Edinburgh FLS1000 Lifetime Spectrometer Standard Operating Procedure

March 9, 2020

Contents

1 Introduction

The Edinburgh FLS1000 Lifetime Spectrometer is designed for fluorescence measurements of liquid and solid samples over excitation and emission wavelengths of 250-900 nm. Variable bandwidth excitation and emission monochromators determine the wavelengths measured. A photon counting detector is used across the entire energy range. Fluorescence lifetime measurements over nanoseconds to microseconds can be made using fixed wavelength pulsed light sources.

2 Acknowledgments

This instrument was purchased with support of the NSF MRSEC program. All publications and patent applications which include work performed on this instrument must acknowledge this support, including the current NSF grant number. For recommended wording, please see `https://www.ccmr.cornell.edu/research/acknowledging-ccmr-funding/`. Use of current grant numbers is vital for funding agency support

3 Safety training

3.1 General Safety Training

Before performing any laboratory work at the Cornell Center for Materials Research Bard Facility, users must have completed the following safety training courses through CULearn:

- EHS 2555 - Laboratory Safety
- EHS 2716 - Chemical Waste Disposal
- EHS 5330 - Fire Safety
- EHS 2397 – Laser Safety

4 Physical Access

Access to Bard Hall room B56 is controlled through a proximity sensor which reads Cornell ID cards. Either before or at the beginning of training, provide the lab manager with a copy of your completed Bard safety form (downloadable from `http://www.ccmr.cornell.edu/ccmraccessform/`). This form includes your ID card number. After completing training, the laboratory manager will confirm that you have completed all necessary safety training courses and then grant you door access. This process may take a few days to complete.

A researcher without a Cornell ID card can request a temporary access card from the laboratory manager.

5 Preparation before coming to the facility

5.1 Liquid samples

Liquid samples are held in cuvettes during fluorescence measurements. These cuvettes are not provided for loan by CCMR, and may be purchased from vendors or from the CCMR. If you need to purchase a quartz cuvette from the CCMR, please consult the laboratory manager in advance. For fluorescence measurements, it is necessary to use 4-side polished cuvettes, since

the Edinburgh FLS1000 spectrometer measures emission at 90° from the direction of the exciting beam to minimize the contribution from scattering. Standard cuvettes hold 2.5 mL - 4.5 mL of liquid, although cuvettes with a smaller sample volume are available. Fused quartz cuvettes can be used over the entire spectral range of the FLS1000. Plastic cuvettes with over 85% transmission over the visible spectrum are available and decrease sharply for wavelengths shorter than 350 nm, as shown in Figure 1. Prices for these items, as purchased through CCMR, can be viewed through FOM using the 'Purchase Supplies' link.

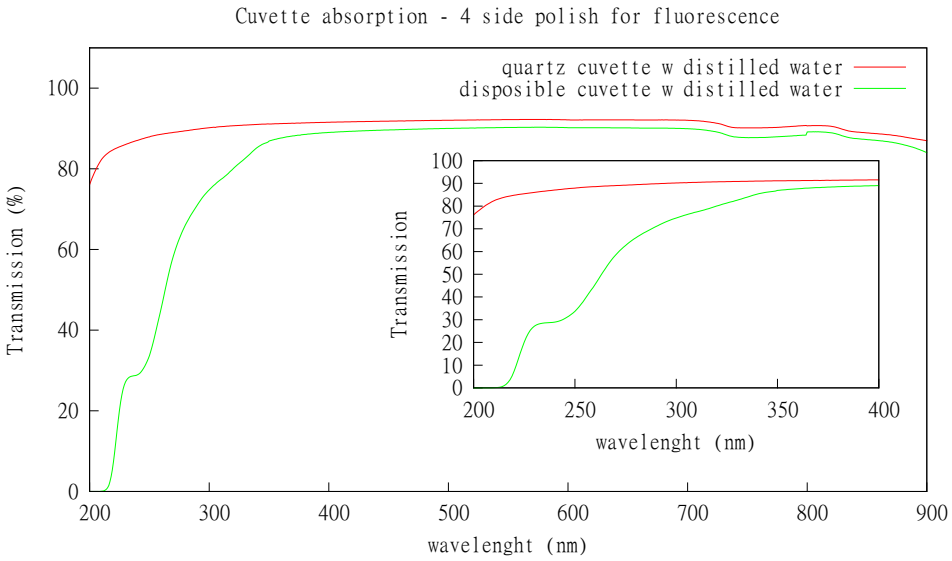

Figure 1: Transmission curves of fluorescence cuvettes measured on Cary 5000 absorption spectrometer. This is the transmission through the entire cuvette at one wavelength. For a fluorescence measurement, you must consider the transmission halfway through the cuvette at two wavelengths, or the product of the square root of the transmission at each wavelength from this plot.

Soda lime glass pipettes and rubber bulbs for sample transfer are available in room B56, located in the back right corner of the room, near the solvent bottles. *If you need to precisely control concentrations, please bring your own micropipette and tips.* Bard Hall room B56 is not a satellite waste accumulation area - you are required to remove any sample material brought

to our lab, in addition to any solvent used to rinse out cuvettes. Isopropanol, Ethanol, and distilled water are available in the lab for cuvette rinsing. DI water is also available if needed for solvating samples.

5.2 Solid samples

A holder designed for samples roughly 20 mm to 27 mm across is available for sample mounting. This provides enough spring force to hold a glass slide. Very thin samples can be either mounted on a glass slide or laid against a slide mounted in the sample holder.

6 Equipment

6.0.1 Liquid sample holder

The sample mount for cuvettes includes integrated thermo-electric heater and cooler, thermocouple, and drive for a mini magnetic stirrer bar (should not be needed in most experiments). A cuvette sits fully within the housing. To remove a cuvette, gently pull the clear plastic peg that extends above the top of the house; this will lift the cuvette so it can be grasped easily. Cuvettes should only be filled a little more than half-way (aim for 2/3 full). Since the light is focused at the sample, only the middle portion is illuminated and overly full cuvettes lead to spills.

6.0.2 Solid sample holder

The solid sample mount holds something of similar dimensions of a cuvette tilted off vertical and rotated so that the a similar view would be seen along either the incident of exit light beam path, but specular reflect won't propagate along the exit path. A holder with a spring-loaded bracket can hold a small frame and slide into the sample mount.

7 Procedure

7.1 Start-up

The power for the system is controlled by a single switch, on the topmost unit labeled 'MAIN POWER SUPPLY'. With the exception of the external

pulsed light sources, this should be the only switch that needs to be flipped. Confirm that the the temperature control unit below the main power supply is on and is displaying the temperature of the detector. This will start out at room temperature and decrease to -15 to -20°C. It takes roughly 15 minutes for the detector to fully cool, which can be determined by looking at the signal rate output from the detector with no incident fluorescent signal on it. This signal is a few thousand counts per second when the detector is warm, and a few hundred counts per second when fully cooled.

7.1.1 Continuous light source

After turning on the power, look at the screen on the lamp housing near the lamp power button. The lamp display next to the power button should say 'Lamp off. Fans on. Ready to start', or just 'Ready to start'. Feel the air temperature at the vent on top of the lamp unit. If this air is noticeably above room temperature, wait until it has cooled sufficiently, which is 15 minutes since the previous user turned off the lamp.

Press the lamp power button to start the Xenon arc lamp. The lamp display should say 'Lamp starting', then switch to show the lamp power, current, voltage and total operating time.

7.1.2 Pulsed light source

If you know that you want to make lifetime measurements and you know what excitation wavelength you want to use (presently 280, 405, and 450 nm available), mount the appropriate source (described below) and switch it on using the key. The laser is available for operation within a few minutes, but it is recommended that users allow 15 minutes for the laser to warm for measurements.

The laser pulse period should be set to 500 ns or longer. The laser is enabled by pressing button on top; this should only be done while making measurements since these lasers have a finite operational life.

7.2 Enabling the control computer

The instrument control computer must be enabled through FOM by a user with an active CCMR FOM account. FOM is accessed through a web in-

terface, and may be accessed through the data analysis computer or any other device with a web browser at `https://fom.ccmr.cornell.edu/fom`. If FOM is accessed through a public computer, you should either use a private browsing session on a browser which is then closed before you finish your experiment. Otherwise, you must log out of FOM through the browser *and* close the browser. Failure to do so may grant another user access to your FOM account through the public instrument.

7.3 Install sample mount

There are three sample mounts, but we will primarily use 2; one for liquids and one for solids. Please read about both mounts since you will need to switch between them. Wear gloves when handling samples or sample mounts, or reaching inside the spectrometer.

7.3.1 Changing from solid sample to liquid sample mount

Remove the lid from the sample chamber, and lift off the panel which makes up the corner on the angled part of the instrument body, to give better access to the sample holder. Lift the sample holder upward. The small square rod connecting the sample stage to the knob on the instrument housing will slide off easily. Remove the holder and place it in the Edinburgh spectrometer drawer below the instrument. Carefully lower the cuvette sample stage from above making sure there is clearance between the large black disk at its base and the lenses. Orient the water fittings on the stage so that they are closest to the edge of the table. When properly aligned, the four reference balls should sit in holes on the base, with the open faces windows of the cuvette holder facing the incident light (to the left) and emitted light (back).

If you plan to use sample cooling, you will need to make cooling water connections and an electrical connection. When making or breaking water connections, first confirm that the chiller and pump are not running. If the system is off, or the Fluoracle software has not been started, then the flow should be off. If Fluoracle is already running on the control computer, select Setup→Temperature Device Setup→Temp. control=Off.

Connections for water are made through quick connect fittings below the housing, which must be accessed through the lowest panel (with the Manufactured in the United Kingdom sticker) on the on the angled side. It is held

in place with magnets and pins, so grip from the side and pull straight out. Place one hand on top of the sample mount to prevent accidentally tipping the sample mount into the lenses. Push the female metal connectors on the plastic hose onto the male fittings on the sample stage, one at a time. You should hear a click when the connection is made.

Make the electronic connection to the thermoelectric heater in the sample stage using the DB15 connection in the compartment below the sample stage.

7.3.2 Changing from liquid sample to solid sample mount

When making or breaking water connections, first confirm that the chiller and pump are not running. If the system is off, or the Fluoracle software has not been started, then the flow should be off. If Fluoracle is already running on the control computer, select Setup→Temperature Device Setup→Temp. control=Off.

Remove the lid from the sample chamber, and lift off the panel which makes up the corner on the angled part of the instrument body, to give better access to the sample holder. To disconnect the water lines, place one hand on top of the sample mount to prevent accidentally tipping the sample mount into the lenses. Reach inside the instrument through the lower opening and grasp the metal collar of a water fitting. Take care to grasp the round metal collar, not hexagonal metal body or the plastic tube. Pull straight down on the collar to disconnect the line.

Disconnect the sample heating/cooling by unplugging the DB15 connection.

Lift the liquid sample mount straight upwards, taking care not to hit the lenses with the bottom of the sample mount. The DB15 connection often catches on the bottom of the chamber; just reach below to free it. Remove the holder and place it in the Edinburgh spectrometer drawer below the instrument. Lower the solid sample mount into the instrument, taking care not to hit the lenses with the bottom of the sample mount. The square rod should be pointed towards the square shaft connected to the black knob on the instrument housing. Tilt the sample mount away from you and slide the square rod into the square shaft. Confirm that the mount is correctly aligned, with 4 reference balls sitting in holes in the housing.

7.4 Fluorescence measurements with Fluoracle software

Start Fluoracle on the instrument control computer if it isn't not already running. Initially the signal rate window should appear. This window, shown in Figure 2, is used for manual control of the instrument and manual observations.

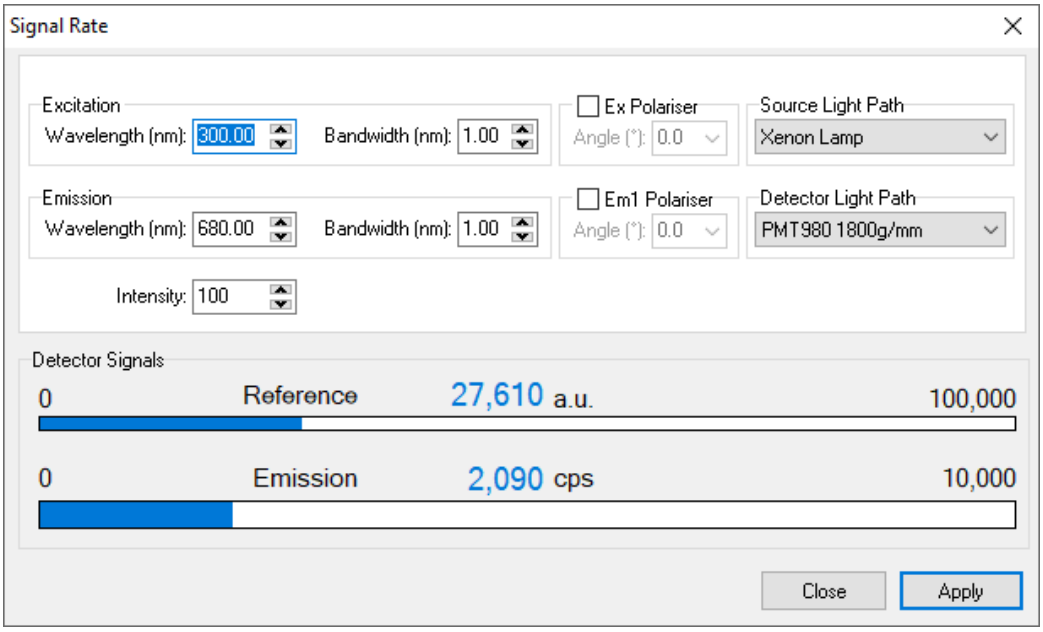

Figure 2: Signal rate window in Fluoracle. The high emission signal with no sample in the compartment indicates that that detector is not fully cooled.

7.4.1 Signal rate window

Changes made in this window will be implemented on the spectrometer either immediately for buttons, checked boxes and drop-down menus, or after clicking apply for numeric changes.

Excitation and emission wavelength The excitation and emission wavelength boxes can be used to control the monochromators to set what emission and excitation wavelengths they pass. All measurements on a fluorescence

spectrophotometer involve two distinct wavelengths; the excitation (incident) and emission.

Excitation and emission bandwidth The monochromators each contain entrance and exit slits which control the bandwidth of the light that they pass. Increased bandwidth results in greater signal, but over a less precise spectral bandwidth. Each bandwidth should be set significantly smaller than the width of any spectral feature that needs to be resolved in an experiment, which is sample dependent. Additionally, during a measurement, the excitation and emission wavelength must be kept roughly 3-5 times further apart than the large bandwidth. The means that if you need emission data close to the excitation wavelength, both bandwidths need to be relatively small.

Excitation and emission polarizers Check boxes are used to insert or retract polarizers in the excitation or emission beam path. When inserted, values polarization orientations of 0°, 35°, 55°, and 90°can be selected.

Source light path This box is used to select the Xenon lamp for continuous wave measurements in which the excitation wavelength is controlled by the monochromator, or a pulsed light source with a fixed wavelength and narrow bandwidth. Changing this field moves a prism in or out of the optical path of the instrument. When the prism is inserted, the light from the lamp is blocked and the light from the laser is directed to the sample.

Detector light path The emission monochromator has two gratings that may be selected from this menu: 1800 lines per millimeter and 600 lines per millimeter. The 1800 l/mm grating can be used for emission measurements from 250 nm to 700 nm. The 600 l/mm grating can be used for measurements 600-900 nm. Even with the 600 l/mm grating, the emission signal becomes very weak over 800 nm because of the efficiency of the detector.

Intensity Variable neutral density filter wheels are located in both the continuous wave and pulsed light source paths. In both cases the attenuation is significantly non-linear, but monotonic, decreasing from full transmission at Intensity=100 (default setting).

Reference signal When operating in continuous wave mode, a partially reflective mirror sends a portion of light from the excitation path (after the monochromator, polarizer, and attenuator) into a reference detector. This signal is used to normalize spectral measurements to account for the spectrum of the source. The signal on this detector must be kept below 4,000,000 arbitrary units to ensure a linear response. Failure to do so will provide an incorrect scaling for portions of the scan. If the signal is above this level, either decrease the excitation bandwidth or the intensity using the filter wheel.

When operating in with a pulsed source, this field displays the pulses per second from the source, as measured via the trigger signal. It should report 0 Hz except when the button on top of the laser has be pressed to trigger the laser. The pulse rate should not be higher than 2 MHz to preserve the longevity of the laser. Furthermore, this rate should not be fast than $1/(10\tau)$ where τ is your fluorescence decay lifetime. Failure to follow this guideline will mean that your sample does not fully relax to the ground electronic state between pulses.

Emission signal The emission signal is measured on a thermoelectrically chilled photomultiplier tube. It is reported in counts per seconds, where each count corresponds an electronic pulse produced when one photon is detected. The maximum rate at which the detector provides a linear response is 1,000,000 cps. Above this rate the counter will not distinguish each pulse as a separate event, and the readings will be too low. This signal can be decreased by decreasing the excitation bandwidth, emission bandwidth, or intensity.

When the system first starts, this detector reads 2,000-4,000 counts per second. When the detector is fully cooled, it reads 200-400 cps with no light on the detector.

7.4.2 Excitation and emission spectra

A typical spectral measurement might proceed as follows:

1. Open the Signal Rate window and set reasonable bandwidths for excitation and emission
2. Set the excitation wavelength to the shortest value (highest energy per

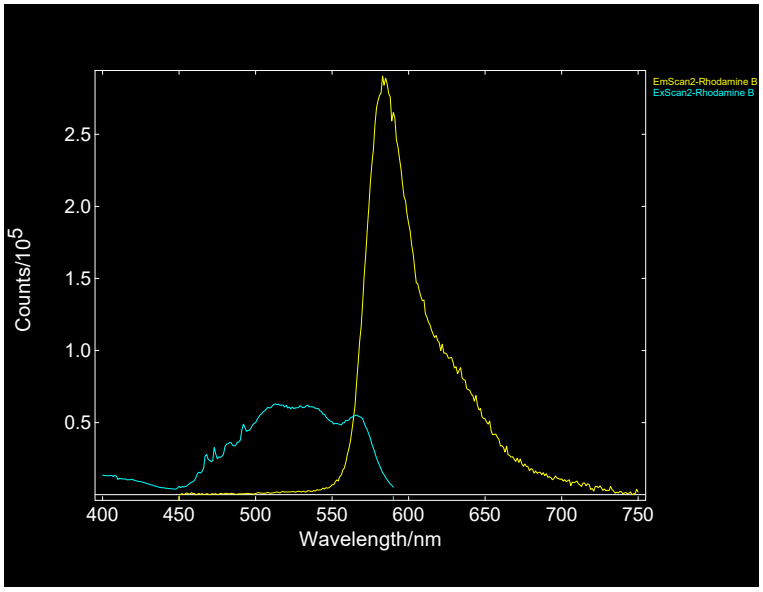

Figure 3: Combined excitation and emission spectra from Rhodamine B dissolved in DI water.

photon) within the instrument's range that you might expect a peak. Make sure that the reference intensity is well below 4,000,000. Close the Signal Rate window.

3. Open an emission scan window by selecting New Spectral→Emission Scan. To ensure that scattered light does not reach the detector, keep the starting wavelength significantly above the excitation wavelength, or $\lambda_{Ex} + \lambda_{offset} \leq \lambda_{Em}$, where $\lambda_{offset} = a(BW_{Ex} + BW_{Em})$ and a is on the order of 3. If sample scattering is strong, then to avoid artificial signal due to second order diffraction from the monochromator, keep the maximum excitation less than twice the source wavelength, or $\lambda_{Em} \leq 2\lambda_{Ex} - \lambda_{offset}$. During the sweep, when near peaks watch the intensity displayed in the lower right hand corner of the screen. This is the raw count rate on the detector, and should stay below 1,000,000. The plotted count rate is corrected based on variation in the monitor intensity and the instrument's efficiency as a function of wavelength.

4. Set the emission wavelength higher than the emission peak ($\geq \lambda_{peak} + \lambda_{offset}$). Select New Spectral→Excitation Scan. Scan the excitation

wavelength from a low value up to the emission peak.

5. With the excitation and emission plots open, click the merge plots button along the top to make a combined graph that has both pieces of information, as shown in Figure 3.

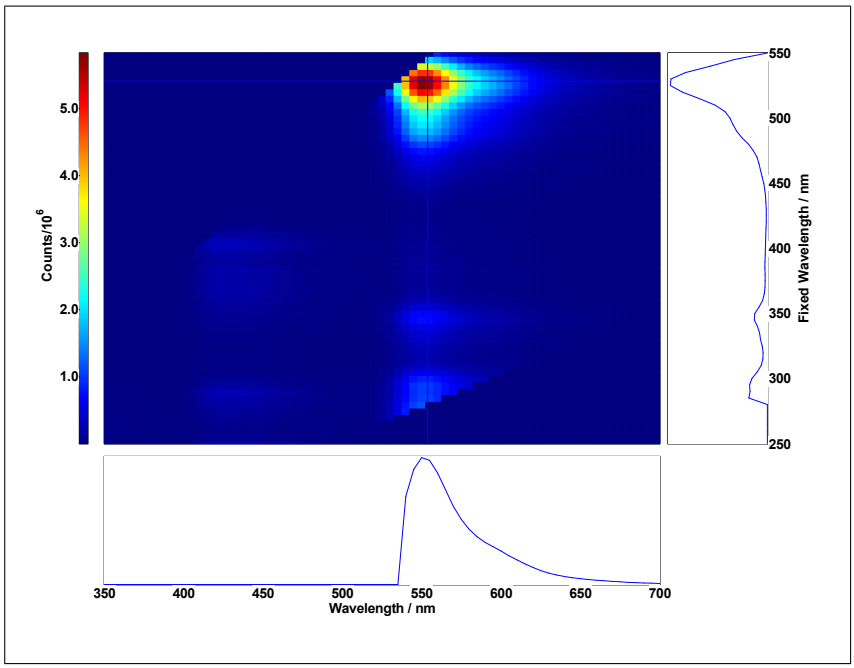

Figure 4: Excitation and emission map from Rhodamine B, Fluoroscein and yellow highlighter dissolved in DI water. The horizontal axis is emission wavelength and the vertical axis is the excitation wavelength. The top left portion of the plot contains no data because of the requirement that $\lambda_{Ex} + \lambda_{offset} \leq \lambda_{Em}$. The lower right portion has no data because of the requirement that $\lambda_{Em} \leq 2\lambda_{Ex} - \lambda_{offset}$. Both of these are enforced by the scan software with λ_{offset} chosen by the user.

Excitation-emission maps can be made, where both wavelengths are scanned. This is more time consuming than individual plots, and is most useful when there are multiple fluorescent compounds present in a sample. The user can specify a width that the emission measurement stays away from the excitation wavelength, so the end result is not a rectangular grid of data, as shown in Figure 4.

7.4.3 Lifetime measurement

The Edinburgh FLS lifetime spectrometer can readily measure decay times from 1 ns to 50 us. Faster decay times, down to 5 ps, can in principle be measured, but great care has to be taken to characterize the pulse shape and fit the decay. Decay measurements are made by recording the time between an excitation pulse and a single emitted photon being detected. Because of the instrument design, only one photon can be measured per pulse. In order to ensure that the all emitted photons have an equal probability of being observed, the count rate on the detector must be <= 0.03 times the pulse rate. A typical procedure follows:

1. Determine which available wavelength source – 280 nm LED, 405 nm laser diode laser, or 450 nm diode laser – is most appropriate for the experiment. This is based on prior experience, literature information, or based on an excitation scan as described above.
2. Install the pulsed source on the side shelf outside the spectrometer. Check that the repetition time is 500 ns or longer. If in doubt, set it to a longer time. Turn on the laser.
3. Wait 5 minutes.
4. In Fluoracle, go to Setup→EPL Lasers Setup and choose the appropriate wavelength source.
5. Open the Signal Rate window, and change the Source Light Path. The top Detector Signal should have changed from Reference to arbitrary units to EPL in Hz.
6. Press the red Laser On/Off button on the laser.
7. With the Emission wavelength at the desired value, check that the emission count rate <0.03xEPL_rate. For example, using the 1 us setting on the laser, the EPL rate should be very close to 1 MHz, and the emission rate should be below 30 kHz. If this is not the case, decrease the emission bandwidth or change the Intensity (attenuate the incident beam). Close the Signal Rate window.
8. Select New Lifetime→Manual. Configure the measurement:
   (a) Time range - this should be much longer than your decay time

(10x) and much shorter than your repetition time (0.1x).

(b) Channels - 512 is usually reasonable.

(c) Peak counts - this determines the statistical quality of the data an how long the measurement takes. The software builds up a histogram of photons vs delay time with a bin width determined by time range/channels, and ends when any single channel gets to 'peak counts'.

(d) Click new. The histogram of events is plotted on a logarithmic plot. Within ten seconds of measuring, you should have an idea of whether the time range chosen was suitable. Example data is shown in Figure 5.

(e) Press the laser of/off button when done with the scan. It does have a finite lifetime.

9. Zoom in on the portion of the scan which appears to have a linear decay on the log plot. This should start a few ns after the peak.

10. Select Analyze-Exponential Tail fit. Put in an initial guess for the decay time tau, and run the fit. Fluoracle generates plots of fit data and measured data vs time and fit error vs time and displays the decay time, as shown in Figure 5.

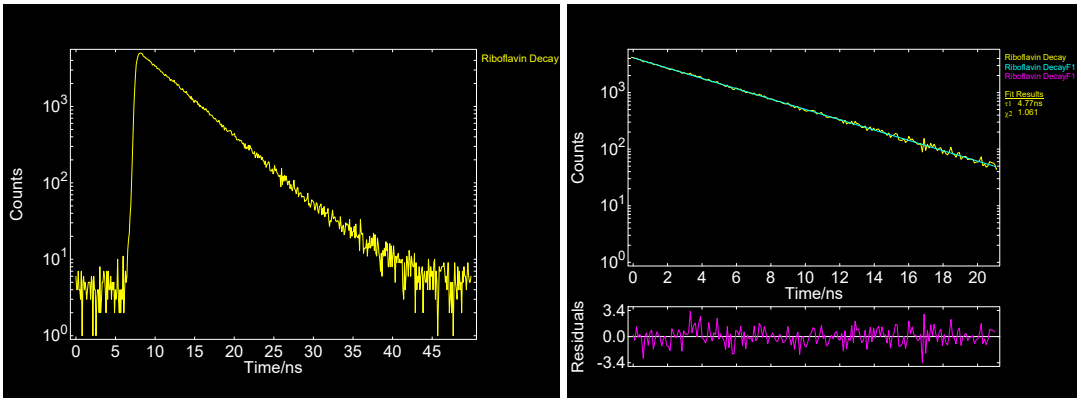

Figure 5: Fluorescent lifetime measurement from Riboflaven B (left) and and exponential tail fit to the data (right) showing a 4.8 ns decay time.

7.5 End of experiment

7.5.1 Cleaning up work space and glassware

This instrument is in a shared laboratory space. It is therefore important that each user take responsibility for cleanliness of the work space. Simply put, leave the lab space as clean as you found it. This includes:

- Clean any glassware or other lab hardware which came into contact with samples. When rinsing glassware, capture any solvents in a container which you remove at the end of the experiment, with the exception of water used for final rinsing, which may go down the drain.
- Dispose of any pipettes (including plastic) in the trash containers labeled 'glass only'. These containers should only be used for pipettes, broken glassware and used slides.
- If you used powder or liquid samples, wipe down any work surfaces where samples were handled or transfered.
- Remove samples. Take all samples and other materials with you.

7.5.2 Shutting down instrument

If there is no user coming after you during the day, and the Xenon arc lamp is on, press the lamp on/off button. The lamp fans stay on for a time (potentially a few minutes), and the display near the button says that the lamp is off and the fans are on. The main power to the unit should not be shut off while the instrument is in this start. Once the fans are off, the display says that the fans are off, and says please wait with a timer counting down for roughly 15 minutes. This is how long one should wait before restarting the lamp. The main power supply can be switched off during the wait period.

If a pulsed source was used, it should be switched off with the key.

7.5.3 Disabling the control computer through FOM

Disable the instrument through FOM. Either go to the instrument schedule page and click on your present use, or go to your User Home and click on the instrument logoff link at the top of the page. A pop-up box will appear. Select the appropriate funding source (if you have more than one option), and then click on the 'Logoff' button. If you access FOM through a shared

computer you must log out of FOM *and* close all instances of the web browser to avoid unintended access of your account by another user. Log our of FOM by clicking on the red 'X' in the top right corner of the page, next to your name, and confirm that you wish to log out.

8 Quick Review

1. Feel the temperature of the source. If it's warm, let it cool for 15 minutes.
2. Turn on the instrument power. Turn on the Xenon lamp if you are measuring spectra. Mount and turn on (but don't pulse) laser if measuring lifetimes.
3. Install solid or liquid sample holder.
4. Mount sample.
5. Set excitation and emission wavelengths to expected peaks and set bandwidths. Confirm Referenence < 4 M cps and Emission < 1 M cps.
6. Configure and run scans.
7. Save data as Fluoracle and .csv files.
8. Transfer data via Box or Dropbox
9. Turn off source.
10. Clean workspace.
11. Turn off instrument (when fans are not running).
12. Disable the instrument through FOM.
13. Make sure you cleaned the work surface and remove all of your materials.