

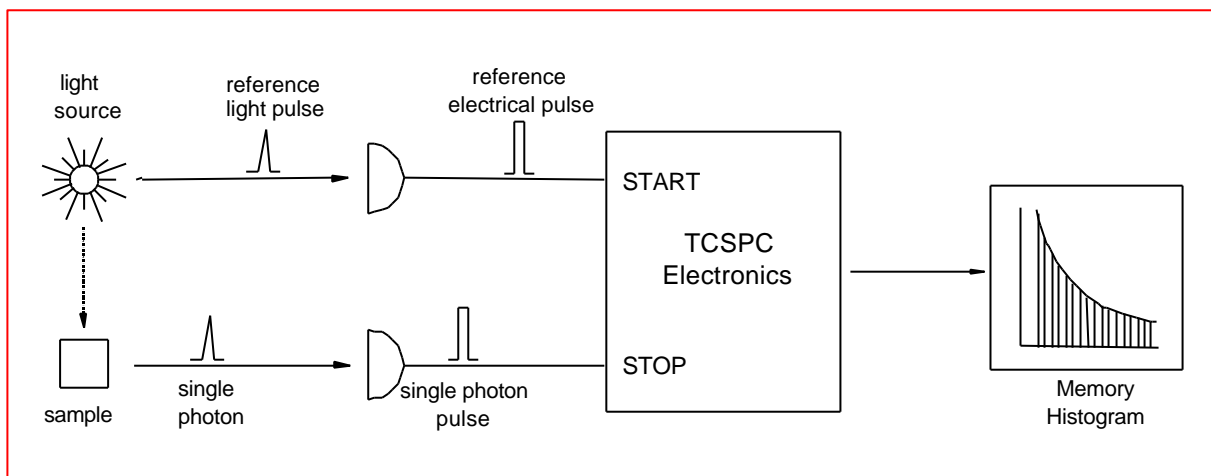
# Technical Note

## What is TCSPC ? (Time Correlated Single Photon Counting)

### 1 PRINCIPLE OF OPERATION

TCSPC is a well established and the most common technique for fluorescence lifetime measurements, but is also becoming increasingly important for photon migration measurements, optical time domain reflectometry measurements and time of flight measurements.

The principle of time correlated single photon counting is the detection of single photons and the measurement of their arrival times in respect to a reference signal, usually the light source. Time correlated single photon counting is a statistical method and a high repetitive light source is needed to accumulate a sufficient number of photon events for a required statistical data precision.

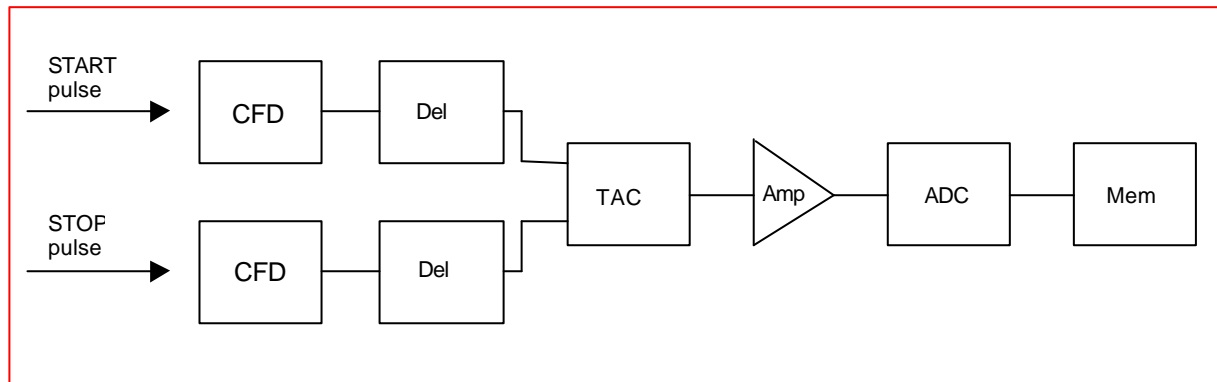


The TCSPC electronics can be compared to a fast stopwatch with two inputs. The clock is started by the START signal pulse and stopped by the STOP signal pulse. The time measured for one START – STOP sequence will be represented by an increase of a memory value in a histogram, in which the channels on the x-axis represent the time. With a high repetitive light source millions of START – STOP sequences can be measured in a short time. The resulting histogram counts versus channels will represent the fluorescence intensity versus time.

Generally, one of the input pulses to the TCSPC electronics (either START and STOP) will be a pulse generated by a single photon. Single photons can be detected with photodetectors having an intrinsic high gain. The majority of those photodetectors are photomultipliers or micro-channel plate photomultipliers, but also single photon avalanche photodiodes. For statistical reasons it is important to ensure no more than one single photon event per light flash is detected. Multi-photon events will affect the histogram statistics and will yield to erroneous measurement results. (This is known in literature as “pulse pile-up problem”.) In order to ensure that only one photon per light flash is detected the photon rate is kept low in comparison to the rate of the exciting lamp, usually 5% or lower.

## 2 TCSPC ELECTRONICS

The main components for signal processing in TCSPC are constant fraction discriminators (CFD), electrical delays (Del), the Time-to-Amplitude Converter (TAC), and Amplifier (Amp), Analogue to Digital Converter (ADC) and digital memory (Mem).



At the input of the electronics, incoming pulses are evaluated in respect to the pulse height. Only pulses higher than a given threshold will be accepted for further signal processing. Thus small amplitude noise pulses are readily eliminated.

The constant fraction discriminators on both the START and the STOP input then analyse the pulse shape of the individual pulses. The portion of the steepest slope of the initial edge on the incoming pulses (generally negative pulses) is taken as a criteria for the temporal position. Which portion of the slope is taken will depend on the fraction, the constant fraction delay (or shaping delay), and the zero crossing level. Threshold, fraction, constant fraction delay, and zero crossing level will depend on the type of detector used and need to be matched to the individual detector.

At the output of the CFD pulses are re-shaped to a standard height and shape. They then can be delayed by an electronic shifting delay. This delay will later result in a left or right shift of the entire measurement on the time axis.

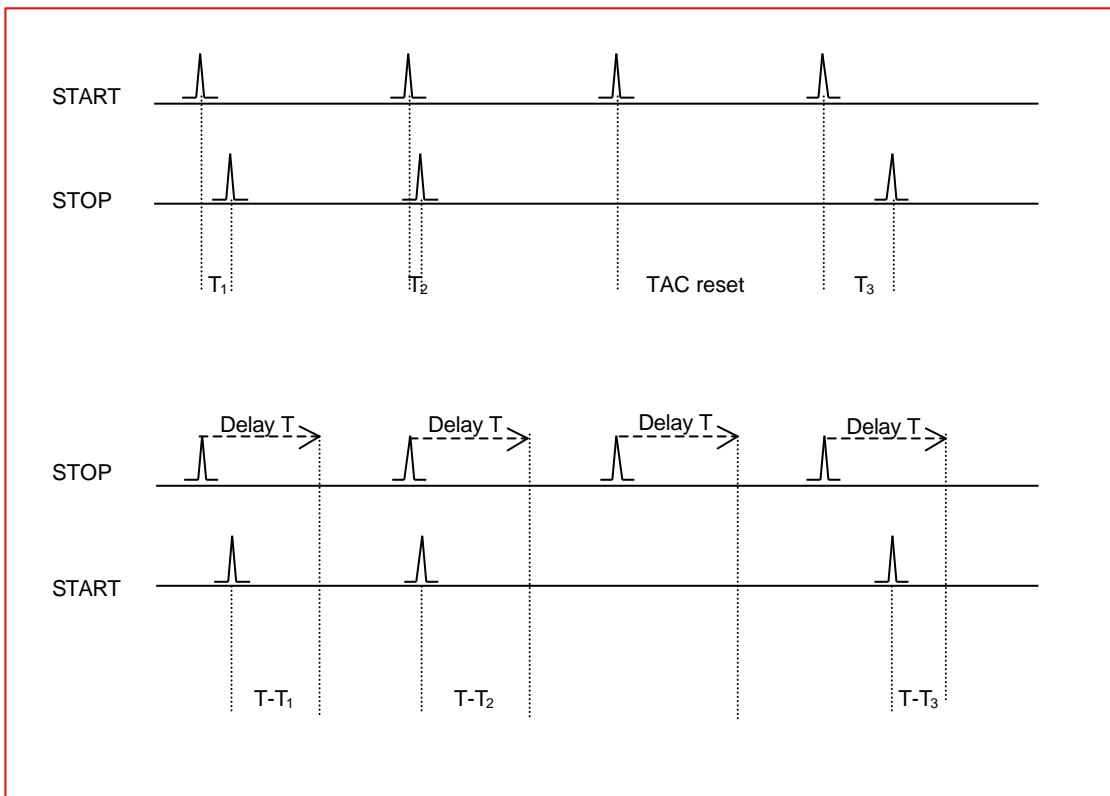
The TAC is the fast clock, started by the START and stopped by the STOP pulse. The START pulse initiates the growth of a ramp signal. Depending on the arrival time of the STOP pulse the ramp will have a lower or higher height. Once the growth of the ramp has been stopped, the level will remain constant for a defined period. The TAC output pulse can then be amplified, thus effectively stretching the time axis. Minimum and maximum available (amplified) TAC amplitude determine the time range.

The amplified TAC output pulse is effectively an analogue pulse of a height corresponding to a measured time of a single START – STOP sequence. For further processing the pulse height will be measured by a digital pulse height measure device, the ADC. The ADC resolution determines, how many discrete time values are possible. All possible measured TAC pulse amplitudes will therefore put into different time bins. The width of the time bin is the ratio of the full time range and the resolution of the ADC in channels. It is the time resolution, usually given in picoseconds / channel or nanoseconds / channel.

### 3 FORWARD AND REVERSE MODE

There are two different operating modes in TCSPC applications: In Forward Mode the pulse rate from the light source is connected to the START input. This rate (with generally equally spaced pulses in time) is substantially higher than the (more or less random) pulses from the detector connected to the STOP input. The advantage of the forward mode is that no shifting delay is required or that it is relatively small and that photon events with long retardation times are represented at the longer time scales, so that an additional inversion of the time scale is not required.

At high rates of the light source, however, the forward mode has a clear disadvantage. Because the vast majority of TAC circles will be started by the START pulse, but never stopped by a STOP signal, it needs to be reset at overflow. The electronics is kept busy more than 20 times than actually needed. For electronic circuits with an upper limit of operating circles per second (given by the dead time of the overall system) this means the highest count rate processed will be reduced by a factor more than 20. To avoid this (and to utilise the full capability of signal processing) TCSPC electronics can be operated in reverse mode, where the signal cable carrying the high count rate from the light source is connected to the STOP input and the low rate is connected to START. The drawback is that the reference pulses from the light source need to be shifted by a usually long shifting delay, so that they arrive at the input of the TAC later than the START pulses from the detector. A rule of thumb is that this delay is slightly longer than the time scale chosen for the measurement. In reverse mode the time axis of the memory histogram is internally reversed in order to see photon events with long retardation times demonstrated at the right part of the time axis.



## 4 TEMPORAL RESOLUTION AND LIFETIME RANGE

TCSPC can be used to measure lifetimes ranging from approx. 5ps to over 50µs (over 7 orders of magnitude). The lower limit is given by the jitter of the TCSPC electronics, while the upper limit arises from the time one can afford to acquire data with reasonable precision.

However, in most cases not the TCSPC technique itself sets the limits for temporal resolution and lifetime range measurable, but the light source and the detector in use.

The lower limit of the lifetime range (or temporal resolution –  $\tau_{min}$ ) can be estimated with the following equation:

$$\tau_{min} = \frac{1}{10} \sqrt{FWHM_{Light\ Source}^2 + FWHM_{TTS\ Detector}^2 + FWHM_{Electronics\ Jitter}^2}$$

The sum of squares is an estimate for the width of the instrumental response function (which can be measured by replacing the fluorescent sample by a scattering medium). The factor 1/10 represents the fact that with numerical reconvolution (see our Technical Note: Numerical Reconvolution of Fluorescence Lifetime Data) lifetimes as short as approximately 10% of the width of the instrumental response can be resolved. Often the width of the exciting light pulses ( $FWHM_{Light\ Source}$ ) and the width of the detector response ( $FWHM_{TTS\ Detector}$ ) set the limits of the temporal resolution.

The upper limit of the lifetime range will be set by the pulse repetition frequency of the exciting light source, but it will also be affected by the dark count rate of the detector. If the fluorescence does not have sufficient time to decay down to Zero before the next excitation pulse hits the sample, than a stationary background signal will build up, thus limiting the dynamic range. If the decay process is allowed to continue until the signal is less than 1/10000 of the peak count, than the longest measurable lifetime ( $\tau_{max}$ ) can be estimated as follows:

$$\tau_{min} = \frac{1}{f_{Light\ Source}}$$

Equally, a background will build up during the time of acquiring the data, when the detector has a high number of dark counts. And similarly to the repetition rate problem this will limit the dynamic range. It is rather complex to cover this dark count rate background with the equation above as several other parameters would need to be considered. In addition, the amount of background signal tolerated by the user is quite often a matter of “taste”. As the rule of thumb: The bigger the number of dark counts, the less accurate measurements in the long time range (particularly with weak emitting samples).

The graph below shows examples for different light source / detector combinations. Please refer also to our detailed technical notes on detectors and light sources for TCSPC.

