Technical Note

Sensitivity of FluoTime Series Lifetime Spectrometers

Peter Kapusta, PicoQuant GmbH, August 2002

Introduction

What does “sensitivity of a lifetime spectrometer” mean? The crucial parameter associated with lifetime spectrometers is the time-resolution, intuitively understood as the shortest decay time that can be resolved (but not necessarily measured!) from the instrument’s response to a short excitation pulse. Time-resolution is a kind of sensitivity as well; it refers to the ability to detect small changes of the decay kinetics. On the other hand, today’s widespread use of Time-Correlated Single Photon Counting (TCSPC) is partially based on the fact, that TCSPC is inherently very sensitive in a classical, spectroscopic sense. No wonder: single photon counting means detection of very low signal intensities. A frequent question of researchers is then: what is the smallest concentration, which allows for lifetime measurement with your instrument? Such a simple question – as usual – has a simple “answer”: it depends on your sample. This application note describes a benchmark test of FluoTime 200 and FluoTime 100 lifetime spectrometers. Various aspects of lifetime measurement of low concentration samples are discussed.

Where does the sensitivity come from?

1) Fluorescence measurements, no matter whether steady state or time-resolved, are sensitive due to the “zero background” principle. They involve absolute measurement of light intensity with virtually no background, in contrast to e.g. absorption spectrometry that measures small difference of two intense signals.

2) Photon counting – the light detection method of choice for timing applications – has excellent signal-to-noise ratio and wide dynamic range.

3) By accumulation of time-correlated photon events, as done by TCSPC, it is possible to record decay curves even in the case, when the intensity of the sample’s response is lower than the detector’s (and the overall electronic) noise. This is one of the greatest and often overlooked advantages of the TCSPC method. The reader is kindly referred to our application note [1] for details.

4) Last but not least, the spectrometer design influences the efficiency of detection. Instruments can be optimized for ultimate time-resolution, ease of use, portability, etc. It is difficult to fulfil various requirements simultaneously. With the FluoTime 100 and 200 we try to provide solutions at both ends of this spectrum. Please refer to the datasheet of the FluoTime 100 and 200 spectrometers or check out our website for details.

Sample related issues

Detectability, or in other words, the minimum detectable concentration (MDC) of a fluorophore is proportional to the product of its fluorescence quantum yield and molar absorptivity (also referred as exctinction coefficient or absorption cross section). The more the better. High absorptivity means more “effective use” of excitation light at a given concentration. High quantum yield means more efficient conversion of the absorbed (usually small) amount of photons to fluorescence response. Popular

fluorophores have absorptivities on the order of 10^4–10^5 liter·mol^{-1}·cm^{-1}, measured in the main absorption maximum. [2] However, the absorptivity value relevant for us, measured at the excitation wavelength, is usually much smaller. This is because the wavelength of the laser (i.e. the preferred excitation source for high sensitivity measurements) is fixed and rarely coincides with the spectral position of the absorption maximum. (PicoQuant offers a broad range of pulsed light sources, please check out our website for up-to-date information.) Finally, the emission spectrum also influences the detectability. Standard FluoTime spectrometers use PMTs that are sensitive up to 650, 820 or 850 nm. Due to photocathode physics, the detector's spectral sensitivity decreases closer to its red limit, and falls down rapidly outside the rated wavelength range.

It follows, that complete evaluation of the spectrometer sensitivity requires measurements of various fluorophores under various, well documented experimental conditions. This goes beyond the scope of this note. For sake of clarity and simplicity, we will restrict ourselves to a comparison of test results obtained with two different spectrometers using the same sample and excitation source.

Experimental procedure

A master solution of Coumarin 6 (LC5370, CAS reg. num. 38215-35-0) in spectral grade ethanol was prepared. The precise starting concentration was determined by absorption spectroscopy. For example, optical density (absorbance) of 0.3 at 455 nm measured against pure solvent corresponds to 5.6 µM. [2] Samples down to sub-picomolar concentrations were prepared by consecutive volumetric dilution. (10 nM Coumarin 6 solution has a theoretical optical density of 0.00025 that cannot be measured with usual absorbtion spectrometers.)

We used the PicoQuant LDH 400 violet laser diode driven by PDL 800-B at 10 MHz repetition rate. The laser delivered 270 µW average output power at 414 nm. It should be noted that the sample absorbance at 414 nm is less than half of that measured at the maximum of the absorption band (see Fig.1).

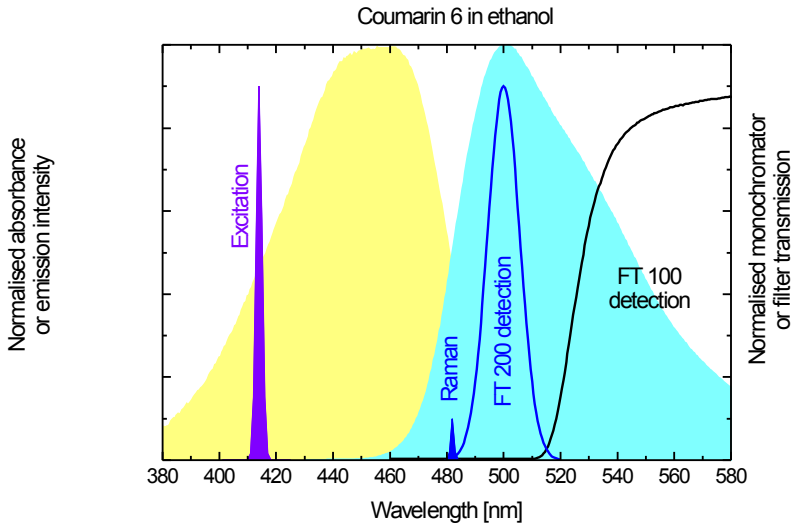

Fig. 1: Absorption and fluorescence emission spectra of Coumarin 6 in ethanol. Excitation and Raman scattering wavelengths are also indicated. Note that the vertical (intensity) scale is normalized and does not correspond to the real intensities. See the text for explanation of FT 100 and FT 200 detection spectral ranges.

Polarization plane of excitation was set to vertical and magic angle polarized emission was detected. The experimental runtime to collect TCSPC histograms were fixed to one minute only, for all samples. Histograms were analyzed by FluoFit release 3.1 lifetime analysis software.

FluoTime 200

This spectrometer uses Glan-Taylor polarizers in both excitation and emission arm, and a 100 mm single monochromator to select the detection wavelength and bandpass. Since we are looking for the sensitivity limit, wide slits (2 mm, providing 16 nm bandpass, see Fig.1) were used and all irises were completely open. The detector was an equivalent of PMA 182 from PicoQuant, based on Hamamatsu H5783-P04 photomultiplier tube.

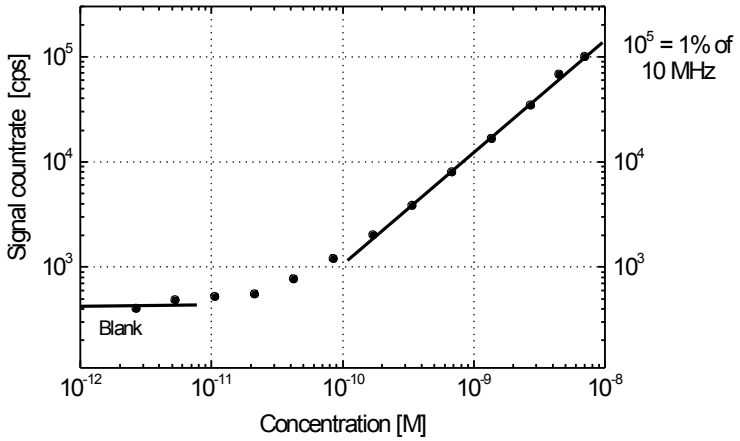

Fig. 2: Signal countrate vs. sample concentration. FluoTime 200 Basic, see the text for experimental conditions.

Looking at Fig.2, the high sensitivity is evident at a first glance. Since the signal countrate must be kept lower than approx. 1 % of the excitation rate (see [1]), it was necessary to dilute the master solution by a factor of more than 100 to reduce the signal intensity. Further dilution down to 0.1 nM leads to almost linear decrease of the fluorescence signal countrate, i.e. exactly how it should be in theory. However, decreasing the sample concentration even further leads to a deviation from the linear intensity-concentration relationship. In this case, the main reason is the finite stray light rejection of the single monochromator and the detector's dark noise. At 10 picomolar (10^{-11} M) concentration, the signal countrate and the blank countrate are equal within the experimental precision. Nevertheless, our main interest is in the decay curves and the lifetimes recovered.

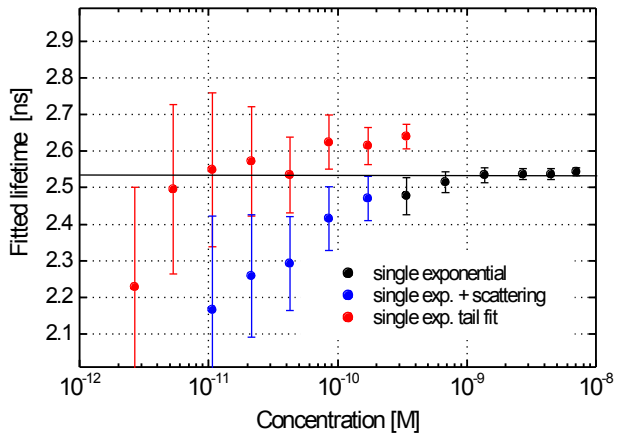

Fig 3: Single exponential fit results vs. sample concentration. FluoTime 200 Basic, see the text for experimental conditions.

Reconvolution fit of 0.7 nM Coumarin 6 solution's decay yields $\chi^2 < 1.2$ and lifetime of 2.52 $\pm$ 0.03 ns, that is still in excellent agreement with the 2.54 ns average lifetime obtained at $c > 10^{-9}$ M, see Fig.3. At lower concentrations, the increasing amount of scattered excitation light manifest itself as an additional ultrafast decay component. Introducing a second exponential term to the reconvolution with a fixed "lifetime" of 1 ps mimics the scattered light. 10^{-10} to 10^{-11} M sample decays can be fitted with this "pseudo" single exponential reconvolution. The χ^2 remain less than 1.2, but the recovered lifetimes systematically deviate to lower values (blue points in Fig.3). An alternative approach is to perform a single exponential tail-fit instead of complicated reconvolution. Cutting off the initial ultrafast portion of the decay effectively "eliminate" the scattered light. The fit results obtained so are plotted on Fig.3 by red.

FluoTime 100

This compact spectrometer uses inexpensive sheet polarizers in both excitation and emission arm. The spectral area of detection is determined by the selected filter. During the test, we used a 530 nm long-pass glass filter (see Fig.1) and the PMA 185 detector.

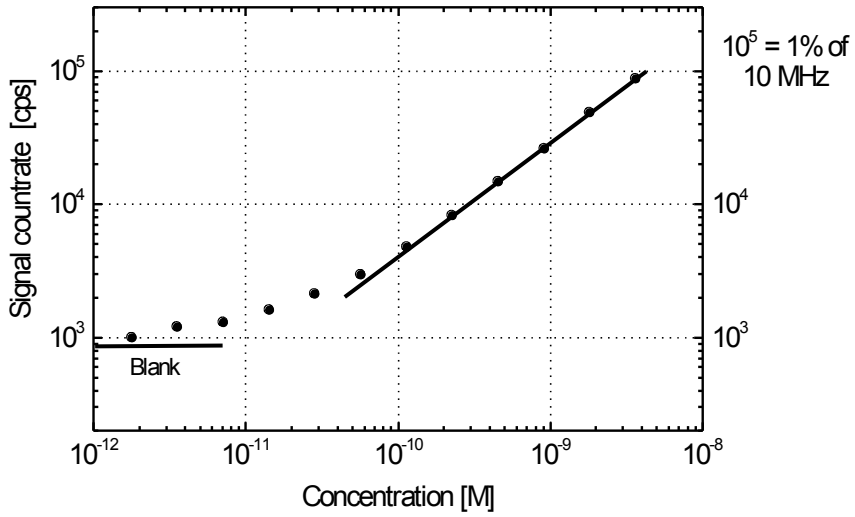

Fig. 4: Signal countrate vs. sample concentration. FluoTime 100, see the text for experimental conditions.

Fig. 4 demonstrates that the sensitivity is even higher. To reach the countrate of 10^5 cps (i.e. the 1% of 10 MHz pile-up limit), dilution down to 3 nM was necessary. The signal intensity is a linear function of the sample concentration down to 10^{-10} M. In comparison to FluoTime 200, the background is now higher, due to contribution of (very weak) parasitic filter luminescence.

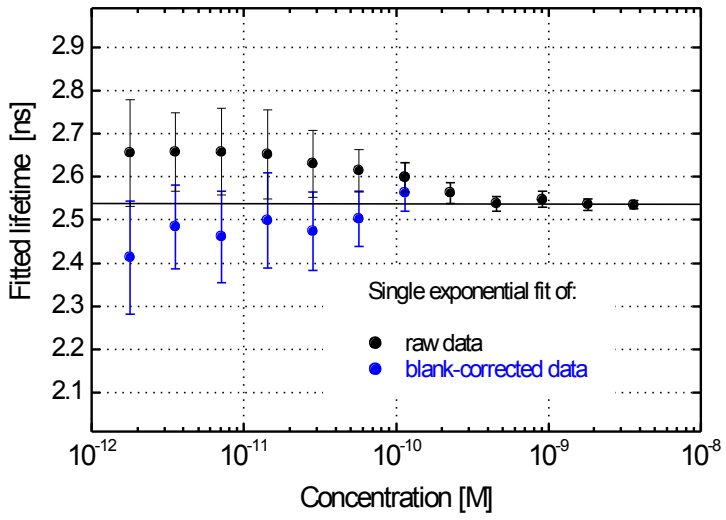

Fig 5: Single exponential reconvolution fit results vs. sample concentration. FluoTime 100, see the text for experimental conditions.

Fitting the raw data to a single exponential model with reconvolution yields lifetime values plotted in black on Fig. 5. Below 10^{-10} M concentration, the quality of the fit (as measured by χ^2 value) deteriorates and the recovered lifetime value is longer that the real one. The reason is the increasing relative

contribution of filter luminescence mentioned above. It is excited by the scattered excitation light and contains long-lifetime components. Mixing these components to the sample's response influences the fit results. Most glass filters and the majority of bandpass filters exhibit such a parasitic luminescence when evaluated at these extreme sensitivities. Although very weak, it becomes significant when the pure signal intensity decreases. Fortunately, this complication can be partially eliminated. Using a blank sample (in our case: pure ethanol in the same type of cuvette) one can record a "pure" filter decay and then subtract it from the Coumarin 6 decay curves. The results obtained by fitting such a blank-corrected decay traces are plotted on Fig.5 as blue circles.

Conclusion

At last, what is the smallest Coumarin concentration, which allows for lifetime measurement with FluoTime? Figures 3 and 5 can be interpreted in various ways. TCSPC perfectionists, insisting on rigorous reconvolution analysis and always expecting $\chi^2 \approx 1$ will say that 500 pM for FT 200 and 100 pM for FT 100. Pragmatic researchers, who merely want to know the lifetime, will answer: some tens of picomoles with relative precision better than 20 % for both spectrometers

References

1. Wahl M.: Time-Correlated Single Photon Counting Tech Note TCSPC 1.1, PicoQuant GmbH, Berlin, Germany 1998

2. Brackmann U.: Lamdachrome® Laser Dyes. 2nd revised ed., Lambda Physik GmbH, Göttingen, Germany, 1997

PicoQuant GmbH
Unternehmen für optoelektronische Forschung und Entwicklung
Rudower Chaussee 29 (IGZ), 12489 Berlin, Germany
Telephone: +49 / (0)30 / 6392 6560
Fax: +49 / (0)30 / 6392 6561
e-mail: photonics@pq.fta-berlin.de
WWW: http://www.picoquant.com