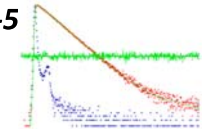


Rapid and efficient fluorescence decay acquisition

***The fast and efficient collection of photons emanating from the fluorescence emission of a sample is a goal of fluorescence lifetime spectroscopy using time-correlated single-photon counting (TCSPC). Historically, TCSPC was considered to be the most sensitive method for fluorescence lifetime determination, but often requiring "long" data acquisition times. This aspect was principally related to source technology (repetition rate limitations) and the 2% start to stop ratio restriction. As excitation source technology has improved, with repetition rates increasing from kHz to many MHz, the dead time of the timing electronics has become significant. Here the coupling of a high repetition rate (up to 100MHz) DeltaDiode lasers with low dead time data acquisition electronics, FluoroHub and DeltaHub, demonstrates the ability to acquire data efficiently and rapidly*.**

TCSPC data acquisition

Time-correlated single-photon counting (TCSPC) can be represented schematically as shown in Fig. 1. The major determining factors in relation to the time resolution are the optical pulse width and the transit time spread of the detector. The timing electronics are also influential and their dead time affects the efficiency of the data collection process.

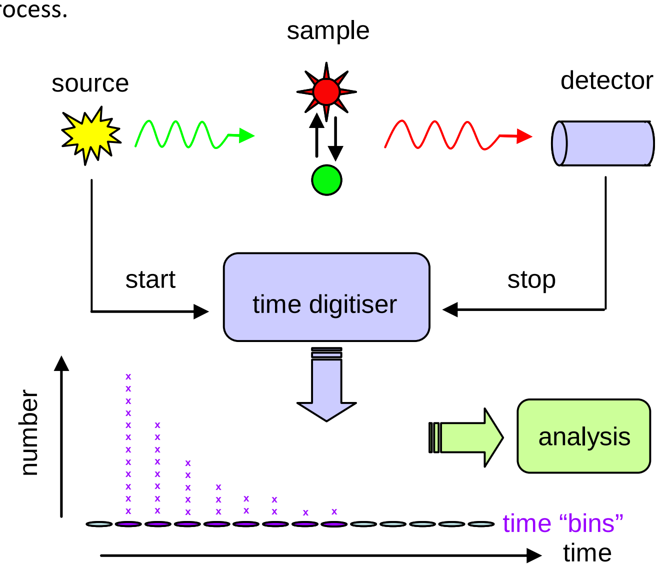


Fig. 1. Schematic representation of a TCSPC set up

The advent of high repetition excitation sources has meant that the processing speed of the timing electronics is now crucial for fast and efficient data collection. The ability of sources, such as the *DeltaDiode*, to operate up to 100MHz requires the timing electronics to be capable of accepting pulses separated by 10ns. This means that the dead time has to be similar, which traditionally has not been the case. Newer electronics, such as the *DeltaHub* and *FluoroHub-B* are examples of units with very short dead time (<10ns). Fig. 2 illustrates the difference in efficiency of converting a "stop" photon into a histogram point for different count (stop) rates with an excitation rate of 5MHz.

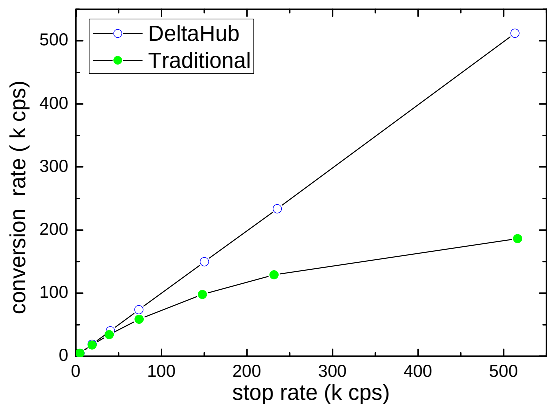
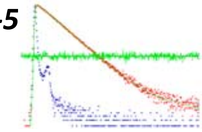


Fig. 2. Difference in converting a stop event into a histogram count, between a traditional time to amplitude converter based electronics and a *DeltaHub*.

From this figure it can be seen that at lower stop rates the two systems are very similar, but at higher rates the traditional system is less efficient which relates to its longer dead time.

Efficient data acquisition with 100MHz excitation rate

A possible application where a fast and efficient data acquisition is necessary is in fluorescence lifetime imaging (FLIM). This enables imaging to be performed on samples that may move position (eg cells in solution) and where photobleaching may be an issue. In this application data is typically not taken to such a high a precision as with cuvette based measurements. At present the longest lifetime that can be measured at this rate is <2ns in order to avoid re-excitation of the sample before it has fully decayed. Previous work has indicated that just under ~200 photons (counts) are required to fit a monoexponential decay.



As a model system, a dye with a lifetime of ~380ps was chosen and the effect of different count rates on the detector ascertained for different collection times. The outcome, along with the recovered lifetime values is shown in Fig. 3, with an excitation rate of 100MHz.

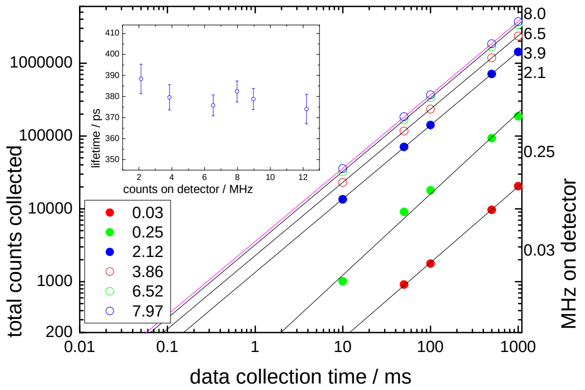


Fig. 3. Effect of collection rate and time on the number of counts obtained and the lifetime (inset). Linear fits are shown for the data points

This shows that within error the lifetime value is the same in all cases. This indicates that, under these conditions for this sample, the influence of pulse pile up is negligible. The linearity also indicates that the collection process is efficient.

Considering the fact that about 200 counts are required to fit a monoexponential decay within a 10% error (see associated references in the paper acknowledged at the end of this note) then it can be estimated that a data collection time of 60μs should be sufficient to model a single exponential decay. To verify this, a decay was measured, see Fig. 4. The lifetime determined using reconvolution analysis and found to be in agreement with the other measurements. This is an example of an extremely rapid TCSPC acquisition and demonstrates the ability of the technique to follow short dynamic processes.

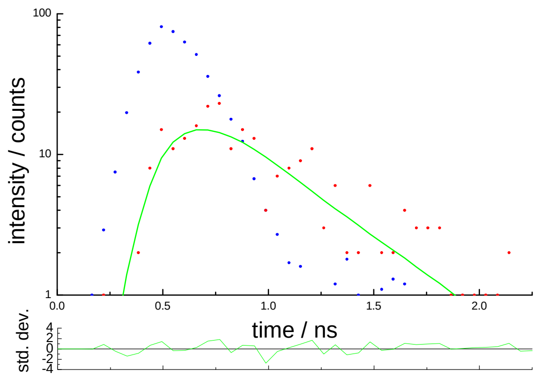


Fig. 4. Fluorescence decay measured in 60μs, along with instrumental response and fitted function. The weighted residuals are also given. The recovered lifetime was 366ps.

Summary

The combination of a high repetition excitation source coupled with low dead time electronics allows the fast and efficient collection of time-resolved fluorescence data. This can be important in the area of microscopy and where fast dynamic processes need to be studied.

The results shown in this note acknowledge the following paper,

D. McLoskey, D. Campbell, A. Allison and G. Hungerford, **2011**. *Fast time-correlated single-photon counting fluorescence lifetime acquisition using a 100 MHz semiconductor excitation source*. Meas. Sci. Technol. 22, 067001.