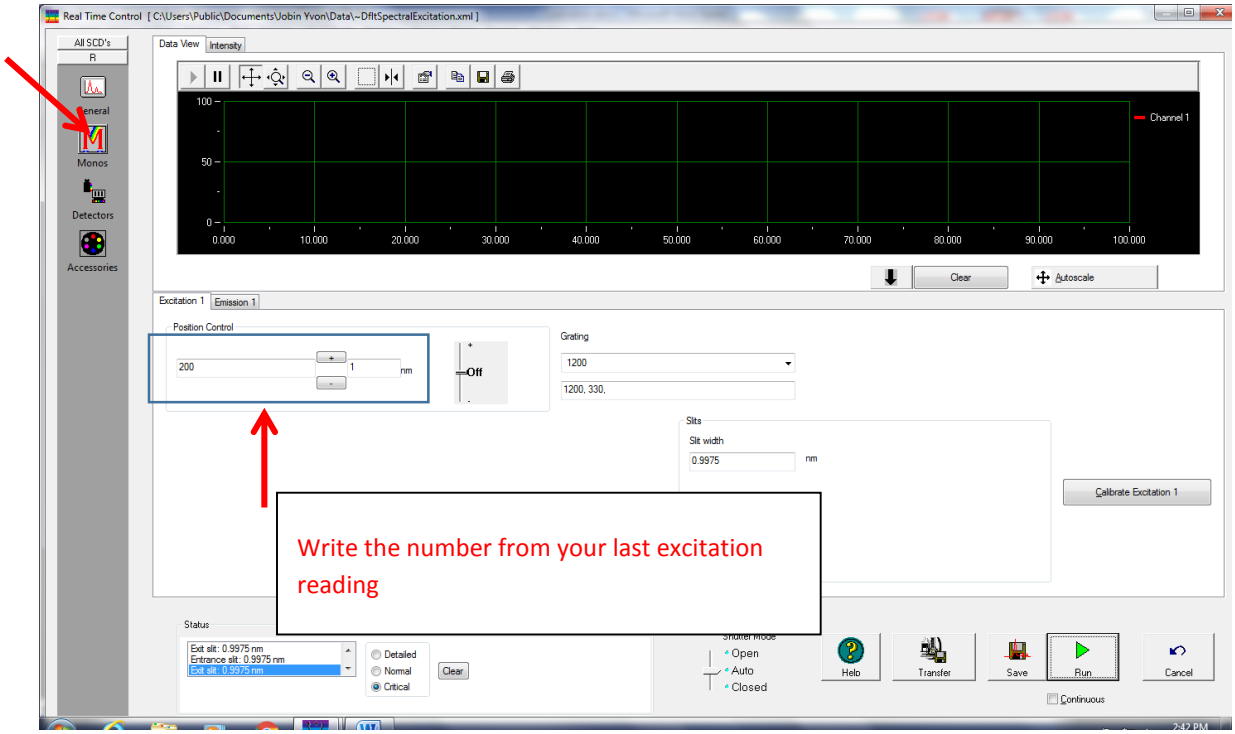

Figure 10 Calibrate excitation

Here we write 467

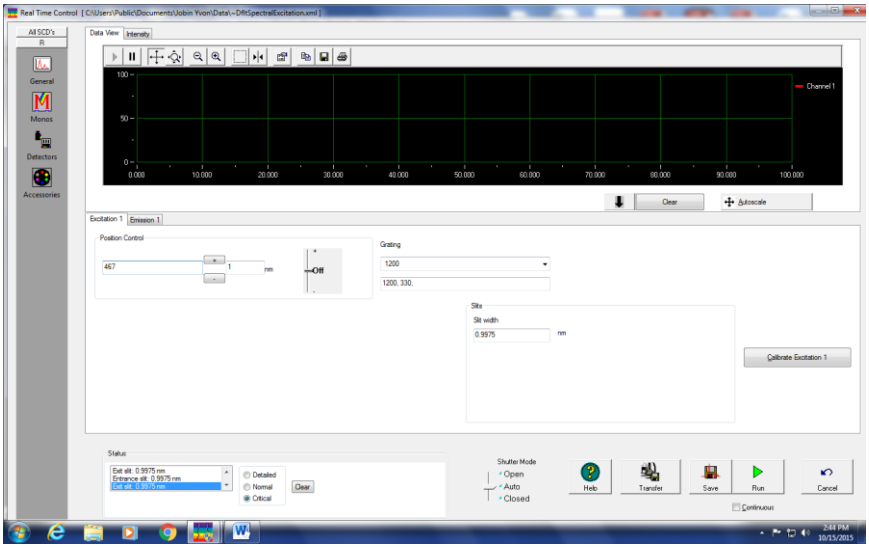

Figure 11 Calibrate excitation

Then hit on calibration excitation 1. Write the number it should be. It should be 467. Then hit ok. Then hit CANCEL from the RTC control window.

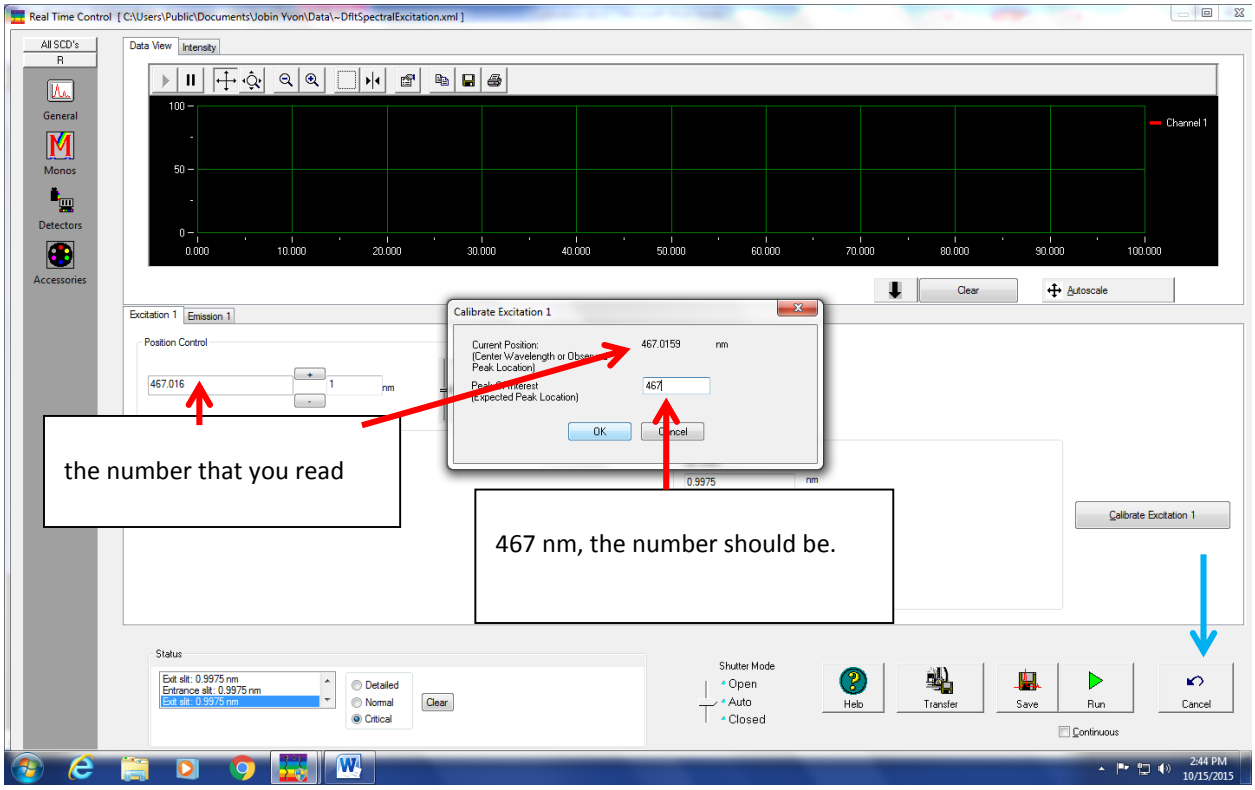

Figure 12 Calibrate excitation

10. Hit the Previous Experiment button. Run again with default settings.

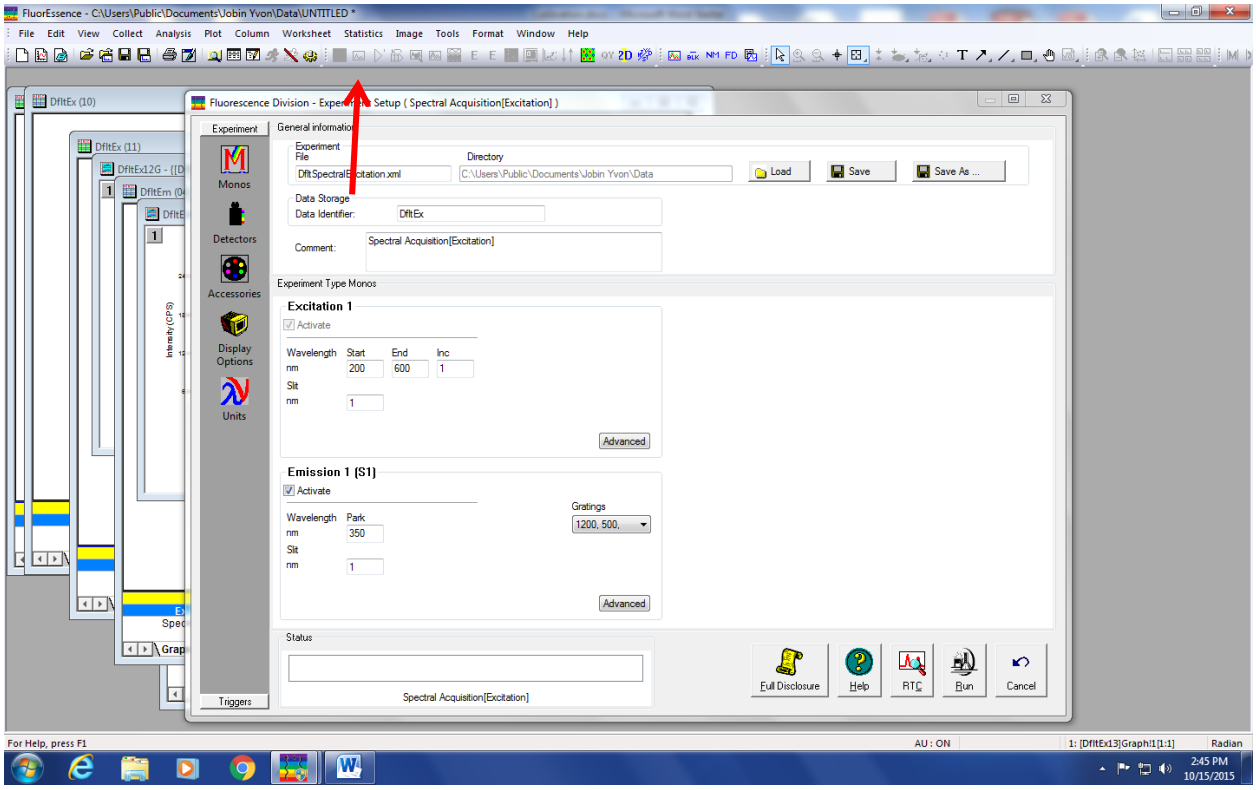

Figure 13 Run PREVIOUS experiment.

11. Confirm the excitation is at 467 nm. The calibration is done.

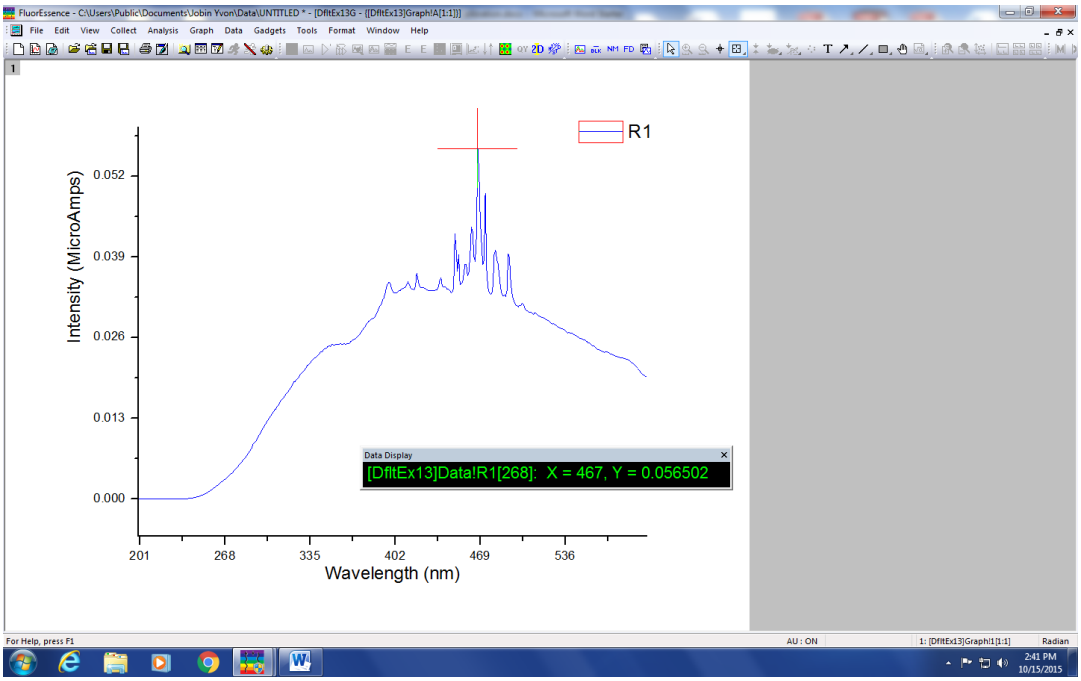

Figure 14 Check the excitation wavelength.

Emission calibration

1. Choose emission. Place MilliQ water sample in sample compartment, and close lid.

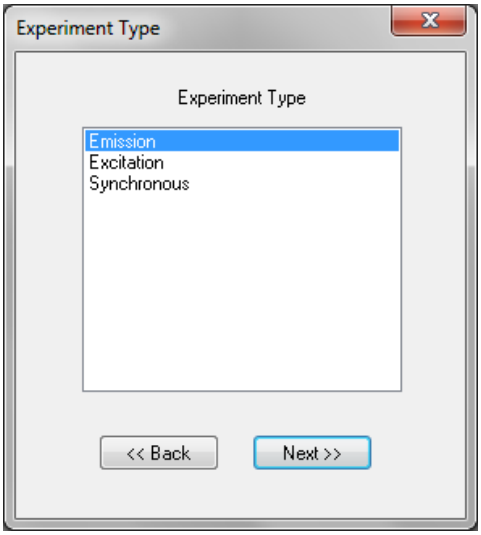

Figure 15 Check Emission

2. Use default settings for the Emission calibration. Hit RUN.

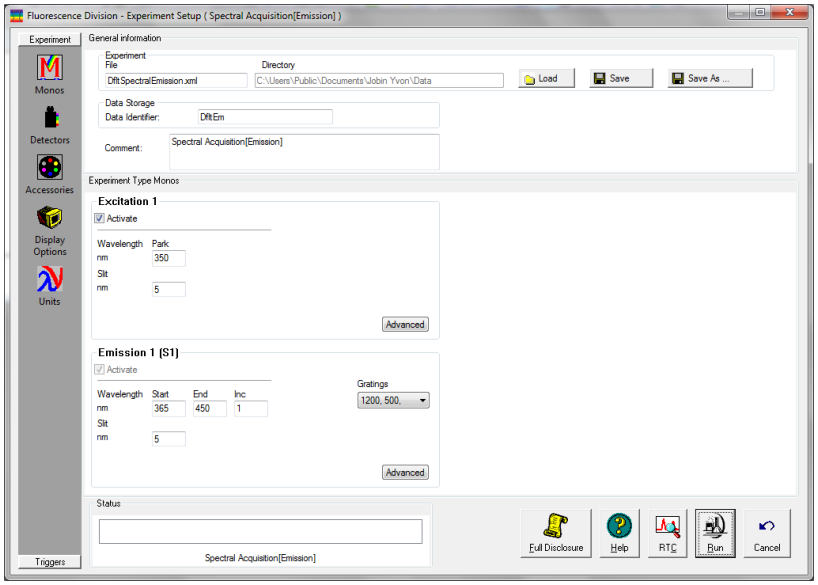

Figure 16 Emission set up window. Use default settings.

3. You will see emission spectra.

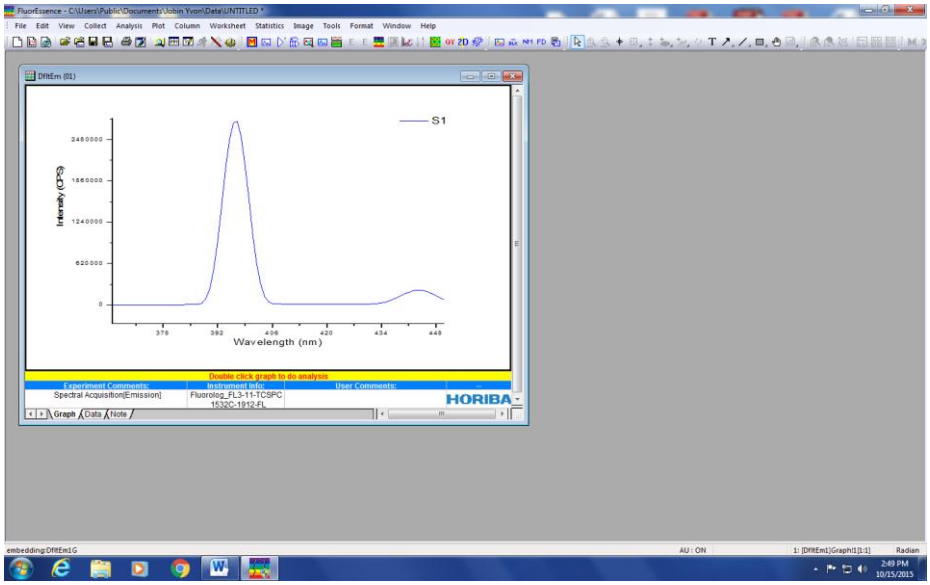

Figure 17 Emission spectra window

4. Put the cursor on the top of the peak. The emission should be 397 nm. If the number is not 397 nm, the emission should be calibrated.

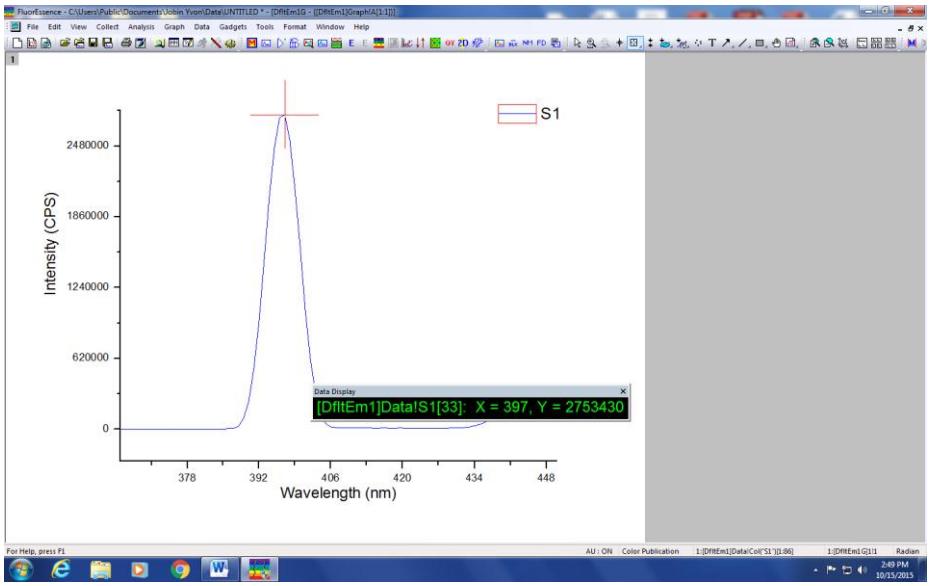

Figure 18 Check emission maximum

5. Click on PREVIOUS experiment button. Click RTC button.

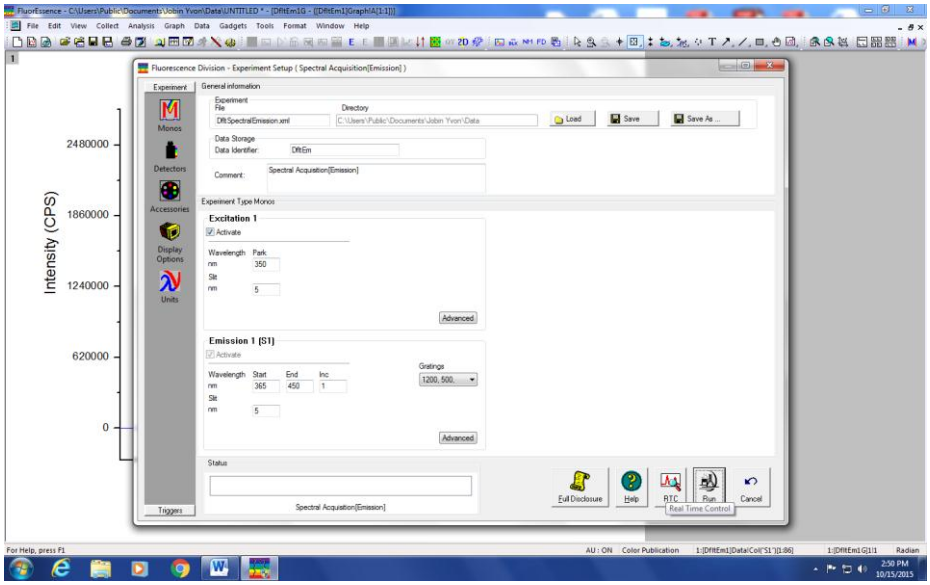

Figure 19 Run RTC

6. Write the number what you have seen in the emission, and write 397 nm in the calibration emission window.

7. Run PREVIOUS experiment again with default settings and then double check the emission wavelength.

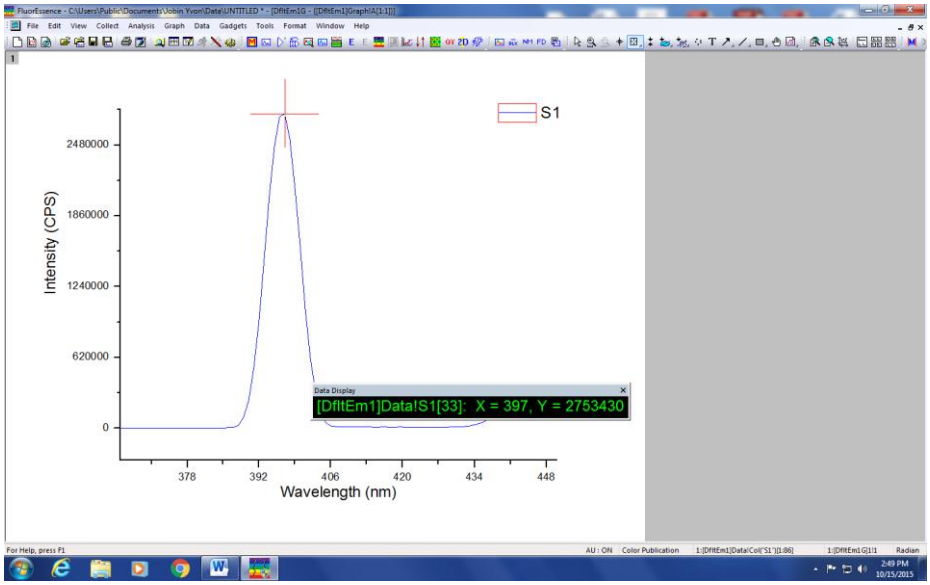

Figure 20 Emission analysis window. Double check the emission is at 397 nm.