

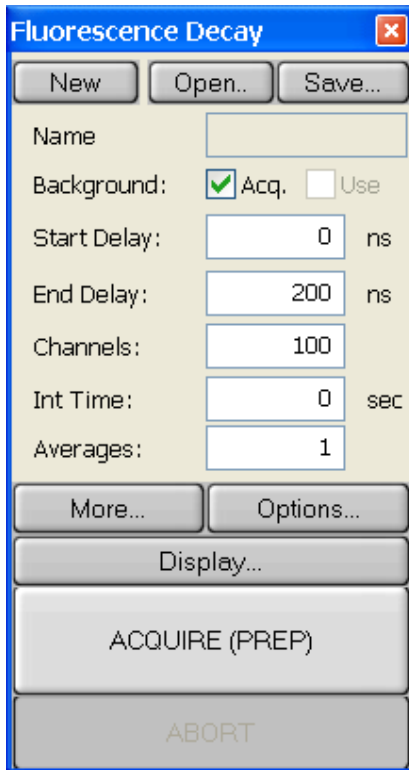


Example Experiment

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- A. You will need the following items to carry out a fluorescence lifetime measurement:
1. Fluorescent sample, which can be one of the following:
 - Solution in a 10 mm x 10 mm cuvette
 - Solution in a microcuvette (510 optional accessory), which is adaptable to 10 mm x 10 mm cuvette holder
 - Solid sample (e.g. slide) mounted on the 503 solid sample holder (optional accessory)
 - Sample placed in the 502 liquid nitrogen dewar (optional accessory)
 2. Scatterer solution (to acquire the instrument response function, IRF), which can be one of the following:
 - Colloidal silica (available as Ludox from Aldrich) highly diluted in water
 - Glycogen in water
 - In an emergency situation, powdered non-dairy creamer in water can also be used
 3. Emission filter (typically a cut-off type, refer to 504, 505 and 506 filter accessory sets) to cut off the excitation light
 4. The 508 set of neutral density filters (optional, but helpful accessory)
- B. To prepare the instrument for the measurement:
1. Turn the EasyLife ON. The red LED power indicator on the EasyLife front panel should be lit
 2. Turn the computer ON.
 3. Make sure the emission slit is partly open, e.g. start with 3-mm opening (2 full turns = 1 mm).
 4. Place the sample in the sample holder
 5. Place the emission filter in the filter holder
- C. Next step is to set up the acquisition parameters in EasyLife V. The following example is based on 1 nM fluorescein solution in 0.1 N NaOH.

1. Click **Acquire** on the menu bar and select **Fluorescence Decay** on the drop down menu. Select **FL Decay** option. This brings up the **Fluorescence Decay** dialog box. Set the required parameters:



- **Background:** Check the Acq box
- **Start Delay** (ns). Enter a value, which is about 3 to 5 ns in front of the excitation pulse. If you don't know where the excitation pulse is, enter 0.
- **End Delay** (ns). Add about 5 to 8 times your sample lifetime to the **Start Delay** and enter here. If you have no idea what the lifetime of your sample is, enter a relatively large value, e.g. 200 ns.
- **Channels.** This is the number of data points defining the decay curve. Enter a desired value, e.g. 200 (use less channels to speed up the experiment and more channels to improve accuracy of the measurement).
- **Integration** (s). This controls the time over which the output signal is averaged at each time delay step. Enter 0.1 s for a quick scan. Use a lower value to speed up the experiment or a larger value to improve signal to noise.
- **Averages.** Number of scans to be acquired and averaged. Enter 1 for a quick scan, use more averages to improve signal to noise.

2. The **FL- Decay** dialog box has three buttons **More...**, **Display...**, and **Options...** which lead to further dialog boxes. See Common Acquisition Controls in the manual for more information.

D. In order to acquire the fluorescence decay:

1. Click **ACQUIRE** and then **START**, which acquires the background. *Note:* the Green Trigger LED on the front panel of the EasyLife should be lit now. The **Background Acq** checkbox will then dim, and the **Use** box will be checked.
2. Click **ACQUIRE** and then **START** again, which initiates the decay curve measurement. It will take a few seconds to complete the scan.

After the scan is completed, you will likely see a decay trace, which will look like one of 3 traces depicted in Fig. 1.

First of all, it's obvious that the time window is much too large. We deliberately selected a broad time range in order to locate the signal. Now we can narrow the time window to a meaningful range. In this case we go back to *Acquire* menu and change *Start Delay* to 65 ns and *End Delay* to 95 ns. A rule of thumb is to use the total time range (i.e. range = *End Delay* - *Start Delay*) of approx. 5 to 8 times the lifetime value. The lifetime can be approximated as the full width of the decay trace at half maximum. The *Start Delay* should be a few nanoseconds in front of the excitation pulse, so the pre-pulse baseline could be determined.

If the intensity is too high, the decay will look like trace A, i.e. it will have a truncated top part. If this is the case, reduce the slit width and repeat the measurement (Step D) with the new *Start Delay* and *End Delay* settings. Alternatively, you may use a neutral density filter (optional) to reduce the intensity. Keep in mind that a filter with optical density (OD) of 0.3 will reduce the intensity approximately 2 times.

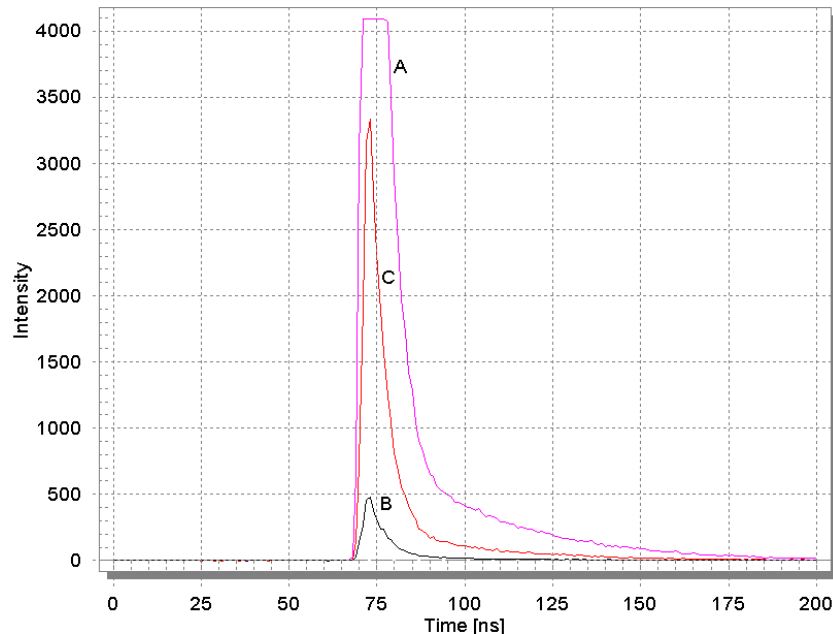


Fig. 1. Three different outcomes of decay curve measurement: A – intensity too high; B – intensity too low; C – good level of intensity. The time range is too broad for all 3 traces.

If the intensity is too low (i.e. Fig. 1 trace B), you may want to increase the slit opening to admit more light to the detector. Open the slit wider and repeat the measurement (Step D) with the new *Start Delay* and *End Delay* settings. If the slit is fully open (6 mm) and the intensity is still low, the way to improve the signal to noise is to increase the integration time, use more averages, or both. If feasible, you may also increase the sample concentration, but be aware of possible inner filter or other effects.

If your decay looks like trace C in Fig. 1, the intensity is adequate, so you can just repeat the measurement (Step D) with the new *Start Delay* and *End Delay* settings. The final decay should look like trace A in Fig. 2.

- E. The next step is to record the Instrument Response Function (IRF) using the scatterer solution. To record the IRF do the following:
1. Remove the sample and place the cuvette with scatterer in the sample holder.
 2. Remove the emission filter.
 3. **DO NOT CHANGE** such parameters as *Channels* or *Start Delay* and *End Delay*. You may change *Integration* or *Averages* if required.
 4. Click *ACQUIRE* and then *START* to measure the IRF.

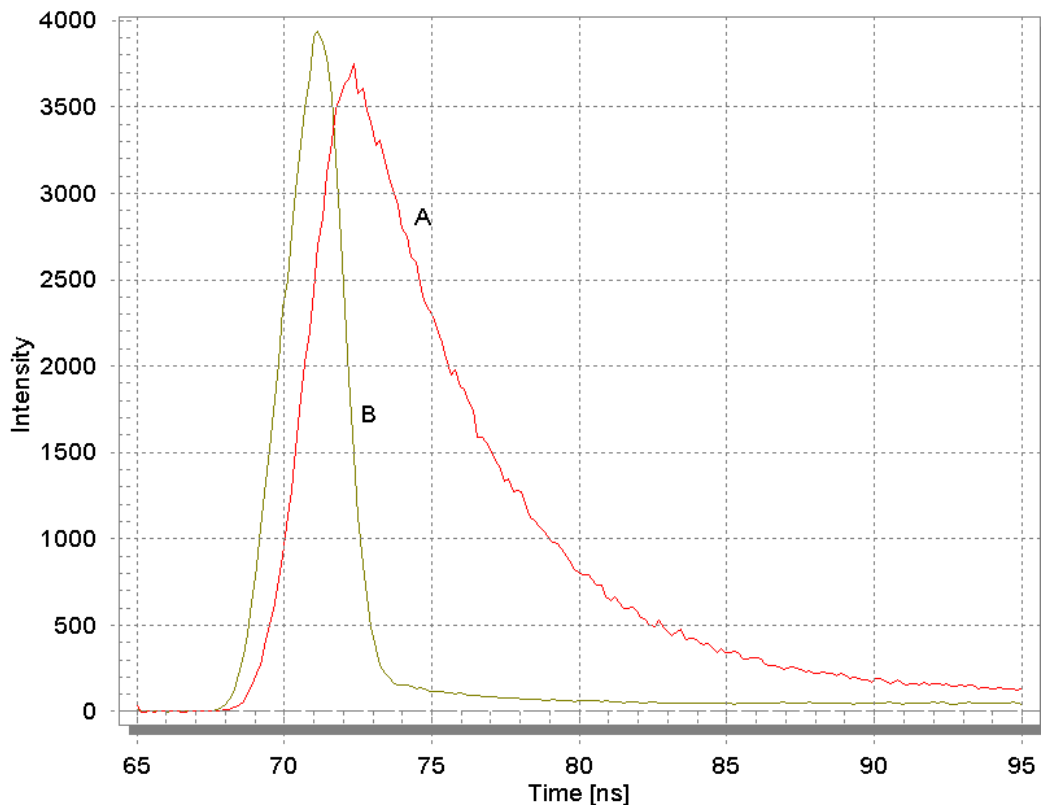


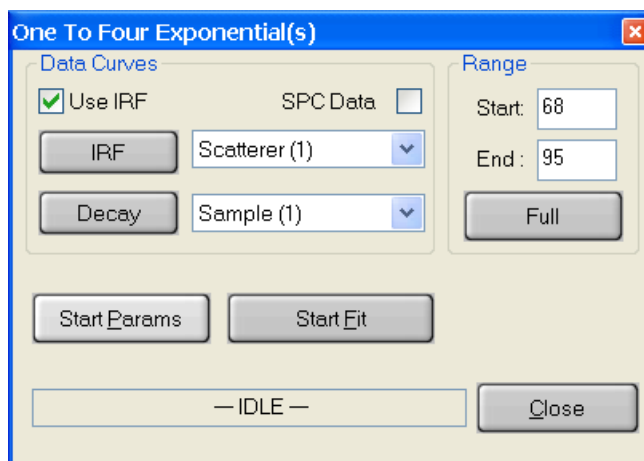
Fig. 2. A typical outcome of correctly conducted fluorescence decay measurement. A – fluorescence decay; B – instrument response function (IRF).

If the intensity is too high (truncated top), lower the concentration of the scatterer or place a neutral density filter in the excitation or emission filter holder and repeat the

measurement. Avoid changing the emission slit opening in order to adjust the intensity of the scatterer, as it may change the size of the illuminated spot on the PMT photocathode leading to potential undesirable artifacts.

If the intensity is too low, increase the concentration of the scatterer and repeat the measurement. As stated above, avoid changing the emission slit opening to adjust the intensity of the scatterer.

- F. Once the fluorescence decay and the IRF have been measured (Fig. 2), the fluorescence lifetime can be determined with the use of analysis software.
 1. Click **Math** on the menu bar, run the pointer down to highlight **Analysis** on the drop down menu and across to highlight **1 To 4 Exp. Lifetime** on the second drop down menu. Click on this to bring up the **One To Four Exponential(s)** dialog box shown below. Make the following selections:



- **Use IRF** – controls whether the instrument response function will be used in the analysis. In most cases the IRF is used except when the lifetime of the sample is very long compared to the temporal width of the LED pulse. Toggle this option on or off by checking/unchecking the box.
- **SPC Data** – Used only when single photon counting data has been imported for analysis. Toggle this option on or off by checking/unchecking the box. Leave it unchecked for EasyLife data.
- **IRF** - Select the scatterer decay curve by clicking on the name of the appropriate curve in the legend. Click on the IRF button. The name of the curve will appear in the window.
- **Decay** - Select the sample decay curve by clicking on the name of the appropriate curve in the legend. Click on the decay button. The name of the curve will appear in the window.
- Click **Start Parameters** to enter the fitting parameters.

- **Number of Lifetimes** – The number of different lifetimes used to analyze the decay curve. Select a number between 1 and 4.
- **Pre-exp., Lifetime, Fix** – For each of the lifetimes to be used in the fit an initial guess for the lifetime and the preexponential factor (**Pre-exp**) must be given. Only the relative values of the preexponential factors are relevant here so that for a single exponential fit the value 1 is normally used. Each of the lifetimes chosen for the analysis may be fixed at the input value or allowed to float in the fit. Toggle this option on or off by clicking on it. Occasionally, the fit will not succeed if the starting values are too far off. If this occurs, try changing the starting values.
- **Fix Shift** – The shift parameter accounts for some instrumental artifacts, such as the ‘color shift’ effect or potential instrumental instabilities. It corrects for a zero-time shift of the excitation pulse relative to the decay (see Data Analysis chapter in EasyLife V Manual for details). The shift parameter may be fixed at the input value (in nanoseconds) or allowed to float in the fit. Toggle this option on or off by clicking on it. A value of 0.0 is usually a good initial guess
- **Fix Offset** – Since fluorescence decays are obtained with an analog measurement, there will always be a small offset over imposed on the decay signal. This offset may be included as a parameter in the analysis. The offset parameter may be fixed at

the input value or allowed to float in the fit. Toggle this option on or off by clicking on it.

- Click **OK** to accept the fitting parameters and exit.
- **Start, End** – Position the mouse pointer at the desired start delay, click and hold down the left mouse button, drag the mouse to the desired end delay and release the button. The selected range will be shown on the screen in a different color. Alternatively, the start delay and end delay for the portion of the sample decay that is to be analyzed may be entered from the keyboard.
- Click **Full** if you want to analyze the decay over the entire time range.
- Click **Start fit** to initiate lifetime analysis.

The progress of the analysis is shown in the box marked idle in the above illustration. The fit may be stopped at any time by clicking the **STOP** button.

The results of the fit are shown in two different ways. The best-fit curve, the residuals and the correlation are displayed as curves on the screen. The values of the parameters found in the fit and some statistical information on the fit are entered into a text editor, which pops up in a window at the end of the fit. Please consult the EasyLife V Manual for details about the fit parameters.

Click **Close** to exit this analysis mode.

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